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(54) **METHOD OF DETECTING ANTIBODIES TO HCV**

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(52) U.S. Cl. **435/5**

(57) **ABSTRACT**

A family of cDNA sequences derived from hepatitis C virus (HCV) are provided. These sequences encode antigens which react immunologically with antibodies present in individuals with non-A non-B hepatitis (NANBH), but which are absent from individuals infected with hepatitis A virus, or hepatitis B virus, and also are absent in control individuals. The HCV cDNA sequences lack substantial homology to the sequences of hepatitis delta virus (HDV) and HBV. A comparison of the sequences of amino acids encoded in the HCV cDNA with the sequences of Flaviviruses indicates that HCV may be related to the Flaviviruses.

The HCV cDNA sequences and the polypeptides encoded therein are useful as reagents for the detection and therapy of HCV. The reagents provided in the invention are also useful for the isolation of NANBH agent(s), for the propagation of these agents in tissue culture, and for the screening of antiviral agents for HCV.

FIG. 1   Translation of DNA 5-1-1

```
       AlaSerCysLeuAsnCysSerAlaSerIleIleProAspArgGluValLeuTyrArgGlu
  1    GGCCTCCTGCTTGAACTGCTCGGCGAGCATCATACCTGACAGGGAAGTCCTCTACCGAGA
       CCGGAGGACGAACTTGACGAGCCGCTCGTAGTATGGACTGTCCCTTCAGGAGATGGCTCT
```

```
       PheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeu
  61   GTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCT
       CAAGCTACTCTACCTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGA
```

```
       AlaGluGlnPheLysGlnLysAlaLeuGlyLeu
  121  CGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCC
       GCGGCTCGTCAAGTTCGTCTTCCGGGAGCCGGAGG
```

FIG. 3   Translation of DNA 5-1-1,81,91&1-2

```
       GlyCysValValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAsp
  1    CTGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAATCATACCTG
       GACCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGAC
```

```
                            T
       ArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyr
  61   ACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGT
       TGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCGTGAATGGCA
                            A
```

```
       IleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGln
  121  ACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGC
       TGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCCGGAGGACG
```

```
       ..ThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeu
  181  AGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTGGCAAAAAC
       TCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTG
```

```
       GluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGly
  241  TCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATACTTGGCGG
       AGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTATGAACCGCC
```

```
       LeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaVal
  301  GCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTG
       CGAACAGTTGCGACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAAATGTCGACGAC
```

```
       ThrSerProLeuThrThrSerGln
  361  TCACCAGCCCACTAACCACTAGCCAAA
       AGTGGTCGGGTGATTGGTGATCGGTTT
```

FIG. 2.

FIG. 4   Translation of DNA 81

```
        SerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMet
  1   GTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGAT
      CAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTA

        GluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPhe
 61   GGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTT
      CCTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAA

        LysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaPro
121   CAAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCC
      GTTCGTCTTCCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGG

        AlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPhe
181   TGCTGTCCAGACCAACTGGCAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTT
      ACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAA

        IleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAla
241   CATCAGTGGGATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGC
      GTAGTCACCCTATGTTATGAACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACG

        SerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln
301   TTCATTGATGGCTTTTACAGCTGCTGTCACCAGCCCACTAACCACTAGCCAAA
      AAGTAACTACCGAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGGTTT
```

FIG. 5   Translation of DNA 36

```
        AspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAla
  1   GATGCCCACTTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCG
      CTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGC

        TyrGlnAlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrp
 61   TACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGG
      ATGGTTCGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACC

        LysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeu
121   AAGTGTTTGATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTG
      TTCACAAACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGAC

        GlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCys
181   GGCGCTGTTCAGAATGAAATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGC
      CCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACG

        MetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAla
241   ATGTCGGCCGACCTGGAGGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCT
      TACAGCCGGCTGGACCTCCAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGA

        AlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArgValValLeu
301   GCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTG
      CGAAACCGGCGCATAACGGACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAAC

        -----------Overlap with 81]-------------------
        SerGlyLysProAlaIleIleProAspArgGluValLeuTyrArg
361   TCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAG
      AGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTC
```

FIG. 6   Combined ORF of DNAs 36 & 81

```
       AspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAla
  1    GATGCCCACTTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCG
       CTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGC

       TyrGlnAlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrp
 61    TACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGG
       ATGGTTCGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACC

       LysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeu
121    AAGTGTTTGATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTG
       TTCACAAACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGAC

       GlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCys
181    GGCGCTGTTCAGAATGAAATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGC
       CCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACG

       MetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAla
241    ATGTCGGCCGACCTGGAGGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCT
       TACAGCCGGCTGGACCTCCAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGA

       AlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArgValValLeu
301    GCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTG
       CGAAACCGGCGCATAACGGACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAAC

       SerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMet
361    TCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATG
       AGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTAC

       GluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPhe
421    GAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTC
       CTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAG

       LysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaPro
481    AAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCT
       TTCGTCTTCCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGA

       AlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPhe
541    GCTGTCCAGACCAACTGGCAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTC
       CGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAG

       IleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAla
601    ATCAGTGGGATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCT
       TAGTCACCCTATGTTATGAACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACGA

       SerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln
661    TCATTGATGGCTTTTACAGCTGCTGTCACCAGCCCACTAACCACTAGCCAAA
       AGTAACTACCGAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGGTTT
```

FIG. 7   Translation of DNA 32

```
      ------Overlap with 81--------------------
      PheThrAlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIleLeu
  1   CTTTTACAGCTGCTGTCACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCAACATAT .
      GAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTATA

      GlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAla
  61  TGGGGGGGTGGGTGGCTGCCCAGCTCGCCGCCCCGGTGCCGCTACTGCCTTTGTGGGCG
      ACCCCCCCACCCACCGACGGGTCGAGCGGCGGGGGCCACGGCGATGACGGAAACACCCGC

      GlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeu
  121 CTGGCTTAGCTGGCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAGACATCC
      GACCGAATCGACCGCGGCGGTAGCCGTCACAACCTGACCCCTTCCAGGAGTATCTGTAGG

      AlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGlu
  181 TTGCAGGGTATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTG
      AACGTCCCATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCAC

      ValProSerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeu
  241 AGGTCCCCTCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCC
      TCCAGGGGAGGTGCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGG

      ValValGlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAla
  301 TCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGG
      AGCATCAGCCGCACCAGACACGTCGTTATGACGCGGCCGTGCAACCGGGCCCGCTCCCCC

      ValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSer
  361 CAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCC
      GTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGG
```

FIG. 8 Translation of DNA 35

```
      SerIleGluThrIleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArg
  1   TCCATTGAGACAATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCAGG
      AGGTAACTCTGTTAGTGCGAGGGGGTCCTACGACAGAGGGCGTGAGTTGCAGCCCCGTCC


      ThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGly
 61   ACTGGCAGGGGGAAGCCAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCGGC
      TGACCGTCCCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCCG


      MetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeu
121   ATGTTCGACTCGTCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTC
      TACAAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAG


      ThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProVal
181   ACGCCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCGTG
      TGCGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCAC


                                                       ------
      CysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAla
241   TGCCAGGACCATCTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCC
      ACGGTCCTGGTAGAACTTAAAACCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGG


      ------------------------------------------------------------
      HisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGln
301   CACTTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAA
      GTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGGTT


      -----------------Overlap with 36--------------------------------
      -AlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCys
361   GCCACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGT
      CGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCACA


      ------------------------------------------------------------------
      LeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAla
421   TTGATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCT
      AACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGCGA
```

FIG. 9-1 Combined ORF of DNAs 35,36,81 & 32

```
        SerIleGluThrIleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArg
   1    TCCATTGAGACAATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCAGG
        AGGTAACTCTGTTAGTGCGAGGGGGTCCTACGACAGAGGGCGTGAGTTGCAGCCCCGTCC

        ThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGly
   61   ACTGGCAGGGGGAAGCCAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCGGC
        TGACCGTCCCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCCG

        MetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeu
   121  ATGTTCGACTCGTCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTC
        TACAAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAG

        ThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProVal
   181  ACGCCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCGTG
        TGCGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCAC

        CysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAla
   241  TGCCAGGACCATCTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCC
        ACGGTCCTGGTAGAACTTAAAACCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGG

        HisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGln
   301  CACTTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAA
        GTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGGTT

        AlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCys
   361  GCCACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGT
        CGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCACA

        LeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAla
   421  TTGATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCT
        AACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGCGA

        ValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSer
   481  GTTCAGAATGAAATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCG
        CAAGTCTTACTTTAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACGTACAGC

        AlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeu
   541  GCCGACCTGGAGGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGCTGCTTTG
        CGGCTGGACCTCCAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGACGAAAC

        AlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArgValValLeuSerGly
   601  GCCGCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCGGG
        CGGCGCATAACGGACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGCCC

        LysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGlu
   661  AAGCCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAG
        TTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTC

        CysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGln
   721  TGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAG
        ACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTC

        LysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaVal
   781  AAGGCCCTCGGCCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTC
        TTCCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAG
```

FIG. 10   Translation of DNA 37b

```
        LeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAsp
    1   CTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGTCTTGAC
        GAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCAGAACTG


        ValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeuMetThr
   61   GTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGTCGTGGCAACCGATGCCCTCATGACC
        CACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAGCACCGTTGGCTACGGGAGTACTGG


        GlyTyrThrGlyAspPheAspSerValIleAspTyrAsnThrCysValThrGlnThrVal
  121   GGCTATACCGGCGACTTCGACTCGGTGATAGACTACAATACGTGTGTCACCCAGACAGTC
        CCGATATGGCCGCTGAAGCTGAGCCACTATCTGATGTTATGCACACAGTGGGTCTGTCAG

                                   ------------------------Overlap with
        AspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAspAlaVal
  181   GATTTCAGCCTTGACCCTACCTTCACCATTGAGACAATCACGCTCCCCCAGGATGCTGTC
        CTAAAGTCGGAACTGGGATGGAAGTGGTAACTCTGTTAGTGCGAGGGGGTCCTACGACAG


        clone 35--------------------
        SerArgThrGlnArgArgGlyArgThr
  241   TCCCGCACTCAACGTCGGGGCAGGACTG
        AGGGCGTGAGTTGCAGCCCCGTCCTGAC
```

FIG. 11   Translation of DNA 33b

```
        -------Overlap with 32----------------------------
        MetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrVal
    1   GATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCCCACGCACTACGT
        CTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCCTTGGTACAAAGGGGGTGCGTGATGCA


        ProGluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGln
   61   GCCGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACCCA
        CGGCCTCTCGCTACGTCGACGGGCGCAGTGACGGTATGAGTCGTCGGAGTGACATTGGGT


        LeuLeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThrProCysSerGlySer
  121   GCTCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGGTTC
        CGAGGACTCCGCTGACGTGGTCACCTATTCGAGCCTCACATGGTGAGGTACGAGGCCAAG


        TrpLeuArgAspIleTrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeu
  181   CTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTGGCT
        GACCGATTCCCTGTAGACCCTGACCTATACGCTCCACAACTCGCTGAAATTCTGGACCGA


        LysAlaLysLeuMetProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyr
  241   AAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCTTTGTGTCCTGCCAGCGCGGGTA
        TTTTCGATTCGAGTACGGTGTCGACGGACCCTAGGGGAAACACAGGACGGTCGCGCCCAT


        LysGlyValTrpArgVal
  301   TAAGGGGGTCTGGCGAGTG
        ATTCCCCCAGACCGCTCAC
```

FIG. 12   Translation of DNA 40b

```
      AlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIle
  1   GGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAAT
      CCGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCCTGGCCCCACTCTTGTTA

      ThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCys
 61   TACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGTG
      ATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCCGCCCAC

      SerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSerThrAspAlaThrSer
121   CTCGGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACATC
      GAGCCCCCCGCGAATACTGTATTATTAAACACTGCTCACGGTGAGGTGCCTACGGTGTAG

      IleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValVal
181   CATCTTGGGCATCGGCACTGTCCTTGACCAAGCAGAGACTGCGGGGGCGAGACTGGTTGT
      GTAGAACCCGTAGCCGTGACAGGAACTGGTTCGTCTCTGACGCCCCCGCTCTGACCAACA

      LeuAlaThrAlaThrProProGlySerValThrValProHisProAsnIleGluGluVal
241   GCTCGCCACCGCCACCCCTCCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGGT
      CGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGTGACACGGGGTAGGGTTGTAGCTCCTCCA

      AlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIle
301   TGCTCTGTCCACCACCGGAGAGATCCCTTTTTACGGCAAGGCTATCCCCCTCGAAGTAAT
      ACGAGACAGGTGGTGGCCTCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATTA

                                                        --------
      LysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAla
361   CAAGGGGGGGAGACATCTCATCTTCTGTCATTCAAAGAAGAAGTGCGACGAACTCGCCGC
      GTTCCCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTCACGCTGCTTGAGCGGCG

      -------------------------Overlap with 37b-------------------------
      LysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerVal
421   AAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGTCTTGACGTGTCCGT
      TTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCA

            --------
      IleProThr
481   CATCCCGACCAG
      GTAGGGCTGGTC
```

FIG. 13 Translation of DNA 25c

```
      ---------------------------------------------------------------
      CysSerLeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSerSerGluCys
  1   ACTGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGT
      TGACGTCGGAGTGACATTGGGTCGAGGACTCCGCTGACGTGGTCACCTATTCGAGCCTCA


      ---------------------------------------------------------------
      ThrThrProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCysGluValLeu
  61  GTACCACTCCATGCTCCGGTTCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGT
      CATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCCTGACCTATACGCTCCACA.


      ----------------Overlap with 33b----------------------------
      SerAspPheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGlyIleProPhe
  121 TGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCT
      ACTCGCTGAAATTCTGGACCGATTTTCGATTCGAGTACGGTGTCGACGGACCCTAGGGGA


      -------------------------------------------------------------
      ValSerCysGlnArgGlyTyrLysGlyValTrpArgGlyAspGlyIleMetHisThrArg
  181 TTGTGTCCTGCCAGCGCGGGTATAAGGGGGTCTGGCGAGGGGACGGCATCATGCACACTC
      AACACAGGACGGTCGCGCCCATATTCCCCCAGACCGCTCCCCTGCCGTAGTACGTGTGAG


      CysHisCysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArgIleValGly
  241 GCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCG
      CGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTTTTGCCCTGCTACTCCTAGCAGC


      ProArgThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGly
  301 GTCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGG
      CAGGATCCTGGACGTCCTTGTACACCTCACCCTGGAAGGGGTAATTACGGATGTGGTGCC


      ProCysThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGlu
  361 GCCCCTGTACCCCCCTTCCTGCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAG
      - CGGGGACATGGGGGGAAGGACGCGGCTTGATGTGCAAGCGCGATACCTCCCACAGACGTC


      GluTyrValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMetThrThrAsp
  421 AGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTG
      TCCTTATACACCTCTATTCCGTCCACCCCCTGAAGGTGATGCACTGCCCATACTGATGAC


      AsnLeuLysCysProCysGlnValProSerProGluPhePheThrGlu
  481 ACAATCTCAAATGCCCGTGCCAGGTCCCATCGCCCGAATTTTTCACAGAAT
      TGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTA
```

FIG. 14-1 Combined ORF of DNAs 40b/37b/35/36/81/32/33b/25c

```
       AlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIle
  1   TGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAAT
      ACGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCCTGGCCCCACTCTTGTTA

       ThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCys
 61   TACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGTG
      ATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCCGCCCAC

       SerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSerThrAspAlaThrSer
121   CTCGGGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACATC
      GAGCCCCCCGCGAATACTGTATTATTAAACACTGCTCACGGTGAGGTGCCTACGGTGTAG

       IleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValVal
181   CATCTTGGGCATCGGCACTGTCCTTGACCAAGCAGAGACTGCGGGGGCGAGACTGGTTGT
      GTAGAACCCGTAGCCGTGACAGGAACTGGTTCGTCTCTGACGCCCCCGCTCTGACCAACA

       LeuAlaThrAlaThrProProGlySerValThrValProHisProAsnIleGluGluVal
241   GCTCGCCACCGCCACCCCTCCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGGT
      CGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGTGACACGGGGTAGGGTTGTAGCTCCTCCA

       AlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIle*
301   TGCTCTGTCCACCACCGGAGAGATCCCTTTTTACGGCAAGGCTATCCCCCTCGAAGTAAT
      ACGAGACAGGTGGTGGCCTCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATTA

       LysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAla
361   CAAGGGGGGGGAGACATCTCATCTTCTGTCATTCAAAGAAGAAGTGCGACGAACTCGCCGC
      GTTCCCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTCACGCTGCTTGAGCGGCG

       LysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerVal
421   AAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGTCTTGACGTGTCCGT
      TTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCA

       IleProThrSerGlyAspValValValValAlaThrAspAlaLeuMetThrGlyTyrThr
481   CATCCCGACCAGCGGCGATGTTGTCGTCGTGGCAACCGATGCCCTCATGACCGGCTATAC
      GTAGGGCTGGTCGCCGCTACAACAGCAGCACCGTTGGCTACGGGAGTACTGGCCGATATG

       GlyAspPheAspSerValIleAspTyrAsnThrCysValThrGlnThrValAspPheSer
541   CGGCGACTTCGACTCGGTGATAGACTACAATACGTGTGTCACCCAGACAGTCGATTTCAG
      GCCGCTGAAGCTGAGCCACTATCTGATGTTATGCACACAGTGGGTCTGTCAGCTAAAGTC

       LeuAspProThrPheThrIleGluThrIleThrLeuProGlnAspAlaValSerArgThr
601   CCTTGACCCTACCTTCACCATTGAGACAATCACGCTCCCCCAGGATGCTGTCTCCCGCAC
      GGAACTGGGATGGAAGTGGTAACTCTGTTAGTGCGAGGGGGTCCTACGACAGAGGGCGTG

       GlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGly
661   TCAACGTCGGGGCAGGACTGGCAGGGGGAAGCCAGGCATCTACAGATTTGTGGCACCGGG
      AGTTGCAGCCCCGTCCTGACCGTCCCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCC

       GluArgProSerGlyMetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCys
721   GGAGCGCCCCTCCGGCATGTTCGACTCGTCCGTCCTCTGTGAGTGCTATGACGCAGGCTG
      CCTCGCGGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGAC

       AlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThr
781   TGCTTGGTATGAGCTCACGCCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACAC
      ACGAACCATACTCGAGTGCGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTG

       ProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeu
841   CCTGGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTTGGGAGGGCGTCTTTACAGGCCT
      GGGCCCCGAAGGGCACACGGTCCTGGTAGAACTTAAAACCCTCCCGCAGAAATGTCCGGA

       ThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyr
901   CACTCATATAGATGCCCACTTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTA
      GTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAAT
```

```
          LeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAsp
  961  CCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGA
       GGACCATCGCATGGTTCGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCCT

          GlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeu
 1021  CCAGATGTGGAAGTGTTTGATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGCT
       GGTCTACACCTTCACAAACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGA

          TyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIle
 1081  ATACAGACTGGGCGCTGTTCAGAATGAAATCACCCTGACGCACCCAGTCACCAAATACAT
       TATGTCTGACCCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGTCAGTGGTTTATGTA

          MetThrCysMetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGly
 1141  CATGACATGCATGTCGGCCGACCTGGAGGTCGTCACGAGCACCTGGGTGCTCGTTGGCGG
       GTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGCTCGTGGACCCACGAGCAACCGCC

          ValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArg
 1201  CGTCCTGGCTGCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCAG
       GCAGGACCGACGAAACCGGCGCATAACGGACAGTTGTCCGACGCACCAGTATCACCCGTC

          ValValLeuSerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPhe
 1261  GGTCGTCTTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAGAGTT
       CCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAA

          AspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAla
 1321  CGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGC
       GCTACTCTACCTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGCG

          GluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluVal
 1381  CGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGT
       GCTCGTCAAGTTCGTCTTCCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCA

          IleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMet
 1441  TATCGCCCCTGCTGTCCAGACCAACTGGCAAAAACTCGAGACCTTCTGGGCGAAGCATAT
       ATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCCGCTTCGTATA

          TrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnPro
 1501  GTGGAACTTCATCAGTGGGATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACCC
       CACCTTGAAGTAGTCACCCTATGTTATGAACCGCCCGAACAGTTGCGACGGACCATTGGG

          AlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln
 1561  CGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTCACCAGCCCACTAACCACTAGCCA
       GCGGTAACGAAGTAACTACCGAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGGT

          ThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAla
 1621  AACCCTCCTCTTCAACATATTGGGGGGGTGGGTGGCTGCCCAGCTCGCCGCCCCCGGTGC
       TTGGGAGGAGAAGTTGTATAACCCCCCCACCCACCGACGGGTCGAGCGGCGGGGGCCACG

          AlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGly
 1681  CGCTACTGCCTTTGTGGGCGCTGGCTTAGCTGGCGCCGCCATCGGCAGTGTTGGACTGGG
       GCGATGACGGAAACACCCGCGACCGAATCGACCGCGGCGGTAGCCGTCACAACCTGACCC

          LysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAla
 1741  GAAGGTCCTCATAGACATCCTTGCAGGGTATGGCGCGGGGCGTGGCGGGAGCTCTTGTGGC
       CTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCGCCCTCGAGAACACCG

          PheLysIleMetSerGlyGluValProSerThrGluAspLeuValAsnLeuLeuProAla
 1801  ATTCAAGATCATGAGCGGTGAGGTCCCCTCCACGGAGGACCTGGTCAATCTACTGCCCGC
       TAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCCTCCTGGACCAGTTAGATGACGGGCG

          IleLeuSerProGlyAlaLeuValValGlyValValCysAlaAlaIleLeuArgArgHis
 1861  CATCCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCCGGCA
       GTAGGAGAGCGGGCCTCGGGAGCATCAGCCGCACCAGACACGTCGTTATGACGCGGCCGT
```

FIG. 14-2

```
          ValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArg
```

```
1921  CGTTGGCCCGGGCGAGGGGGCAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCTCCCG
      GCAACCGGGCCCGCTCCCCCGTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGC


      GlyAsnHisValSerProThrHisTyrValProGluSerAspAlaAlaAlaArgValThr
1981  GGGGAACCATGTTTCCCCCACGCACTACGTGCCGGAGAGCGATGCAGCTGCCCGCGTCAC
      CCCCTTGGTACAAAGGGGGTGCGTGATGCACGGCCTCTCGCTACGTCGACGGGCGCAGTG


      AlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSer
2041  TGCCATACTCAGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTGCACCAGTGGATAAG
      ACGGTATGAGTCGTCGGAGTGACATTGGGTCGAGGACTCCGCTGACGTGGTCACCTATTC


      SerGluCysThrThrProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCys
2101  CTCGGAGTGTACCACTCCATGCTCCGGTTCCTGGCTAAGGGACATCTGGGACTGGATATG ·
      GAGCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCCTGACCTATAC


      GluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGly
2161  CGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATGCCACAGCTGCCTGG
      GCTCCACAACTCGCTGAAATTCTGGACCGATTTTCGATTCGAGTACGGTGTCGACGGACC


      IleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArgValAspGlyIleMet
2221  GATCCCCTTTGTGTCCTGCCAGCGCGGGTATAAGGGGGTCTGGCGAGTGGACGGCATCAT
      CTAGGGGAAACACAGGACGGTCGCGCCCATATTCCCCCAGACCGCTCACCTGCCGTAGTA


      HisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArg
2281  GCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGAG
      CGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTTTTGCCCTGCTACTC


      IleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyr
2341  GATCGTCGGTCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTCCCCATTAATGCCTA
      CTAGCAGCCAGGATCCTGGACGTCCTTGTACACCTCACCCTGGAAGGGGTAATTACGGAT


      ThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgVal
2401  CACCACGGGCCCCTGTACCCCCCCTTCCTGCGCCGAACTACACGTTCGCGCTATGGAGGGT
      GTGGTGCCCGGGGACATGGGGGGGAAGGACGCGGCTTGATGTGCAAGCGCGATACCTCCCA


      SerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMet
2461  GTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCACTACGTGACGGGTAT
      CAGACGTCTCCTTATACACCTCTATTCCGTCCACCCCCTGAAGGTGATGCACTGCCCATA


      ThrThrAspAsnLeuLysCysProCysGlnValProSerProGluPhePheThrGlu
2521  GACTACTGACAATCTCAAATGCCCGTGCCAGGTCCCATCGCCCGAATTTTTCACAGAAT
      CTGATGACTGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTA
```

FIG. 14-3

FIG. 15   Translation of DNA 33c

```
       AlaValAspPheIleProValGluAsnLeuGluThrThrMetArgSerProValPheThr
  1    GGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCAC
       CCGCCACCTGAAATAGGGACACCTCTTGGATCTCTGTTGGTACTCCAGGGGCCACAAGTG

       AspAsnSerSerProProValValProGlnSerPheGlnValAlaHisLeuHisAlaPro
  61   GGATAACTCCTCTCCACCAGTAGTGCCCCAGAGCTTCCAGGTGGCTCACCTCCATGCTCC
       CCTATTGAGGAGAGGTGGTCATCACGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGG

       ThrGlySerGlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysVal
  121  CACAGGCAGCGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGT
       GTGTCCGTCGCCGTTTTCGTGGTTCCAGGGCCGACGTATACGTCGAGTCCCGATATTCCA
                                                              _____

       LeuValLeuAsnProSerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAla
  181  GCTAGTACTCAACCCCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCAAGGC
       CGATCATGAGTTGGGGAGACAACGACGTTGTGACCCGAAACCACGAATGTACAGGTTCCG
       _____Overlap with 40b_____
       HisGlyIleAspProAsnIleArgThrGlyValArgThrIleThrThrGlySerProIle
  241  TCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCCCCAT
       AGTACCCTAGCTAGGATTGTAGTCCTGGCCCCACTCTTGTTAATGGTGACCGTCGGGGTA .
       _____

       ThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAsp
  301  CACGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGCGCTTATGA
       GTGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCCGCCCACGAGCCCCCCGCGAATACT
       _____

       IleIleIleCysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThr
  361  CATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATTGGCAC
       GTATTATTAAACACTGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTAACCGTG
       _____

       ValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrPro
  421  TGTCCTTGACCAAGCAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCACCCC
       ACAGGAACTGGTTCGTCTCTGACGCCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGG
       _____

       ProGlySerValThrValProHisProAsnIleGluGluValAlaLeuSerThrThrGly
  481  TCCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCACCGG
       AGGCCCGAGGCAGTGACACGGGGTAGGGTTGTAGCTCCTCCAACGAGACAGGTGGTGGCC
       _____

       GluIleProPheTyrGlyLysAlaIleProLeuGluValIleLysGlyGlyArgHisLeu
  541  AGAGATCCCTTTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGGAGACATCT
       TCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCCCTCTGTAGA
       _____

       IlePheCysHisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGly
  601  CATCTTCTGTCATTCAAAGAAGAAGTGCGACGAACTCGCCGCAAAGCTGGTCGCATTGGG
       GTAGAAGACAGTAAGTTTCTTCTTCACGCTGCTTGAGCGGCGTTTCGACCAGCGTAACCC
       _____

       IleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAsp
  661  CATCAATGCCGTGGCCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCGGCGA
       GTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTCGCCGCT
       _____

       ValValValValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerVal
  721  TGTTGTCGTCGTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACTCGGT
       ACAACAGCAGCACCGTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCA

       IleAspCysAsnThrCys
  781  GATAGACTGCAATACGTGTG
       CTATCTGACGTTATGCACAC
```

FIG. 16   Translation of DNA 8h

```
      ProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIlePro
  1   CTCCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATTC
      GAGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAGTGCTCCGTGCGGCTACAGTAAG
```

```
      ValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyrLeu
 61   CCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACT
      GGCACGCGGCCGCCCCACTATCGTCCCCGTCGGACGACAGCGGGGCCGGGTAAAGGATGA
```

```
      LysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePheArg
121   TGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTTA
      ACTTTCCGAGGAGCCCCCCAGGCGACAACACGGGGCGCCCCGTGCGGCACCCGTATAAAT
```

```
                                              ─────────────────Overlap with
      AlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsnLeu
181   GGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAACC .
      CCCGGCGCCACACGTGGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTGG *
```

```
      33c─────────────────────────────────────
      GluThrThrMetArgSerProValPheThrAspAsnSer
241   TAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTCCTC
      ATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAG
```

FIG. 17   Translation of DNA 7e

```
      GlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeuGly
  1   GGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGG
      CCCCACCTCCAACGACCGCGGGTAGTGCCGCATGCGGGTCGTCTGTTCCCCGGAGGATCC
```

```
      CysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGlnIle
 61   GTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGAT
      CACGTATTAGTGGTCGGATTGACCGGCCCTGTTTTTGGTTCACCTCCCACTCCAGGTCTA
```

```
      ValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThrVal
121 - TGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACTGT
      ACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCACACGACCTGACA
```

```
      TyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMetTyr
181   CTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATGTA
      GATGGTGCCCCGGCCTTGCTCCTGGTAGCGCAGTGGGTTCCCAGGACAGTAGGTCTACAT
```

```
      ThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeuThr
241   TACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCGCAAGGTAGCCGCTCATTGAC
      ATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGGCGAGTAACTG
```

```
      ─────────Overlap with 8h─────────────────────
      ProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHis
301   ACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACG
      TGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAGTGCTCCGTGC
```

FIG. 18 Translation of DNA 14c

```
       AsnMetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGlyProCysThrProLeu
  1    GACATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCTGTACCCCCCT
       CTTGTACACCTCACCCTGGAAGGGGTAATTACGGATGTGGTGCCCGGGGACATGGGGGGA


       ----------------Overlap with 25c----------------
       ProAlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyrValGluIle
 61    TCCTGCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAAATACGTGGAGAT
       AGGACGCGGCTTGATGTGCAAGCGCGATACCTCCCACAGACGTCTCCTTATGCACCTCTA


       ArgGlnValGlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeuLysCysPro
121    AAGGCAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATCTTAAAGCCC
       TTCCGTCCACCCCCTGAAGGTGATGCACTGCCCATACTGATGACTGTTAGAATTTACGGG


       CysGlnValProSerProGluPhePheThrGluLeuAspGlyValArgLeuHisArgPhe
181    GTGCCAGGTCCCATCGCCCGAATTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTT
       CACGGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAA


       AlaProProCysLysProLeuLeuArgGluGluValSerPheArgValGlyLeuHisGlu
241    TGCGCCCCCCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGA
       ACGCGGGGGGACGTTCGGGAACGACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCT


       TyrProValGlySerGlnLeuProCysGluProGluProAspValAlaValLeuThrSer
301    ATACCCGGTAGGGTCGCAATTACCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTC
       TATGGGCCATCCCAGCGTTAATGGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAG


       MetLeuThrAspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGly
361    CATGCTCACTGATCCCTCCCATATAACAGCAGAGGCGGCCGGGCGAAGGTTGGCGAGGGG
       GTACGAGTGACTAGGGAGGGTATATTGTCGTCTCCGCCGGCCCGCTTCCAACCGCTCCCC


       SerProProSerValAlaSerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAla
421    ATCACCCCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGC
       TAGTGGGGGGAGACACCGGTCGAGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCG


       ThrCysThrAlaAsnHisAspSerProAsp
481    AACTTGCACCGCTAACCATGACTCCCTGAT
       TTGAACGTGGCGATTGGTACTGAGGGACTA
```

FIG. 19   Translation of DNA 8f

```
                        ----------------------------------Overlap with 14c----------------
        SerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHis
   1    AGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCGCTAACCAT
        TCGAGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGTGGCGATTGGTA


        AspSerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGly
  61    GACTCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGGCGGC
        CTGAGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCTCTACCCGCCG


        AsnIleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeu
 121    AACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTT
        TTGTAGTGGTCCCAACTCAGTCTTTTGTTTCACCACTAAGACCTGAGGAAGCTAGGCGAA


        ValAlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArg
 181    GTGGCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAATCCTGCGGAAGTCTCGG
        CACCGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCCTTCAGAGCC


        ArgPheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGlu
 241    AGATTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAACCCCCCGCTAGTGGAG
        TCTAAGCGGGTCCGGGACGGGCAAACCCGCGCCGGCCTGATATTGGGGGGCGATCACCTC


        ThrTrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProPro
 301    ACGTGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTGTCCGCTTCCACCTCCA
        TGCACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGCGAAGGTGGAGGT


        LysSerProProValPro
 361    AAGTCCCCTCCTGTGCCG
        TTCAGGGGAGGACACGGC
```


FIG. 20   Translation of DNA 33f

```
        ValTrpAlaArgProAspTyrAsnProProLeuValGluThrTrpLysLysProAspTyr
   1    CGTTTGGGCGCGGCCGGACTATAACCCCCCGCTAGTGGAGACGTGGAAAAAGCCCGACTA
        GCAAACCCGCGCCGGCCTGATATTGGGGGGCGATCACCTCTGCACCTTTTTTGGGCTGAT


                        -----------------Overlap with 8f-----------------------------
        GluProProValValHisGlyCysProLeuProProProLysSerProProValProPro
  61    CGAACCACCTGTGGTCCATGGCTGCCCGCTTCCACCTCCAAAGTCCCCTCCTGTGCCTCC
        GCTTGGTGGACACCAGGTACCGACGGGCGAAGGTGGAGGTTTCAGGGGAGGACACGGAGG


        ProArgLysLysArgThrValValLeuThrGluSerThrLeuSerThrAlaLeuAlaGlu
 121    GCCTCGGAAGAAGCGGACGGTGGTCCTCACTGAATCAACCCTATCTACTGCCTTGGCCGA
        CGGAGCCTTCTTCGCCTGCCACCAGGAGTGACTTAGTTGGGATAGATGACGGAACCGGCT


        LeuAlaThrArgSerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThrThr
 181    GCTCGCCACCAGAAGCTTTGGCAGCTCCTCAACTTCCGGCATTACGGGCGACAATACGAC
        CGAGCGGTGGTCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAATGCCCGCTGTTATGCTG


        ThrSerSerGluProAlaProSerGlyCysProProAspSerAspAlaGluSerPhe
 241    AACATCCTCTGAGCCCGCCCCTTCTGGCTGCCCCCCCGACTCCGACGCTGAGTCCTTTGC
        TTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGGCTGAGGCTGCGACTCAGGAAACG
```

FIG. 21   Translation of DNA 33g

```
        AlaSerArgSerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThrThrThr
  1     GCCTCCAGAAGCTTTGGCAGCTCCTCAACTTCCGGCATTACGGGCGACAATACGACAACA
        CGGAGGTCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAATGCCCGCTGTTATGCTGTTGT

               ————————Overlap with 33f————————
        SerSerGluProAlaProSerGlyCysProProAspSerAspAlaGluSerTyrSerSer
 61     TCCTCTGAGCCCGCCCCTTCTGGCTGCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCC
        AGGAGACTCGGGCGGGGAAGACCGACGGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGG

        MetProProLeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrpSerThr
121     ATGCCCCCCCTGGAGGGGGGAGCCTGGGGATCCGGATCTTAGCGACGGGTCATGGTCAACG
        TACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAATCGCTGCCCAGTACCAGTTGC

        ValSerSerGluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSerTrpThr
181     GTCAGTAGTGAGGCCAACGCGGAGGATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACA
        CAGTCATCACTCCGGTTGCGCCTCCTACAGCACACGACGAGTTACAGAATGAGAACCTGT

        GlyAlaLeuValThrProCysAlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSer
241     GGCGCACTCGTCACCCCGTGCGCCGCGGAAGAACAGAAACTGCCCATCAATGCACTAAGC
        CCGCGTGAGCAGTGGGGCACGCGGCGCCTTCTTGTCTTTGACGGGTAGTTACGTGATTCG

        AsnSerLeuLeuArgHisHisAsnLeuValTyrSerThrThrSerArgSer
301     AACTCGTTGCTACGTCACCACAATTTGGTGTATTCCACCACCTCACGCAGTG
        TTGAGCAACGATGCAGTGGTGTTAAACCACATAAGGTGGTGGAGTGCGTCAC
```

FIG. 22   Translation of DNA 7f

```
        GlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAlaHisAsnGlyLeuArg
  1     GGCACCTATGTTTATAACCATCTGACTCCTCTTCGGGACTGGGCGCACAACGGCTTGCGA
        CCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCCGCGTGTTGCCGAACGCT

        AspLeuAlaValAlaValGluProValValPheSerGlnMetGluThrLysLeuIleThr
 61     GATCTGGCCGTGGCTGTAGAGCCAGTCGTCTTCTCCCAAATGGAGACCAAGCTCATCACG
        CTAGACCGGCACCGACATCTCGGTCAGCAGAAGAGGGTTTACCTCTGGTTCGAGTAGTGC

        TrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeuProValSerAlaArg
121     TGGGGGGCAGATACCGCCGCGTGCGGTGACATCATCAACGGCTTGCCTGTTTCCGCCCGC
        ACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAACGGACAAAGGCGGGCG

        ArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSerLysGlyTrpArgLeu
181     AGGGGCCGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCCAAGGGTTGGAGGTTG
        TCCCCGGCCCTCTATGACGAGCCCGGTCGGCTACCTTACCAGAGGTTCCCAACCTCCAAC

        LeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeuGlyCysIleIleThr
241     CTGGCGCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGGGTGCATAATCACC
        GACCGCGGGTAGTGCCGCATGCGGGTCGTCTGTTCCCCGGAGGATCCCACGTATTAGTGG

               ————————————Overlap with 7e————————————————————
        SerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGlnIleValSerThrAla
301     AGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCT
        TCGGATTGACCGGCCCTGTTTTTGGTTCACCTCCCACTCCAGGTCTAACACAGTTGACGA

        AlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrp
361     GCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGG
        CGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCACACGACC
```

FIG. 23 Translation of DNA 11b

```
    GlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyr
  1 GGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATATTACAAGCGCTAT
    CCGCCACAACAAGAGCAGCCCAACTACCGCGACTGAGACAGTGGTATAATGTTCGCGATA

    IleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHis
 61 ATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGAAGCGCAACTGCAC
    TAGTCGACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTG

    ValTrpIleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCys*
121 GTGTGGATTCCCCCCCTCAACGTCCGAGGGGGGCGCGACGCCGTCATCTTACTCATGTGT
    CACACCTAAGGGGGGGAGTTGCAGGCTCCCCCCGCGCTGCGGCAGTAGAATGAGTACACA

    AlaValHisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyPro
181 GCTGTACACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCCGTCTTCGGACCC
    CGACATGTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGGCAGAAGCCTGGG

    LeuTrpIleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeu
241 CTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTTGTGCGCGTCCAAGGCCTT
    GAAACCTAAGAAGTTCGGTCAAACGAATTTCATGGGATGAAACACGCGCAGGTTCCGGAA

    LeuArgPheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIle
301 CTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAATGGTCATC
    GAGGCCAAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCACGTTTACCAGTAG

                  -------------------------------------------------
    IleLysLeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAsp
361 ATTAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACTCCTCTTCGGGAC
    TAATTCAATCCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTG

    -----------------------Overlap with 7f -------------------------
    TrpAlaHisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGln
421 TGGGCGCACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTTCTCCCAA
    ACCCGCGTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCAGCAGAAGAGGGTT

    -------------------------------------------
    MetGluThrLysLeuIleThrTrpGly
481 ATGGAGACCAAGCTCATCACGTGGGGGGGC
    TACCTCTGGTTCGAGTAGTGCACCCCCCG
```

FIG. 24   Translation of DNA 141

```
        GluTyrValValLeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrp
    1   GGGGAGTACGTCGTTCTCCTGTTCCTTCTGCTTGCAGACGCGCGCGTCTGCTCCTGCTTGT
        CCCTCATGCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACGAACA

        MetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAla
   61   GGATGATGCTACTCATATCCCAAGCGGAGGCGGCTTTGGAGAACCTCGTAATACTTAATG
        CCTACTACGATGAGTATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGAATTAC

        AlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrp
  121   CAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCGTGTTCTTCTGCTTTGCAT
        GTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAACGTA

        TyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeu
  181   GGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTC
        CCATAAACTTCCCATTCACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACACCGGAG

        LeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAla
  241   TCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGTGGCCG
        AGGAGGACGAGGACAACCGCAACGGGGTCGCCCGCATGCGCGACCTGTGCCTCCACCGGC

        -----------------------Overlap with 11b -----------------  .
        SerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLys
  301   CGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATATTACA
        GCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGACTGAGACAGTGGTATAATGT

        ------------------------------------------------
        ArgTyrIleSerTrpCysLeuTrpTrpLeuGln
  361   AGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGAA
        TCGCGATATAGTCGACCACGAACACCACCGAAGTCTT
```

FIG. 25   Translation of DNA 39c

```
        ------------------------------------------------------------
        ProAlaProSerGlyCysProProAspSerAspAlaGluSerTyrSerSerMetProPro·
    1   CCAGCCCCTTCTGGCTGCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCCATGCCCCCC
        GGTCGGGGAAGACCGACGGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGGGG

        ------------------------------------------------------------
        LeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrpSerThrValSerSer
   61   CTGGAGGGGGAGCCTGGGGATCCGGATCTTAGCGACGGGTCATGGTCAACAGTCAGTAGT
        GACCTCCCCCTCGGACCCCTAGGCCTAGAATCGCTGCCCAGTACCAGTTGTCAGTCATCA

        -----------------------Overlap with 33g -----------------------
        GluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeu
  121   GAGGCCAACGCGGAGGATGTCGTGTGCTGCTCAATGTCCTACTCTTGGACAGGCGCACTC
        CTCCGGTTGCGCCTCCTACAGCACACGACGAGTTACAGGATGAGAACCTGTCCGCGTGAG

        ------------------------------------------------------------
        ValThrProCysAlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeu
  181   GTCACCCCGTGCGCCGCGGAAGAACAGAAACTGCCCATCAATGCACTGAGCAACTCGTTG
        CAGTGGGGCACGCGGCGCCTTCTTGTCTTTGACGGGTAGTTACGTGACTCGTTGAGCAAC

        ------------------------------------------------------------
        LeuArgHisHisAsnLeuValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLys
  241   CTACGTCACCACAATTTGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGAAG
        GATGCAGTGGTGTTAAACCACATAAGGTGGTGGAGTGCGTCACGAACGGTTTCCGTCTTC

        ------------------------------------------------------------
        LysValThrPheAspArgLeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGlu
  301   AAAGTCACATTTGACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAG
        TTTCAGTGTAAACTGTCTGACGTTCAAGACCTGTCGGTAATGGTCCTGCATGAGTTCCTC

        ValLysAlaAlaAlaSerThrValValLysAlaAsnPhe
  361   GTTAAAGCAGCGGCGTCAAAAGTGAAGGCTAACTTC
        CAATTTCGTCGCCGCAGTTTTCACTTCCGATTGAAG
```

FIG. 26-1 COMBINED ORF OF DNAs
14l/11b/7f/7e/8h/33c/40b/37b/35/36/81/32/33b/25c/14c/8f/33f/33g/39c

```
         GluTyrValValLeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrp
    1    GGGAGTACGTCGTTCTCCTGTTCCTTCTGCTTGCAGACGCGCGCGTCTGCTCCTGCTTGT
         CCCTCATGCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACGAACA

         MetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAla
   61    GGATGATGCTACTCATATCCCAAGCGGAGGCGGCTTTGGAGAACCTCGTAATACTTAATG
         CCTACTACGATGAGTATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGAATTAC

         AlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrp
  121    CAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCGTGTTCTTCTGCTTTGCAT
         GTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAACGTA

         TyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeu
  181    GGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTC
         CCATAAACTTCCCATTCACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACACCGGAG

         LeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAla
  241    TCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGTGGCCG
         AGGAGGACGAGGACAACCGCAACGGGGTCGCCCGCATGCGCGACCTGTGCCTCCACCGGC

         SerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLys
  301    CGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATATTACA
         GCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGACTGAGACAGTGGTATAATGT

         ArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGln
  361    AGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGAAGCGC
         TCGCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTTCGCG

         LeuHisValTrpIleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeu
  421    AACTGCACGTGTGGATTCCCCCCCTCAACGTCCGAGGGGGGCGCGACGCCGTCATCTTAC
         TTGACGTGCACACCTAAGGGGGGGAGTTGCAGGCTCCCCCCGCGCTGCGGCAGTAGAATG

         MetCysAlaValHisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPhe
  481    TCATGTGTGCTGTACACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCCGTCT
         AGTACACACGACATGTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGGCAGA

         GlyProLeuTrpIleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGln
  541    TCGGACCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTTGTGCGCGTCC
         AGCCTGGGGAAACCTAAGAAGTTCGGTCAAACGAATTTCATGGGATGAAACACGCGCAGG

         GlyLeuLeuArgPheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMet
  601    AAGGCCTTCTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAA
         TTCCGGAAGAGGCCAAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCACGTTT

         ValIleIleLysLeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeu
  661    TGGTCATCATTAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACTCCTC
         ACCAGTAGTAATTCAATCCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAG

         ArgAspTrpAlaHisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPhe
  721    TTCGGGACTGGGCGCACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCT
         AAGCCCTGACCCGCGTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCAGCAGA

         SerGlnMetGluThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIle
  781    TCTCCCAAATGGAGACCAAGCTCATCACGTGGGGGGCAGATACCGCCGCGTGCGGTGACA
         AGAGGGTTTACCTCTGGTTCGAGTAGTGCACCCCCCGTCTATGGCGGCGCACGCCACTGT

         IleAsnGlyLeuProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAsp
  841    TCATCAACGGCTTGCCTGTTTCCGCCCGCAGGGGCCGGGAGATACTGCTCGGGCCAGCCG
         AGTAGTTGCCGAACGGACAAAGGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGGTCGGC

         GlyMetValSerLysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThr
```

```
901  ATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCAGCAGA
     TACCTTACCAGAGGTTCCCCACCTCCAACGACCGCGGGTAGTGCCGCATGCGGGTCGTCT

              ArgGlyLeuLeuGlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGlu
961  CAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGG
     GTTCCCCGGAGGATCCCACGTATTAGTGGTCGGATTGACCGGCCCTGTTTTTGGTTCACC

              GlyGluValGlnIleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGly
1021 AGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATG
     TCCCACTCCAGGTCTAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAGTTAC

              ValCysTrpThrValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyPro
1081 GGGTGTGCTGGACTG---TACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTC
     CCCACACGACCTGA-  -ATGGTGCCCCGGCCTTGCTCCTGGTAGCGCAGTGGGTTCCCAG

              ValIleGlnMetTyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGly
1141 CTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCGCAAG
     GACAGTAGGTCTACATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGCGTTC

              SerArgSerLeuThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHis
1201 GTAGCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGC
     CATCGGCGAGTAACTGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAGTGCTCCG

              AlaAspValIleProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArg
1261 ACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCC
     TGCGGCTACAGTAAGGGCACGCGGCCGCCCCACTATCGTCCCCGTCGGACGACAGCGGGG

              ProIleSerTyrLeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAla
1321 GGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACG
     CCGGGTAAAGGATGAACTTTCCGAGGAGCCCCCCAGGCGACAACACGGGGCGCCCCGTGC

              ValGlyIlePheArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIle
1381 CCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTA
     GGCACCCGTATAAATCCCGGCGCCACACGTGGGCACCTCACCGATTCCGCCACCTGAAAT

              ProValGluAsnLeuGluThrThrMetArgSerProValPheThrAspAsnSerSerPro
1441 TCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTCCTCTC
     AGGGACACCTCTTGGATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAGAG

              ProValValProGlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLys
1501 CACCAGTAGTGCCCCAGAGCTTCCAGGTGGCTCACCTCCATGCTCCCACAGGCAGCGGCA
     GTGGTCATCACGGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTCGCCGT

              SerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnPro
1561 AAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACC
     TTTCGTGGTTCCAGGGCCGACGTATACGTCGAGTCCCGATATTCCACGATCATGAGTTGG

              SerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspPro
1621 CCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATCGATC
     GGAGACAACGACGTTGTGACCCGAAACCACGAATGTACAGGTTCCGAGTACCCTAGCTAG

              AsnIleArgThrGlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyr
1681 CTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTCCACCT
     GATTGTAGTCCTGGCCCCACTCTTGTTAATGGTGACCGTCGGGGTAGTGCATGAGGTGGA

              GlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAsp
1741 ACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGCGCTTATGACATAATAATTTGTG
     TGCCGTTCAAGGAACGGCTGCCGCCCACGAGCCCCCCGCGAATACTGTATTATTAAACAC

              GluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAla
1801 ACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCGGCACTGTCCTTGACCAAG
     TGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAACTGGTTC

              GluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThr
1861 CAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTCCGTCA
     GTCTCTGACGCCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGT
```

FIG. 26-2

```
         ValProHisProAsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyr
1921     CTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCCTTTTT
         GACACGGGGTAGGGTTGTAGCTCCTCCAACGAGACAGGTGGTGGCCTCTCTAGGGAAAAA

         GlyLysAlaIleProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSer
1981     ACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGGAGACATCTCATCTTCTGTCATT
         TGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCCCTCTGTAGAGTAGAAGACAGTAA

         LysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAla
2041     CAAAGAAGAAGTGCGACGAACTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGCCGTGG
         GTTTCTTCTTCACGCTGCTTGAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACGGCACC

         TyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAspValValValValAla
2101     CCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGTCGTGG
         GGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAGCACC

         ThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThr
2161     CAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTGCAATA
         GTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTAT

         CysValThrGlnThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThr
2221     CGTGTGTCACCCAGACAGTCGATTTCAGCCTTGACCCTACCTTCACCATTGAGACAATCA
         GCACACAGTGGGTCTGTCAGCTAAAGTCGGAACTGGGATGGAAGTGGTAACTCTGTTAGT

         LeuProGlnAspAlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysPro
2281     CGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGGAAGC
         GCGAGGGGGTCCTACGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCCTTCG

         GlyIleTyrArgPheValAlaProGlyGluArgProSerGlyMetPheAspSerSerVal
2341     CAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCGGCATGTTCGACTCGTCCG
         GTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGCAGGC

         LeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThr
2401     TCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCCGCCGAGACTA
         AGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAGTGCGGGCGGCTCTGAT

         ValArgLeuArgAlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGlu
2461     CAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCGTGTGCCAGGACCATCTTG
         GTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCACACGGTCCTGGTAGAAC

         PheTrpGluGlyValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThr
2521     AATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCCCACTTTCTATCCCAGA
         TTAAAACCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGGTCT

         LysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArg
2581     CAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTA
         GTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACGCGAT

         AlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysPro
2641     GGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGTTTGATTCGCCTCAAGC
         CCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCACAAACTAAGCGGAGTTCG

         ThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThr
2701     CCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGAAATCA
         GGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGCGACAAGTCTTACTTTAGT

         LeuThrHisProValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValVal
2761     CCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGAGGTCG
         GGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGC

         ThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSer
2821     TCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTGCCTGT
         AGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGACGAAACCGGCGCATAACGGACA

         ThrGlyCysValValIleValGlyArgValValLeuSerGlyLysProAlaIleIlePro
```

FIG. 26-3

```
2881  CAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAATCATAC
      GTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTAGTATG
```

```
           AspArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuPro
2941  CTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCACTTAC
      GACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCGTGAATG
```

```
           TyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeu
3001  CGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCC
      GCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCCGGAGG
```

```
           GlnThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLys
3061  TGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTGGCAAA
      ACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTT
```

```
           LeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAla
3121  AACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATACTTGG
      TTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTATGAACC
```

```
           GlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAla
3181  CGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTG
      GCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAAATGTCGAC
```

```
           ValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpVal
3241  CTGTCACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGGGTGGG
      GACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCCACCC
```

```
           AlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGly
3301  TGGCTGCCCAGCTCGCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTTAGCTG
      ACCGACGGGTCGAGCGGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAATCGAC
```

```
           AlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGly
3361  GCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGGGTATG
      CGCGGCGGTAGCCGTCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATAC
```

```
           AlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThr
3421  GCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCCTCCA
      CGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGGAGGT
```

```
           GluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyVal
3481  CGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGTCGGCG
      GCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGC
```

```
           ValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMet
3541  TGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCAGTGGA
      ACCAGACACGTCGTTATGACGCGGCCGTGCAACCGGGCCCGCTCCCCCGTCACGTCACCT
```

```
           AsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValPro
3601  TGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCCCACGCACTACGTGC
      ACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCGTGATGCACG
```

```
           GluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeu
3661  CGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACCCAGC
      GCCTCTCGCTACGTCGACGGGCGCAGTGACGGTATGAGTCGTCGGAGTGACATTGGGTCG
```

```
           LeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrp
3721  TCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGGTTCCT
      AGGACTCCGCTGACGTGGTCACCTATTCGAGCCTCACATGGTGAGGTACGAGGCCAAGGA
```

```
           LeuArgAspIleTrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLys
3781  GGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTGGCTAA
      CCGATTCCCTGTAGACCCTGACCTATACGCTCCACAACTCGCTGAAATTCTGGACCGATT
```

```
           AlaLysLeuMetProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLys
3841  AAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCTTTGTGTCCTGCCAGCGCGGGTATA
      TTCGATTCGAGTACGGTGTCGACGGACCCTAGGGGAAACACAGGACGGTCGCGCCCATAT
```

FIG. 26-4

```
           GlyValTrpArgValAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThr
3901   AGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCA
       TCCCCCAGACCGCTCACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGT

           GlyHisValLysAsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrp
3961   CTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAACATGT
       GACCTGTACAGTTTTTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTGTACA

           SerGlyThrPheProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaPro
4021   GGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCTGTACCCCCCTTCCTGCGC
       CCTCACCCTGGAAGGGGTAATTACGGATGTGGTGCCCGGGGACATGGGGGGAAGGACGCG

           AsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnVal
4081   CGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGG
       GCTTGATGTGCAAGCGCGATACCTCCCACAGACGTCTCCTTATACACCTCTATTCCGTCC

           GlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnVal
4141   TGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTGCCAGG
       ACCCCCTGAAGGTGATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGCACGGTCC

           ProSerProGluPhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProPro
4201   TCCCATCGCCCGAATTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGCGCCCC
       AGGGTAGCGGGCTTAAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACGCGGGG  .

           CysLysProLeuLeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProVal
4261   CCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATACCCGG
       GGACGTTCGGGAACGACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTATGGGCC

           GlySerGlnLeuProCysGluProGluProAspValAlaValLeuThrSerMetLeuThr
4321   TAGGGTCGCAATTACCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCATGCTCA
       ATCCCAGCGTTAATGGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTACGAGT

           AspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProPro
4381   CTGATCCCTCCCATATAACAGCAGAGGCGGCCGGGCGAAGGTTGGCGAGGGGATCACCCC
       GACTAGGGAGGGTATATTGTCGTCTCCGCCGGCCCGCTTCCAACCGCTCCCCTAGTGGGG

           SerValAlaSerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThr
4441   CCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGCA
       GGAGACACCGGTCGAGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGT

           AlaAsnHisAspSerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGlu
4501   CCGCTAACCATGACTCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGG
       GGCGATTGGTACTGAGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCC

           MetGlyGlyAsnIleThrArgValGluSerGluAsnLysValValIleLeuAspSerPhe
4561   AGATGGGCGGCAACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGACTCCT
       TCTACCCGCCGTTGTAGTGGTCCCAACTCAGTCTTTTGTTTCACCACTAAGACCTGAGGA

           AspProLeuValAlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArg
4621   TCGATCCGCTTGTGGCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAATCCTGC
       AGCTAGGCGAACACCGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGACG

           LysSerArgArgPheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProPro
4681   GGAAGTCTCGGAGATTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAACCCCC
       CCTTCAGAGCCTCTAAGCGGGTCCGGGACGGGCAAACCCGCGCCGGCCTGATATTGGGGG

           LeuValGluThrTrpLysLysProAspTyrGluProProValValHisGlyCysProLeu
4741   CGCTAGTGGAGACGTGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTGTCCGC
       GCGATCACCTCTGCACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGCG

           ProProProLysSerProProValProProProArgLysLysArgThrValValLeuThr
4801   TTCCACCTCCAAAGTCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGTCCTCA
       AAGGTGGAGGTTTCAGGGGAGGACACGGAGGCGGAGCCTTCTTCGCCTGCCACCAGGAGT

           GluSerThrLeuSerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSer
```

FIG. 26-5

```
4861  CTGAATCAACCCTATCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCT
      GACTTAGTTGGGATAGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGA

      ThrSerGlyIleThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCys
4921  CAACTTCCGGCATTACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCCTTCTGGCT
      GTTGAAGGCCGTAATGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGACCGA

      ProProAspSerAspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGly
4981  GCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCCATGCCCCCCCTGGAGGGGGAGCCTG
      CGGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTCGGAC  *

      AspProAspLeuSerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAsp
5041  GGGATCCGGATCTTAGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGCGGAGG
      CCCTAGGCCTAGAATCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCGCCTCC

      ValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAla
5101  ATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGCGCCG
      TACAGCACACGACGAGTTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCACGCGGC

      GluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeu
5161  CGGAAGAACAGAAACTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCACAATT
      GCCTTCTTGTCTTTGACGGGTAGTTACGTGATTCGTTGAGCAACGATGCAGTGGTGTTAA

      ValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArg
5221  TGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATTTGACA
      ACCACATAAGGTGGTGGAGTGCGTCACGAACGGTTTCCGTCTTCTTTCAGTGTAAACTGT

      LeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSer
5281  GACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCGGCGT
      CTGACGTTCAAGACCTGTCGGTAATGGTCCTGCATGAGTTCCTCCAATTTCGTCGCCGCA

      LysValLysAlaAsnLeu
5341  CAAAAGTGAAGGCTAACTTG
      GTTTTCACTTCCGATTGAAC
```

FIG. 26-6

FIG. 27 Translation of DNA   12f

```
        IlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsn
  1  CCATATTTAAAATCAGGATGTACGTGGGAGGGGTCGAACACAGGCTGGAAGCTGCCTGCA
     GGTATAAATTTTAGTCCTACATGCACCCTCCCCAGCTTGTGTCCGACCTTCGACGGACGT

        TrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeu
 61  ACTGGACGCGGGGCGAACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGT
     TGACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCA

        LeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeu
121  TACTGCTGACCACTACACAGTGGCAGGTCCTCCCGTGTTCCTTCACAACCCTACCAGCCT
     ATGACGACTGGTGATGTGTCACCGTCCAGGAGGGCACAAGGAAGTGTTGGGATGGTCGGA

        SerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyVal
181  TGTCCACCGGCCTCATCCACCTCCACCAGAACATTGTGGACGTGCAGTACTTGTACGGGG
     ACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAACACCTGCACGTCATGAACATGCCCC

        --------------------------------------------------------
        GlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeu
241  TGGGGTCAAGCATCGCGTCCTGGGCCATTAAGTGGGAGTACGTCGTTCTCCTGTTCCTTC
     ACCCCAGTTCGTAGCGCAGGACCCGGTAATTCACCCTCATGCAGCAAGAGGACAAGGAAG

        --------------------------------------------------------
        LeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGlu
301  TGCTTGCAGACGCGCGCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGG
     ACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCC

        ----------------Overlap with 14i------------------------
        AlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeu
361  AGGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGCACGGTC
     TCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCGTAGGGACCGGCCCTGCGTGCCAG

        -------
        Val
421  TTGTATC
     AACATAG
```

FIG. 28   Translation of DNA 35f

```
----------Overlap with 39c----------------------
      LeuLysGluValLysAlaAlaAlaSerLysValLysAlaAsnLeuLeuSerValGluGlu
  1   TGCTCAAGGAGGTTAAAGCAGCGGCGTCAAAAGTGAAGGCTAACTTGCTATCCGTAGAGG
      ACGAGTTCCTCCAATTTCGTCGCCGCAGTTTTCACTTCCGATTGAACGATAGGCATCTCC

      AlaCysSerLeuThrProProHisSerAlaLysSerLysPheGlyTyrGlyAlaLysAsp
 61   AAGCTTGCAGCCTGACGCCCCCACACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAAG  .
      TTCGAACGTCGGACTGCGGGGGTGTGAGTCGGTTTAGGTTCAAACCAATACCCCGTTTTC

      ValArgCysHisAlaArgLysAlaValThrHisIleAsnSerValTrpLysAspLeuLeu
121   ACGTCCGTTGCCATGCCAGAAAGGCCGTAACCCACATCAACTCCGTGTGGAAAGACCTTC
      TGCAGGCAACGGTACGGTCTTTCCGGCATTGGGTGTAGTTGAGGCACACCTTTCTGGAAG

      GluAspAsnValThrProIleAspThrThrIleMetAlaLysAsnGluValPheCysVal
181   TGGAAGACAATGTAACACCAATAGACACTACCATCATGGCTAAGAACGAGGTTTTCTGCG
      ACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGATTCTTGCTCCAAAAGACGC

      GlnProGluLysGlyGlyArgLysProAlaArgLeuIleValPheProAspLeuGlyVal
241   TTCAGCCTGAGAAGGGGGGTCGTAAGCCAGCTCGTCTCATCGTGTTCCCCGATCTGGGCG
      AAGTCGGACTCTTCCCCCCAGCATTCGGTCGAGCAGAGTAGCACAAGGGGCTAGACCCGC

      ArgValCysGluLysMetAlaLeuTyrAspValValThrLysLeuProLeuAlaValMet
301   TGCGCGTGTGCGAAAAGATGGCTTTGTACGACGTGGTTACAAAGCTCCCCTTGGCCGTGA
      ACGCGCACACGCTTTTCTACCGAAACATGCTGCACCAATGTTTCGAGGGGAACCGGCACT

      GlySerSerTyrGlyPheGlnTyrSerProGlyGlnArgValGluPheLeuValGlnAla
361   TGGGAAGCTCCTACGGATTCCAATACTCACCAGGACAGCGGGTTGAATTCCTCGTGCAAG
      ACCCTTCGAGGATGCCTAAGGTTATGAGTGGTCCTGTCGCCCAACTTAAGGAGCACGTTC

      TrpLysSerLysLysThrProMetGlyPheSerTyrAspThrArgCysPheAspSerThr
421   CGTGGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGATACCCGCTGCTTTGACTCCA
      GCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTATGGGCGACGAAACTGAGGT

      ValThrGluSerAspIleArgThrGluGluAla
481   CAGTCACTGAGAGCGACATCCGTACGGAGGAGGCA
      GTCAGTGACTCTCGCTGTAGGCATGCCTCCTCCGT
```

FIG. 29 Translation of DNA 19g

```
      ------------------------------------------------------------
      GluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAspThr
   1  GAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGATACC
      CTTAAGGAGCACGTTCGCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTATGC●


      --------------Overlap with 35f----------------------
      ArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyrGln
  61  CGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTACCAA
      GCGACGAAACTGAGGTGTCAGTGACTCTCGCTGTAGGCATGCCTCCTCCGTTAGATGGTT


      CysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeuTyr
 121  TGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAAGTCCCTCACCGAGAGGCTTTAT
      ACAACACTGGAGCTGGGGGTTCGGGCGCACCGGTAGTTCAGGGAGTGGCTCTCCGAAATA


      ValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArgAla
 181  GTTGGGGGCCCTCTTACCAATTCAAGGGGGGAGAACTGCGGCTATCGCAGGTGCCGCGCG
      CAACCCCCGGGAGAATGGTTAAGTTCCCCCCTCTTGACGCCGATAGCGTCCACGGCGCGC


      SerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArgAla
 241  AGCGGCGTACTGACAACTAGCTGTGGTAACACCCTCACTTGCTACATCAAGGCCCGGGCA
      TCGCCGCATGACTGTTGATCGACACCATTGTGGGAGTGAACGATGTAGTTCCGGGCCCGT


      AlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeuVal
 301  GCCTGTCGAGCCGCAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTAGTC
      CGGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGAGCACACACCGCTGCTGAATCAG


      ValIleCysGluSerAlaGlyValGlnGluAspAlaAla
 361  GTTATCTGTGAAAGCGCGGGGGTCCAGGAGGACGCGGCGAG
      CAATAGACACTTTCGCGCCCCCAGGTCCTCCTGCGCCGCTC
```

FIG. 30 Translation of DNA 26g

```
          ---------------------------------------------------------------
          GlyGlyGluAsnCysGlyTyrArgArgCysArgAlaSerGlyValLeuThrThrSerCys
    1     GGGGGGGAGAACTGCGGCTATCGCAGGTGCCGCGCAAGCGGCGTACTGACAACTAGCTGT
          CCCCCCCTCTTGACGCCGATAGCGTCCACGGCGCGTTCGCCGCATGACTGTTGATCGACA


          ---------------------------------------------------------------
          GlyAsnThrLeuThrCysTyrIleLysAlaArgAlaAlaCysArgAlaAlaGlyLeuGln
    61    GGTAACACCCTCACTTGTTACATCAAGGCCCGAGCAGCCTGTCGAGCCGCAGGGCTCCAG
          CCATTGTGGGAGTGAACAATGTAGTTCCGGGCTCGTCGGACAGCTCGGCGTCCCGAGGTC


          -----------Overlap with 19g----------------------------------
          AspCysThrMetLeuValCysGlyAspAspLeuValValIleCysGluSerAlaGlyVal
    121   GACTGCACCATGCTCGTGTGTGGCGACGACTTAGTCGTTATCTGTGAAAGCGCGGGGGTC
          CTGACGTGGTACGAGCACACACCGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCCAG


          -------------------------------
          GlnGluAspAlaAlaSerLeuArgAlaPheThrGluAlaMetThrArgTyrSerAlaPro
    181   CAGGAGGACGCGGCGAGCCTGAGAGCCTTCACGGAGGCTATGAC.AGGTACTCCGCCCCC
          GTCCTCCTGCGCCGCTCGGACTCTCGGAAGTGCCTCCGATACTG..CCATGAGGCGGGGG


          ProGlyAspProProGlnProGluTyrAspLeuGluLeuIleThrSerCysSerSerAsn
    241   CCTGGGGACCCCCCACAACCAGAATACGACTTGGAGCTCATAACATCATGCTCCTCCAAC
          GGACCCCTGGGGGGTGTTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGAGGTTG


          ValSerValAlaHisAspGlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThr
    301   GTGTCAGTCGCCCACGACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACA
          CACAGTCAGCGGGTGCTGCCGCGACCTTTCTCCCAGATGATGGAGTGGGCACTGGGATGT


          ThrProLeuAlaArgAlaAlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeu
    361   ACCCCCCTCGCGAGAGCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTA
          TGGGGGGAGCGCTCTCGACGCACCCTCTGTCGTTCTGTGTGAGGTCAGTTAAGGACCGAT


          GlyAsnIleIleMetPheAlaProThrLeuTrpAla
    421   GGCAACATAATCATGTTTGCCCCCACACTGTGGGCG
          CCGTTGTATTAGTACAAACGGGGGTGTGACACCCGC
```

FIG. 31 Translation of DNA 15e

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          ---------------------------------------------------------------
          GlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAla
    1 --  CGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAGAGC
          GCCGCGACCTTTCTCCCAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCTCG


          ---------------------Overlap with 26g------------------------
          AlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPhe
    61    TGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATGTT
          ACGCACCCTCTGTCGTTCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAA


          -----------------------------
          AlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAla
    121   TGCCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTATAGC
          ACGGGGGTGTGACACCCGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCG


          ArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGlu
    181   CAGGGACCAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGA
          GTCCCTGGTCGAACTTGTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTATCT


          ProLeuAspLeuProProIleIleGlnArgLeu
    241   ACCACTTGATCTACCTCCAATCATTCAAAGACTC
          TGGTGAACTAGATGGAGGTTAGTAAGTTTCTGAG
```

FIG. 32-1 COMBINED ORF OF DNAs 12f through 15e

```
         IlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsn
   1   CCATATTTAAAATCAGGATGTACGTGGGAGGGGTCGAACACAGGCTGGAAGCTGCCTGCA
       GGTATAAATTTTAGTCCTACATGCACCCTCCCCAGCTTGTGTCCGACCTTCGACGGACGT

         TrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeu
  61   ACTGGACGCGGGGCGAACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGT
       TGACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCA

         LeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeu
 121   TACTGCTGACCACTACACAGTGGCAGGTCCTCCCGTGTTCCTTCACAACCCTACCAGCCT
       ATGACGACTGGTGATGTGTCACCGTCCAGGAGGGCACAAGGAAGTGTTGGGATGGTCGGA

         SerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyVal
 181   TGTCCACCGGCCTCATCCACCTCCACCAGAACATTGTGGACGTGCAGTACTTGTACGGGG
       ACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAACACCTGCACGTCATGAACATGCCCC

         GlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeu
 241   TGGGGTCAAGCATCGCGTCCTGGGCCATTAAGTGGGAGTACGTCGTTCTCCTCTTCCTTC
       ACCCCAGTTCGTAGCGCAGGACCCGGTAATTCACCCTCATGCAGCAAGAGGACAAGGAAG

         LeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGlu
 301   TGCTTGCAGACGCGCGCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGG
       ACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCC

         AlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeu
 361   AGGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGGACGCACGGTC
       TCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCGTAGGGACCGGCCCTGCGTGCCAG

         ValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGly
 421   TTGTATCCTTCCTCGTGTTCTTCTGCTTTGCATGGTATTTGAAGGGTAAGTGGGTGCCCG
       AACATAGGAAGGAGCACAAGAAGACGAAACGTACCATAAACTTCCCATTCACCCACGGGC

         AlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeuLeuLeuAlaLeuProGln
 481   GAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTGCTCCTGTTGGCGTTGCCCC
       CTCGCCAGATGTGGAAGATGCCCTACACCGGAGAGGAGGACGAGGACAACCGCAACGGGG

         ArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGlyValValLeuValGly
 541   AGCGGGCCGTACGCGCTGGACACGGAGGTGGCCGCGTCGTGTGGCGGTGTTGTTCTCGTCG
       TCGCCCGCATGCGCGACCTGTGCCTCCACCGGCGCAGCACACCGCCACAACAAGAGCAGC

         LeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrp
 601   GGTTGATGGCGCTGACTCTGTCACCATATTACAAGCGCTATATCAGCTGGTGCTTGTGGT
       CCAACTACCGCGACTGAGACAGTGGTATAATGTTCGCGATATAGTCGACCACGAACACCA

         LeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsn
 661   GGCTTCAGTATTTTCTGACCAGAGTGGAAGCGCAACTGCACGTGTGGATTCCCCCCCTCA
       CCGAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTGCACACCTAAGGGGGGGAGT

         ValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaValHisProThrLeuVal
 721   ACGTCCGAGGGGGGCGCGACGCCGTCATCTTACTCATGTGTGCTGTACACCCGACTCTGG
       TGCAGGCTCCCCCCGCGCTGCGGCAGTAGAATGAGTACACACGACATGTGGGCTGAGACC

         PheAspIleThrLysLeuLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAlaSer
 781   TATTTGACATCACCAAATTGCTGCTGGCCGTCTTCGGACCCCTTTGGATTCTTCAAGCCA
       ATAAACTGTAGTGGTTTAACGACGACCGGCAGAAGCCTGGGGAAACCTAAGAAGTTCGGT

         LeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArgPheCysAlaLeuAla
 841   GTTTGCTTAAAGTACCCTACTTTGTGCGCGTCCAAGGCCTTCTCCGGTTCTGCGCGTTAG
       CAAACGAATTTCATGGGATGAAACACGCGCAGGTTCCGGAAGAGGCCAAGACGCGCAATC

         ArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLysLeuGlyAlaLeuThr
 901   CGCGGAAGATGATCGGAGGCCATTACGTGCAAATGGTCATCATTAAGTTAGGGGCGCTTA
       GCGCCTTCTACTAGCCTCCGGTAATGCACGTTTACCAGTAGTAATTCAATCCCCGCGAAT
```

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           GlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAlaHisAsnGlyLeuArg
 961  CTGGCACCTATGTTTATAACCATCTCACTCCTCTTCGGGACTGGGCGCACAACGGCTTGC
      GACCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCCGCGTGTTGCCGAACG

           AspLeuAlaValAlaValGluProValValPheSerGlnMetGluThrLysLeuIleThr
1021  GAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTTCTCCCAAATGGAGACCAAGCTCATCA
      CTCTAGACCGGCACCGACATCTCGGTCAGCAGAAGAGGGTTTACCTCTGGTTCGAGTAGT

           TrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeuProValSerAlaArg
1081  CGTGGGGGGCAGATACCGCCGCGTGCGGTGACATCATCAACGGCTTGCCTGTTTCCGCCC
      GCACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAACGGACAAAGGCGGG

           ArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSerLysGlyTrpArgLeu
1141  GCAGGGGCCGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCCAAGGGGTGGAGGT
      CGTCCCCGGCCCTCTATGACGAGCCCGGTCGGCTACCTTACCAGAGGTTCCCCACCTCCA

           LeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeuGlyCysIleIleThr
1201  TGCTGGCGCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGGGTGCATAATCA
      ACGACCGCGGGTAGTGCCGCATGCGGGTCGTCTGTTCCCCGGAGGATCCCACGTATTAGT

           SerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGlnIleValSerThrAla
1261  CCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTG
      GGTCGGATTGACCGGCCCTGTTTTTGGTTCACCTCCCACTCCAGGTCTAACACAGTTGAC

           AlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThrValTyrHisGlyAla
1321  CTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACTGTCTACCACGGGG
      GACGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCACACGACCTGACAGATGGTGCCCC

           GlyThrArgThrIleAlaSerProLysGlyProValIleGlnMetTyrThrAsnValAsp
1381  CCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATGTATACCAATGTAG
      GGCCTTGCTCCTGGTAGCGCAGTGGGTTCCCAGGACAGTAGGTCTACATATGGTTACATC

           GlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeuThrProCysThrCys
1441  ACCAAGACCTTGTGGGCTGGCCCCGCTCCGCAAGGTAGCCGCTCATTGACACCCTGCACTT
      TGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGGCGAGTAACTGTGGGACGTGAA

           GlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIleProValArgArgArg
1501  GCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATTCCCGTGCGCCGGC
      CGCCGAGGAGCCTGGAAATGGACCAGTGCTCCGTGCGGCTACAGTAAGGGCACGCGGCCG

           GlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyrLeuLysGlySerSer
1561  GGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAAGGCTCCT
      CCCCACTATCGTCCCCGTCGGACGACAGCGGGGCCGGGTAAAGGATGAACTTTCCGAGGA

           GlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePheArgAlaAlaValCys
1621  CGGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTTAGGGCCGCGGTGT
      GCCCCCCAGGCGACAACACGGGGCGCCCCGTGCGGCACCCGTATAAATCCCGGCGCCACA

           ThrArgGlyValAlaLysAlaValAspPheIleProValGluAsnLeuGluThrThrMet
1681  GCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCA
      CGTGGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTGGATCTCTGTTGGT

           ArgSerProValPheThrAspAsnSerSerProProValValProGlnSerPheGlnVal
1741  TGAGGTCCCCGGTGTTCACGGATAACTCCTCTCCACCAGTAGTGCCCCAGAGCTTCCAGG
      ACTCCAGGGGCCACAAGTGCCTATTGAGGAGAGGTGGTCATCACGGGGTCTCGAAGGTCC

           AlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysValProAlaAlaTyrAla
1801  TGGCTCACCTCCATGCTCCCACAGGCAGCGGCAAAAGCACCAAGGTCCCGGCTGCATATG
      ACCGAGTGGAGGTACGAGGGTGTCCGTCGCCGTTTTCGTGGTTCCAGGGCCGACGTATAC

           AlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAlaThrLeuGlyPheGly
1861  CAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTTGCTGCAACACTGGGCTTTG
      GTCGAGTCCCGATATTCCACGATCATGAGTTGGGGAGACAACGACGTTGTGACCCGAAAC

           AlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIle
```

FIG. 32-2

1921   GTGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAA
        CACGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCCTGGCCCCACTCTTGTT

        ThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCys
1981   TTACCACTGGCAGCCCATCACGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGT
        AATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCCGCCCA

        SerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSerThrAspAlaThrSer
2041   GCTCGGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACAT
        CGAGCCCCCCGCGAATACTGTATTATTAAACACTGCTCACGGTGAGGTGCCTACGGTGTA

        IleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValVal
2101   CCATCTTGGGCATCGGCACTGTCCTTGACCAAGCAGAGACTGCGGGGGGCGAGACTGGTTG
        GGTAGAACCCGTAGCCGTGACAGGAACTGGTTCGTCTCTGACGCCCCCGCTCTGACCAAC

        LeuAlaThrAlaThrProProGlySerValThrValProHisProAsnIleGluGluVal
2161   TGCTCGCCACCGCCACCCCTCCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGG
        ACGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGTGACACGGGGTAGGGTTGTAGCTCCTCC

        AlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIle
2221   TTGCTCTGTCCACCACCGGAGAGATCCCTTTTTACGGCAAGGCTATCCCCCTCGAAGTAA
        AACGAGACAGGTGGTGGCCTCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATT

        LysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAla
2281   TCAAGGGGGGGGAGACATCTCATCTTCTGTCATTCAAAGAAGAAGTGCGACGAACTCGCCG
        AGTTCCCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTCACGCTGCTTGAGCGGC

        LysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerVal
2341   CAAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGTCTTGACGTGTCCG
        GTTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGC

        IleProThrSerGlyAspValValValValAlaThrAspAlaLeuMetThrGlyTyrThr
2401   TCATCCCGACCAGCGGCGATGTTGTCGTCGTGGCAACCGATGCCCTCATGACCGGCTATA
        AGTAGGGCTGGTCGCCGCTACAACAGCAGCACCGTTGGCTACGGGAGTACTGGCCGATAT

        GlyAspPheAspSerValIleAspCysAsnThrCysValThrGlnThrValAspPheSer
2461   CCGGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTCACCCAGACAGTCGATTTCA
        GGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTCTGTCAGCTAAAGT

        LeuAspProThrPheThrIleGluThrIleThrLeuProGlnAspAlaValSerArgThr
2521   GCCTTGACCCTACCTTCACCATTGAGACAATCACGCTCCCCCAGGATGCTGTCTCCCGCA
        CGGAACTGGGATGGAAGTGGTAACTCTGTTAGTGCGAGGGGGTCCTACGACAGAGGGCGT

        GlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGly
2581   CTCAACGTCGGGGCAGGACTGGCAGGGGGGAAGCCAGGCATCTACAGATTTGTGGCACCGG
        GAGTTGCAGCCCCGTCCTGACCGTCCCCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCC

        GluArgProSerGlyMetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCys
2641   GGGAGCGCCCCTCCGGCATGTTCGACTCGTCCGTCCTCTGTGAGTGCTATGACGCAGGCT
        CCCTCGCGGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGA

        AlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThr
2701   GTGCTTGGTATGAGCTCACGCCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACA
        CACGAACCATACTCGAGTGCGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGT

        ProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeu
2761   CCCCGGGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTTGGGAGGGCGTCTTTACAGGCC
        GGGGCCCCGAAGGGCACACGGTCCTGGTAGAACTTAAAACCCTCCCGCAGAAATGTCCGG

        ThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyr
2821   TCACTCATATAGATGCCCACTTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTT
        AGTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAA

        LeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAsp
2881   ACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCA CGTGGG
        TGGACCATCGCATGGTTCGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCC

FIG. 32-3

```
          GlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeu
     2941 ACCAGATGTGGAAGTGTTTGATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGC
          TGGTCTACACCTTCACAAACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGACG

          TyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIle
     3001 TATACAGACTGGGCGCTGTTCAGAATGAAATCACCCTGACGCACCCAGTCACCAAATACA
          ATATGTCTGACCCGCGACAAGTCTTACTTTAGTGGGACTGCCTGGGTCAGTGGTTTATGT

          MetThrCysMetSerAlaAspLeuGluValValThrSerThrTrpValLeuValGlyGly
     3061 TCATGACATGCATGTCGGCCGACCTGGAGGTCGTCACGAGCACCTGGGTGCTCGTTGGCG
          AGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGCTCGTGGACCCACGAGCAACCGC

          ValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArg
     3121 GCGTCCTGGCTGCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCA
          CGCAGGACCGACGAAACCGGCGCATAACGGACAGTTGTCCGACGCACCAGTATCACCCGT

          ValValLeuSerGlyLysProAlaIleIleProAspArgGluValLeuTyrArgGluPhe
     3181 GGGTCGTCTTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAGAGT
          CCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCA

          AspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAla
     3241 TCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCG
          AGCTACTCTACCTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGC

          GluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluVal
     3301 CCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCGTCCCGTCAGGCAGAGG
          GGCTCGTCAAGTTCGTCTTCCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCC

          IleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMet
     3361 TTATCGCCCCTGCTGTCCAGACCAACTGGCAAAAACTCGAGACCTTCTGGGCGAAGCATA
          AATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCCGCTTCGTAT

          TrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnPro
     3421 TGTGGAACTTCATCAGTGGGATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACC
          ACACCTTGAAGTAGTCACCCTATGTTATGAACCGCCCGAACAGTTGCGACGGACCATTGG

          AlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGln
     3481 CCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTCACCAGCCCACTAACCACTAGCC
          GGCGGTAACGAAGTAACTACCGAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGG

          ThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAla
     3541 AAACCCTCCTCTTCAACATATTGGGGGGGTGGGTGGCTGCCCAGCTCGCCGCCCCCGGTG
          TTTGGGAGGAGAAGTTGTATAACCCCCCCACCCACCGACGGGTCGAGCGGCGGGGGCCAC

          AlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGly
     3601 CCGCTACTGCCTTTGTGGGCGCTGGCTTAGCTGGCGCCGCCATCGGCAGTGTTGGACTGG
          GGCGATGACGGAAACACCCGCGACCGAATCGACCGCGGCGGTAGCCGTCACAACCTGACC

          LysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAla
     3661 GGAAGGTCCTCATAGACATCCTTGCAGGGTATGGCGCGGGCGTGGCGGGAGCTCTTGTGG
          CCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCGCCCTCGAGAACACC

          PheLysIleMetSerGlyGluValProSerThrGluAspLeuValAsnLeuLeuProAla
     3721 CATTCAAGATCATGAGCGGTGAGGTCCCCTCCACGGAGGACCTGGTCAATCTACTGCCCG
          GTAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCCTCCTGGACCAGTTAGATGACGGGC

          IleLeuSerProGlyAlaLeuValValGlyValValCysAlaAlaIleLeuArgArgHis
     3781 CCATCCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCCGGC
          GGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGCACCAGACACGTCGTTATGACGCGGCCG

          ValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArg
     3841 ACGTTGGCCCCGGGCGAGGGGGGCAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCTCCC
          TGCAACCGGGGCCCGCTCCCCCGTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGG

          GlyAsnHisValSerProThrHisTyrValProGluSerAspAlaAlaAlaArgValThr
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FIG. 32-4

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3901  GGGGGAACCATGTTTCCCCCACGCACTACGTGCCGGAGAGCGATGCAGCTGCCCGCGTCA
      CCCCCTTGGTACAAAGGGGGTGCGTGATGCACGGCCTCTCGCTACGTCGACGGGCGCAGT


         AlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSer
3961  CTGCCATACTCAGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTGCACCAGTGGATAA
      GACGGTATGAGTCGTCGGAGTGACATTGGGTCGAGGACTCCGCTGACGTGGTCACCTATT


         SerGluCysThrThrProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCys
4021  GCTCGGAGTGTACCACTCCATGCTCCGGTTCCTGGCTAAGGGACATCTGGGACTGGATAT
      CGAGCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCCTGACCTATA


         GluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGly
4061  GCGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATGCCACAGCTGCCTG
      CGCTCCACAACTCGCTGAAATTCTGGACCGATTTTCGATTCGAGTACGGTGTCGACGGAC


         IleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArgValAspGlyIleMet
4141  GGATCCCCTTTGTGTCCTGCCAGCGCGGGTATAAGGGGGTCTGGCGAGTGGACGGCATCA
      CCTAGGGGAAACACAGGACGGTCGCGCCCATATTCCCCCAGACCGCTCACCTGCCGTAGT


         HisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArg
4201  TGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGA
      ACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTTTTGCCCTGCTACT


         IleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyr.
4261  GGATCGTCGGTCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTCCCCATTAATGCCT
      CCTAGCAGCCAGGATCCTGGACGTCCTTGTACACCTCACCCTGGAAGGGGTAATTACGGA


         ThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgVal
4321  ACACCACGGGCCCCTGTACCCCCCTTCCTGCGCCGAACTACACGTTCGCGCTATGGAGGG
      TGTGGTGCCCGGGGACATGGGGGGAAGGACGCGGCTTGATGTGCAAGCGCGATACCTCCC


         SerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMet
4381  TGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCACTACGTGACGGGTA
      ACAGACGTCTCCTTATACACCTCTATTCCGTCCACCCCCTGAAGGTGATGCACTGCCCAT


         ThrThrAspAsnLeuLysCysProCysGlnValProSerProGluPhePheThrGluLeu
4441  TGACTACTGACAATCTCAAATGCCCGTGCCAGGTCCCATCGCCCGAATTTTTCACAGAAT
      ACTGATGACTGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTA


         AspGlyValArgLeuHisArgPheAlaProProCysLysProLeuLeuArgGluGluVal
4501  TGGACGGGGTGCGCCTACATAGGTTTGCGCCCCCCTGCAAGCCCTTGCTGCGGGAGGAGG
      ACCTGCCCCACGCGGATGTATCCAAACGCGGGGGGACGTTCGGGAACGACGCCCTCCTCC


         SerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeuProCysGluProGlu
4561  TATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGGTCGCAATTACCTTGCGAGCCCG
      ATAGTAAGTCTCATCCTGAGGTGCTTATGGGCCATCCCAGCGTTAATGGAACGCTCGGGC


         ProAspValAlaValLeuThrSerMetLeuThrAspProSerHisIleThrAlaGluAla
4621  AACCGGACGTGGCCGTGTTGACGTCCATGCTCACTGATCCCTCCCATATAACAGCAGAGG
      TTGGCCTGCACCGGCACAACTGCAGGTACGAGTGACTAGGGAGGGTATATTGTCGTCTCC


         AlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSerSerSerAlaSerGln
4681  CGGCCGGGCGAAGGTTGGCGAGGGGATCACCCCCCTCTGTGGCCAGCTCCTCGGCTAGCC
      GCCGGCCCGCTTCCAACCGCTCCCCTAGTGGGGGGAGACACCGGTCGAGGAGCCGATCGG


         LeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAspSerProAspAlaGlu
4741  AGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCGCTAACCATGACTCCCCTGATGCTG
      TCGATAGGCGAGGTAGAGAGTTCCGTTGAACGTGGCGATTGGTACTGAGGGGACTACGAC


         LeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsnIleThrArgValGlu
4801  AGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGGCGGCAACATCACCAGGGTTG
      TCGAGTATCTCCGGTTGGAGGATACCTCCGTCCTCTACCCGCCGTTGTAGTGGTCCCAAC


         SerGluAsnLysValValIleLeuLeuAspSerPheAspProLeuValAlaGluGluAspGlu
4861  AGTCAGAAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTGGTGGCGGAGGAGGACG
      TCAGTCTTTTGTTTCACCACTAAGACCTGAGGAAGCTAGGCGAACACCGCCTCCTCCTGC
```

FIG. 32-5

```
          ArgGluIleSerValProAlaGluIleLeuArgLysSerArgArgPheAlaGlnAlaLeu
4921  AGCGGGAGATCTCCGTACCCGCAGAAATCCTGCGGAAGTCTCGGAGATTCGCCCAGGCCC
      TCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCCTTCAGAGCCTCTAAGCGGGTCCGGG

          ProValTrpAlaArgProAspTyrAsnProProLeuValGluThrTrpLysLysProAsp
4981  TGCCCGTTTGGGCGCGGCCGGACTATAACCCCCCGCTAGTGGAGACGTGGAAAAAGCCCG
      ACGGGCAAACCCGCGCCGGCCTGATATTGGGGGGCGATCACCTCTGCACCTTTTTCGGGC

          TyrGluProProValValHisGlyCysProLeuProProProLysSerProProValPro
5041  ACTACGAACCACCTGTGGTCCATGGCTGTCCGCTTCCACCTCCAAAGTCCCCTCCTGTGC
      TGATGCTTGGTGGACACCAGGTACCGACAGGCGAAGGTGGAGGTTTCAGGGGAGGACACG

          ProProArgLysLysArgThrValValLeuThrGluSerThrLeuSerThrAlaLeuAla
5101  CTCCGCCTCGGAAGAAGCGGACGGTGGTCCTCACTGAATCAACCCTATCTACTGCCTTGG
      GAGGCGGAGCCTTCTTCGCCTGCCACCAGGAGTGACTTAGTTGGGATAGATGACGGAACC

          GluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThr
5161  CCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCTCAACTTCCGGCATTACGGGCGACAATA
      GGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAATGCCCGCTGTTAT

          ThrThrSerSerGluProAlaProSerGlyCysProProAspSerAspAlaGluSerTyr
5221  CGACAACATCCTCTGAGCCCGCCCCTTCTGGCTGCCCCCCCGACTCCGACGCTGAGTCCT
      GCTGTTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGGCTGAGGCTGCGACTCAGGA

          SerSerMetProProLeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrp
5281  ATTCCTCCATGCCCCCCCTGGAGGGGGAGCCTGGGGATCCGGATCTTAGCGACGGGTCAT
      TAAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAATCGCTGCCCAGTA

          SerThrValSerSerGluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSer
5341  GGTCAACGGTCAGTAGTGAGGCCAACGCGGAGGATGTCGTGTGCTGCTCAATGTCTTACT
      CCAGTTGCCAGTCATCACTCCGGTTGCGCCTCCTACAGCACACGACGAGTTACAGAATGA

          TrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLysLeuProIleAsnAla
5401  CTTGGACAGGCGCACTCGTCACCCCGTGCGGCCGCGGAAGAACAGAAACTGCCCATCAATG
      GAACCTGTCCGCGTGAGCAGTGGGGCACGCGGCGCCTTCTTGTCTTTGACGGGTAGTTAC

          LeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThrThrSerArgSerAla
5461  CACTAAGCAACTCGTTGCTACGTCACCACAATTTGGTGTATTCCACCACCTCACGCAGTG
      GTGATTCGTTGAGCAACGATGCAGTGGTGTTAAACCACATAAGGTGGTGGAGTGCGTCAC

          CysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeuAspSerHisTyrGln
5521  CTTGCCAAAGGCAGAAGAAAGTCACATTTGACAGACTGCAAGTTCTGGACAGCCATTACC
      GAACGGTTTCCGTCTTCTTTCAGTGTAAACTGTCTGACGTTCAAGACCTGTCGGTAATGG

          AspValLeuLysGluValLysAlaAlaAlaSerLysValLysAlaAsnLeuLeuSerVal
5581  AGGACGTACTCAAGGAGGTTAAAGCAGCGGCGTCAAAAGTGAAGGCTAACTTGCTATCCG
      TCCTGCATGAGTTCCTCCAATTTCGTCGCCGCAGTTTTCACTTCCGATTGAACGATAGGC

          GluGluAlaCysSerLeuThrProProHisSerAlaLysSerLysPheGlyTyrGlyAla
5641  TAGAGGAAGCTTGCAGCCTGACGCCCCCACACTCAGCCAAATCCAAGTTTGGTTATGGGG
      ATCTCCTTCGAACGTCGGACTGCGGGGGTGTGAGTCGGTTTAGGTTCAAACCAATACCCC

          LysAspValArgCysHisAlaArgLysAlaValThrHisIleAsnSerValTrpLysAsp
5701  CAAAAGACGTCCGTTGCCATGCCAGAAAGGCCGTAACCCACATCAACTCCGTGTGGAAAG
      GTTTTCTGCAGGCAACGGTACGGTCTTTCCGGCATTGGGTGTAGTTGAGGCACACCTTTC

          LeuLeuGluAspAsnValThrProIleAspThrThrIleMetAlaLysAsnGluValPhe
5761  ACCTTCTGGAAGACAATGTAACACCAATAGACACTACCATCATGGCTAACAACGAGGTTT
      TGGAAGACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGATTCTTGCTCCAAA

          CysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIleValPheProAspLeu
5821  TCTGCGTTCAGCCTGAGAAGGGGGGTCGTAAGCCAGCTCGTCTCATCGTGTTCCCCGATC
      AGACGCAAGTCGGACTCTTCCCCCCAGCATTCGGTCGAGCAGAGTAGCACAAGGGGCTAG

          GlyValArgValCysGluLysMetAlaLeuTyrAspValValThrLysLeuProLeuAla
```

FIG. 32-6

```
5881  TGGGCGTGCGCGTGTGCGAAAAGATGGCTTTGTACGACGTGGTTACAAAGCTCCCCTTGG
      ACCCGCACGCGCACACGCTTTTCTACCGAAACATGCTGCACCAATGTTTCGAGGGGAACC

          ValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArgValGluPheLeuVal
5941  CCGTGATGGGAAGCTCCTACGGATTCCAATACTCACCAGGACAGCGGGTTGAATTCCTCG
      GGCACTACCCTTCGAGGATGCCTAAGGTTATGAGTGGTCCTGTCGCCCAACTTAAGGAGC

          GlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAspThrArgCysPheAsp
6001  TGCAAGCGTGGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGATACCCGCTGCTTTG
      ACGTTCGCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTATGGGCGACGAAAC

          SerThrValThrGluSerAspIleArgThrGluGluAlaIleTyrGlnCysCysAspLeu
6061  ACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTACCAATGTTGTGACC
      TGAGGTGTCAGTGACTCTCGCTGTAGGCATGCCTCCTCCGTTAGATGGTTACAACACTGG

          AspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeuTyrValGlyGlyPro
6121  TCGACCCCCAAGCCCGCGTGGCCATCAAGTCCCTCACCGAGAGGCTTTATGTTGGGGGCC
      AGCTGGGGGTTCGGGCGCACCGGTAGTTCAGGGAGTGGCTCTCCGAAATACAACCCCCGG

          LeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArgAlaSerGlyValLeu
6181  CTCTTACCAATTCAAGGGGGGAGAACTGCGGCTATCGCAGGTGCCGCGCGAGCGGCGTAC  .
      GAGAATGGTTAAGTTCCCCCCTCTTGACGCCGATAGCGTCCACGGCGCGCTCGCCGCATG  '

          ThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArgAlaAlaCysArgAla
6241  TGACAACTAGCTGTGGTAACACCCTCACTTGCTACATCAAGGCCCGGGCAGCCTGTCGAG
      ACTGTTGATCGACACCATTGTGGGAGTGAACGATGTAGTTCCGGGCCCGTCGGACAGCTC

          AlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeuValValIleCysGlu
6301  CCGCAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTAGTCGTTATCTGTG
      GGCGTCCCGAGGTCCTGACGTGGTACGAGCACACACCGCTGCTGAATCAGCAATAGACAC

          SerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThrGluAlaMetThrArg
6361  AAAGCGCGGGGGGTCCAGGAGGACGCGGCGAGCCTGAGAGCCTTCACGGAGGCTATGACCA
      TTTCGCGCCCCCAGGTCCTCCTGCGCCGCTCGGACTCTCGGAAGTGCCTCCGATACTGGT

          TyrSerAlaProProGlyAspProProGlnProGluTyrAspLeuGluLeuIleThrSer
6421  GGTACTCCGCCCCCCCTGGGGACCCCCCACAACCAGAATACGACTTGGAGCTCATAACAT
      CCATGAGGCGGGGGGGACCCCTGGGGGGTGTTGGTCTTATGCTGAACCTCGAGTATTGTA

          CysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArgValTyrTyrLeuThr
6481  CATGCTCCTCCAACGTGTCAGTCGCCCACGACGGCGCTGGAAAGAGGGTCTACTACCTCA
      GTACGAGGAGGTTGCACAGTCAGCGGGTGCTGCCGCGACCTTTCTCCCAGATGATGGAGT

          ArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAlaArgHisThrProVal
6541  CCCGTGACCCTACAACCCCCCTCGCGAGAGCTGCGTGGGAGACAGCAAGACACACTCCAG
      GGGCACTGGGATGTTGGGGGGAGCGCTCTCGACGCACCCTCTGTCGTTCTGTGTGAGGTC

          AsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrpAlaArgMetIleLeu
6601  TCAATTCCTGGCTAGGCAACATAATCATGTTTGCCCCCACACTGTGGGCGAGGATGATAC
      AGTTAAGGACCGATCCGTTGTATTAGTACAAACGGGGGTGTGACACCCGCTCCTACTATG

          MetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGluGlnAlaLeuAspCys
6661  TGATGACCCATTTCTTTAGCGTCCTTATAGCCAGGGACCAGCTTGAACAGGCCCTCGATT
      ACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCGAACTTGTCCGGGAGCTAA

          GluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuProProIleIleGlnArg
6721  GCGAGATCTACGGGGCCTGCTACTCCATAGAACCACTTGATCTACCTCCAATCATTCAAA
      CGCTCTAGATGCCCCGGACGATGAGGTATCTTGGTGAACTAGATGGAGGTTAGTAAGTTT

          Leu
6781  GACTC
      CTGAG
```

FIG. 32-7

FIG. 33   LEGEND

Lane Number	Chimp Reference Number	Infection Type	Sample date (days) (0=inoculation day)	ALT (alanine) aminotransferase level in sera) mu/ml)
1	1	NANB	0	9
2	1	NANB	76	71
3	1	NANB	118	19
4	1	NANB	154	N/A
5	2	NANB	0	5
6	2	NANB	21	52
7	2	NANB	73	13
8	2	NANB	138	N/A
9	3	NANB	0	8
10	3	NANB	43	205
11	3	NANB	53	13
12	3	NANB	159	6
13	4	NANB	-3	11
14	4	NANB	55	132
15	4	NANB	83	N/A
16	4	NANB	140	N/A
17	5	HAV	0	4
18	5	HAV	25	143
19	5	HAV	40	18
20	5	HAV	268	5
21	6	HAV	-8	N/A
22	6	HAV	15	106
23	6	HAV	41	10
24	6	HAV	129	N/A
26	7	HAV	0	7
27	7	HAV	22	83
28	7	HAV	115	5
29	7	HAV	139	N/A
30	8	HAV	0	15
31	8	HAV	26	130
32	8	HAV	74	8
33	8	HAV	205	5
34	9	HBV	-290	N/A
35	9	HBV	379	9
36	9	HBV	435	6
37	10	HBV	0	8
38	10	HBV	111-118 (pool)	96-156 (pool)
39	10	HBV	205	9
40	10	HBV	240	13
41	11	HBV	0	11
42	11	HBV	28-56 (pool)	8-100 (pool)
43	11	HBV	169	9
44	11	HBV	223	10
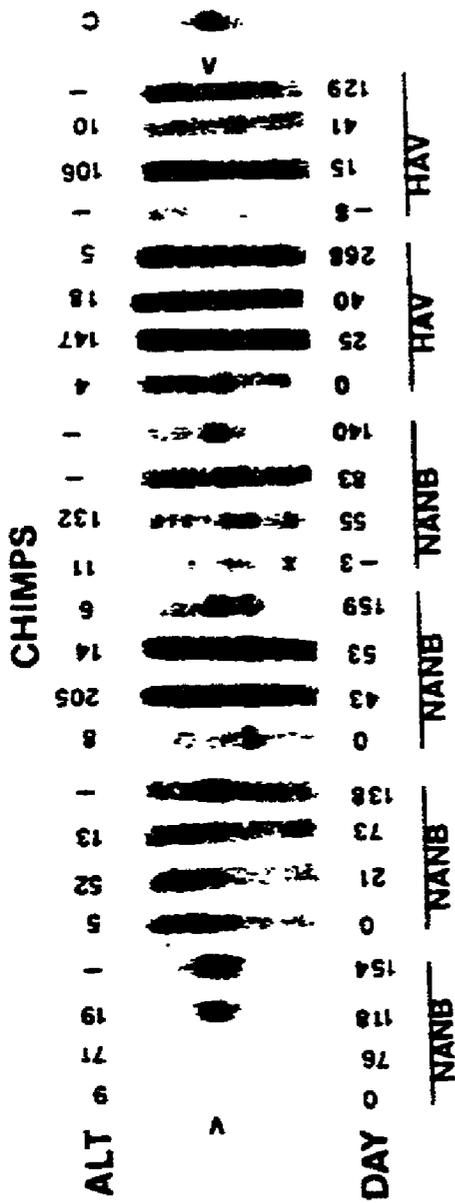
FIG. 33-1
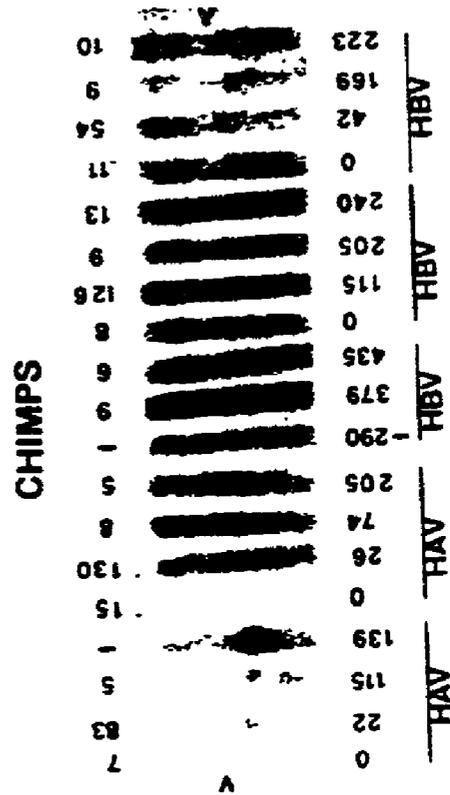
FIG. 33-2

FIG. 34   LEGEND

Lane Number	Patient Reference Number	Diagnosis	ALT Level (mu/ml)
1	1[1]	NANB	1354
2	1[1]	NANB	31
3	2[1]	NANB	14
4	2[1]	NANB	79
5	2[1]	NANB	26
6	3[1]	NANB	78
7	3[1]	NANB	87
8	3[1]	NANB	25
9	4[1]	NANB	60
10	4[1]	NANB	13
11	5[1]	NANB	298
12	5[1]	NANB	101
13	6[1]	NANB	474
14	6[1]	NANB	318
15	7[1]	NANB	20
16	7[1]	NANB	163
17	8[1]	NANB	44
18	8[1]	NANB	50
19	9	NANB	N/A
20	10	NANB	N/A
21	11	NANB	N/A
22	12	Normal	N/A
23	13	Normal	N/A
24	14	Normal	N/A
26	30174	Normal	N/A
27	30105	Normal	N/A
28	30072	Normal	N/A
29	30026	Normal	N/A
30	30146	Normal	N/A
31	30250	Normal	N/A
32	30071	Normal	N/A
33	15	AcuteHAV	N/A
34	16	AcuteHAV	N/A
35	17	AcuteHAV	N/A
36	18	AcuteHAV	N/A
37	48088	AcuteHAV	N/A
38	47288	AcuteHAV	N/A
39	47050	AcuteHAV	N/A
40	46997	AcuteHAV	N/A
41	19	Convalescent HBV	N/A
42	20	(anti-HBSag+ve;	N/A
43	21	anti-HBCag+ve)	N/A
44	22	(anti-HBSag+ve;	N/A
45	23	anti-HBCag+ve)	N/A
46	24	(anti-HBSag+ve;	N/A
47	25	anti-HBCag+ve)	N/A
48	26	(anti-HBSag+ve;	N/A
49	27	anti-HBSag+ve)	N/A

[1]Sequential serum samples were assayed from these patients

FIG. 34-1

FIG. 34-2

FIG. 35

FIG. 36-I COOH-terminus of SOD-C100 Fusion Polypeptide

```
        --------SOD-----------COOH] [--adaptor----] {NANBHpolypeptide}
        AlaCysGlyValIleGlyIleAlaGlnAsnLeuGlyIleArgAspAlaHisPheLeuSer
    1   GCTTGTGGTGTAATTGGGATCGCCCAGAATTTGGGAATTCGGGATGCCCACTTTCTATCC
        CGAACACCACATTAACCCTAGCGGGTCTTAAACCCTTAAGCCCTACGGGTGAAAGATAGG


        >>>>>>>>>>>>>>>>>>>>>
        GlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCys
   61   CAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGC
        GTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACG


        AlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeu
  121   GCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGTTTGATTCGCCTC
        CGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCACAAACTAAGCGGAG


        LysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGlu
  181   AAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGAA
        TTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGCGACAAGTCTTACTT


        IleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSerAlaAspLeuGlu
  241   ATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGAG
        TAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTC


        ValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCys
  301   GTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTGC
        CAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGACGAAACCGGCGCATAACG


        LeuSerThrGlyCysValValIleValGlyArgValValLeuSerGlyLysProAlaIle
  361   CTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAATC
        GACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTAG


        IleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHis
  421   ATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCAC
        TATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCGTG


        LeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGly
  481   TTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGC
        AATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCCG


        LeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrp
  541   CTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTGG
        GAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACC


        GlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyr
  601   CAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATAC
        GTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTATG


        LeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThr
  661   TTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACA
        AACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAAATGT


        AlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGly
  721   GCTGCTGTCACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGGG
        CGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCC


        TrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeu
  781   TGGGTGGCTGCCCAGCTCGCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTTA
        ACCCACCGACGGGTCGAGCGGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAAT


        AlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGly
  841   GCTGGCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGGG
```

```
      CGACCGCGGCGGTAGCCGTCACAACCTGACCCCCTTCCAGGAGTATCTGTAGGAACGTCCC

          TyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGluValPro
  901   TATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCC
        ATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGG

          SerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValVal
  961   TCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGTC
        AGGTGCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAG

          GlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAlaValGln
 1021   GGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCAG
        CCGCACCAGACACGTCGTTATGACGCGGCCGTGCAACCGGGCCCGCTCCCCCGTCACGTC

                            <<<<<<<<<<<<<<<<<<<<<<<NANBH] [---extra
          TrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProValHisHis
 1081   TGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCCAGTCCATCAT
        ACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGTCAGGTAGTA

          -----]
          LysArgOP
 1141   AAGCGTTGACGCTCCCTACGGGTGGACTGTGGAGAGACAGGGCACTGCTAAGGCCCAAAT
        TTCGCAACTGCGAGGGATGCCCACCTGACACCTCTCTGTCCCGTGACGATTCCGGGTTTA

 1201   CTCAGCCATGCATCGAGGGGTACAATCCGTATGGCCAACAACTAGCGCGTACGTAAAGTC
        GAGTCGGTACGTAGCTCCCCATGTTAGGCATACCGGTTGTTGATCGCGCATGCATTTCAG

 1261   TCCTTTCTCGATGGTCCATACCTTAGATGCGTTAGCATTAATCCGAATTC
        AGGAAAGAGCTACCAGGTATGGAATCTACGCAATCGTAATTAGGCTTAAG
```

FIG. 36-2

FIG. 37 a

FIG. 37 b

FIG. 39

FIG. 38

FIG. 40

FIG. 41a

FIG. 41b

Homology between the HCV polypeptide encoded by combined ORF of clones
----
141 through 39c) and the non-structural protein of the Dengue flavi-
----
virus(MNWVD1).

```
                  10        20        30        40        50
HCV       EYVVLLFLLLADARVCSCLWMMLLISQAEAALENLVILNAASLAGTHGLVSFLVFFCFA

MNWVD1    AVSFVTLITGNMSFRDLGRVMVMVGATMTDDIGMGVTYLALLAAFKVRPTFAAGILLRKL
             130       140       150       160       170      180
```

```
           60        70        80        90        100       110
HCV       WYLKGKWVPGAVYTFYGMWPLILLLLALPQRAYALDTEVAASCGGVVLVGLMALTLSPYY

MNWVD1    TSKELMMTTIGIVLLSQSTIPETILELTDALALGMMVLKMVRKMEKYQLAVTIMAILCVP
             190       200       210       220       230      240
```

```
        120       130       140       150       160       170
HCV       KRYISWCLWWLQYFLTRVEAQLHVWIPPLNVRGGRDAVILLMCAVHPTLVFDITKLLLAV
          :    .  .  :      .  ....  .. : .. : ..:: .: .:.:  .
MNWVD1    NAVILQNAWKVSCTILAVVSVSPLFLTSSQQKADWIPLALTIKGLNPTAIF-LTTLSRTN
             250       260       270       280       290
```

```
        180       190       200       210       220       230
HCV       FGPLWILQASLLKVPYF-VRVQGLLRF-CALARKMIGGHYVQMVIIKLGALTGTYVYNHL
          . : :.....   :       . ..::  ...     .. ... .:: ...  ..
MNWVD1    KKRSWPLNEAIMAVGMVSILASSLLKNDIPMTGPLVAGGLLTVCYV-LTGRSADLELERA
          300       310       320       330       340       350
```

```
        240       250       260       270       280       290
HCV       TPLRDWAHNGLRDLAVAVEPVVFSQMETKLITWGADTAACGDIINGLPVSARRGREILLG
          . ... :..... .. ..  ...:..  .... .... .  :. . ...: . :.
MNWVD1    ADVK-WEDQAEISGSSPILSITISE-DGSMSIKNEEEEQTLTILIRTGLLVISG---LFP
          360       370       380       390       400       410
```

```
        300       310       320       330       340       350
HCV       PADGMVSKGWRLLAPITAYAQQTRGLLGCIITSLTGRDKNQVEGEVQIVSTAAQTFLATC
          . ... .:  :.    . :     . .:    . ..   ..  . . . .  .  ...
MNWVD1    VSIPITAAAWYLWEVKKQRAGVLWDVPSPPPVGKAELEDGAYRIKQKGILGYSQIGAGVY
          420       430       440       450       460       470
```

```
        360       370       380       390       400       410
HCV       INGVCWTVYHGAGTRTIASPKGPVIQMYTNVDQDLV----GWPAPQGSRSLTPCTCGSSD
          .:.  :..:.  . ..       . ...:..::: ::        .. ....     .
MNWVD1    KEGTFHTMWHVTRGAVLMHKGKRIEPSWADVKKDLVSCGGGWKLEGEWKEGEEVQVLALE
          480       490       500       510       520       530
```

```
        420       430       440       450       460       470
HCV       LYLVTRHADVIPVRRRGDSRGSLLSPRPISYLKGSSGGPLLCPAGHAVGIFRAAVCTRGV
          .:  ... :      ...  .. ....   . :.::.:.     :..::.  ...  ::.
MNWVD1    PGKNPRAVQTKPGLFKTN--AGTIGAVSLDFSPGTSGSPIIDKKGKVVGLYGNGVVTRSG
          540       550       560       570       580       590
```

```
        480       490       500       510       520       530
HCV       AKAVDFIPVENLETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKS--TKVPAAYAAQ
          :    .. ..:.   .. .. .:  ..:.       ... .. .::::. .:.:.  .  .
MNWVD1    AYVSAIAQTEK--SIEDNPEIEDDIFRK---RKLTIMDLHPGAGKTKRYLPAIVRGAIKR
          600       610       620       630       640
```

```
          540       550       560       570       580
```

```
HCV     GYKVLVLNPS--VAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFLADGGC
        : ..:.:.  ::::..  .  . .:.:.:  . . . .:..: . : . .
MNWVD1  GLRTLILAPTRVVAAEMEEALRGLPIRYQTPAIRAEHTGREIVDLMCHATFTMRLL-SPV
        650       660       670       680       690       700


        590       600       610       620       630       640
HCV     SGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATATPPGSVTVPHPNIEEV
        .  .X..::  ::  :  ::..::  . :  ....:  :.     .. :::::::  .  :  :  V.
MNWVD1  RVPNYNLIIMDEAHFTDPASIAARGYISTRVE-MGEAAGIFMTATPPGSRD-PFPQSNAP
        710       720       730       740       750       760


        650       660       670       680       690       700
HCV     ALSTTGEIPFYGKAIPLEVIKGGRHLIFCHSKKKCDELAAKLVALGINAVAYYRGLDVSV
        ....  :::
MNWVD1  IMDEEREIPERSWSSGHEWVTDFKGKTVWFVPSIKAGNDTAACLRKNGKKVTQLSRKTFD
        770       780       790       800       810       820


        710       720       730       740·      750       760
HCV     IPTSGDVVVVVATDALMTGYTGDFDSVIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRT
MNWVD1  SEYVKTRTNDWNFVVTTDISEMGANFKAERVIDPRRCMKPVILTDGEERVILAGPMPVTH
        830       840       850       860       870       880


        770       780       790·      800       810       820
HCV     QRRGRTGRGKPGIYRFVAPGERPSGMFDSSVLCECYDAGCAWYELTPAETTVRLRAYMNT
MNWVD1  SS
```

FIGURE 42

FIG. 43

DISTRIBUTION OF RANDOM SAMPLES

C100-3 Ag ELISA Preclinical Kit
416ng C100/WELL, 2 HRS 37°C, 20ul SAMPLE

OD @ 492nm
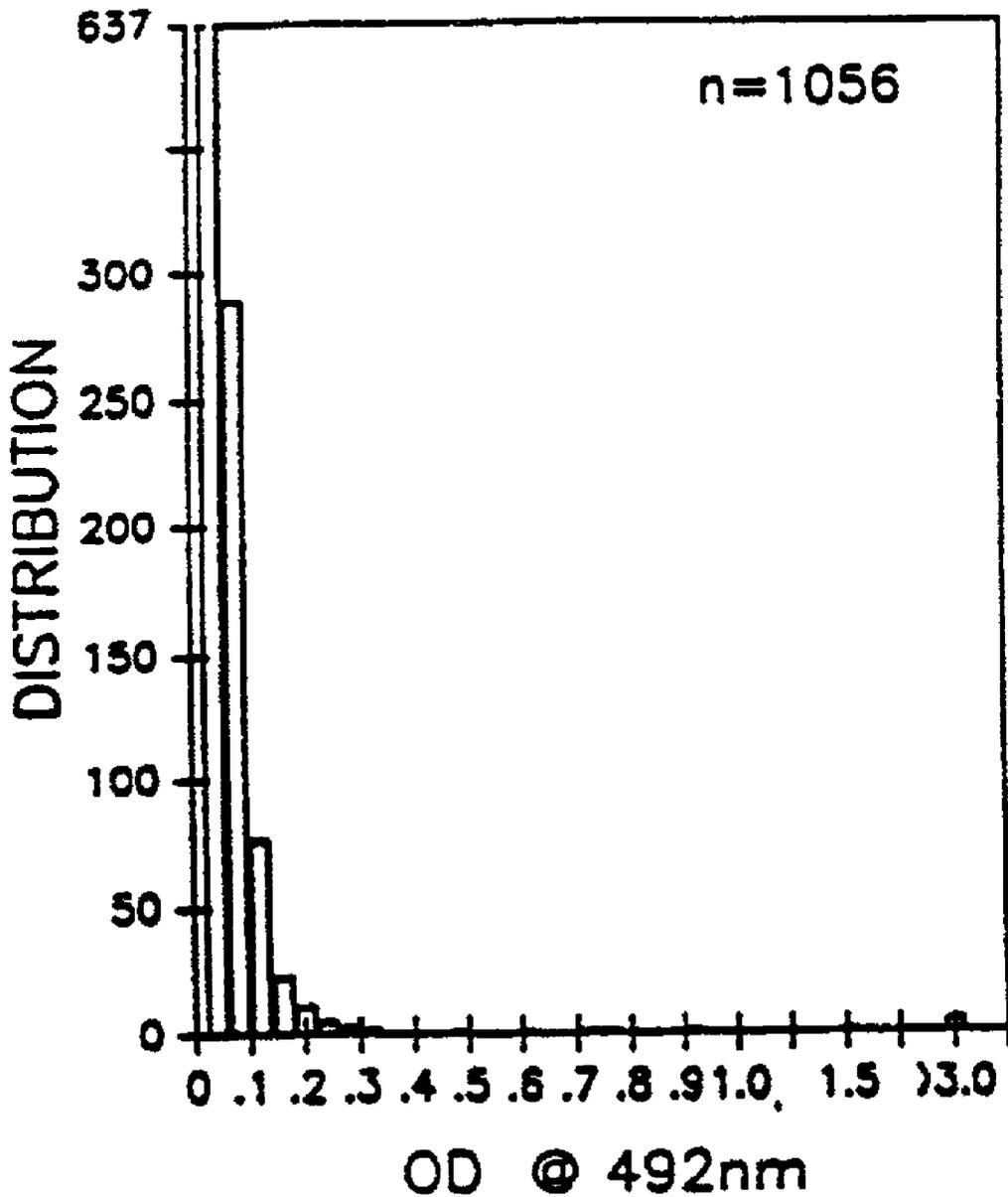
FIG. 44

Distribution of O.D. Values for

Random Blood Donor Samples Tested with Two ELISA

Configurations

C100-3 Ag ELISA MoAB vs Polyclonal
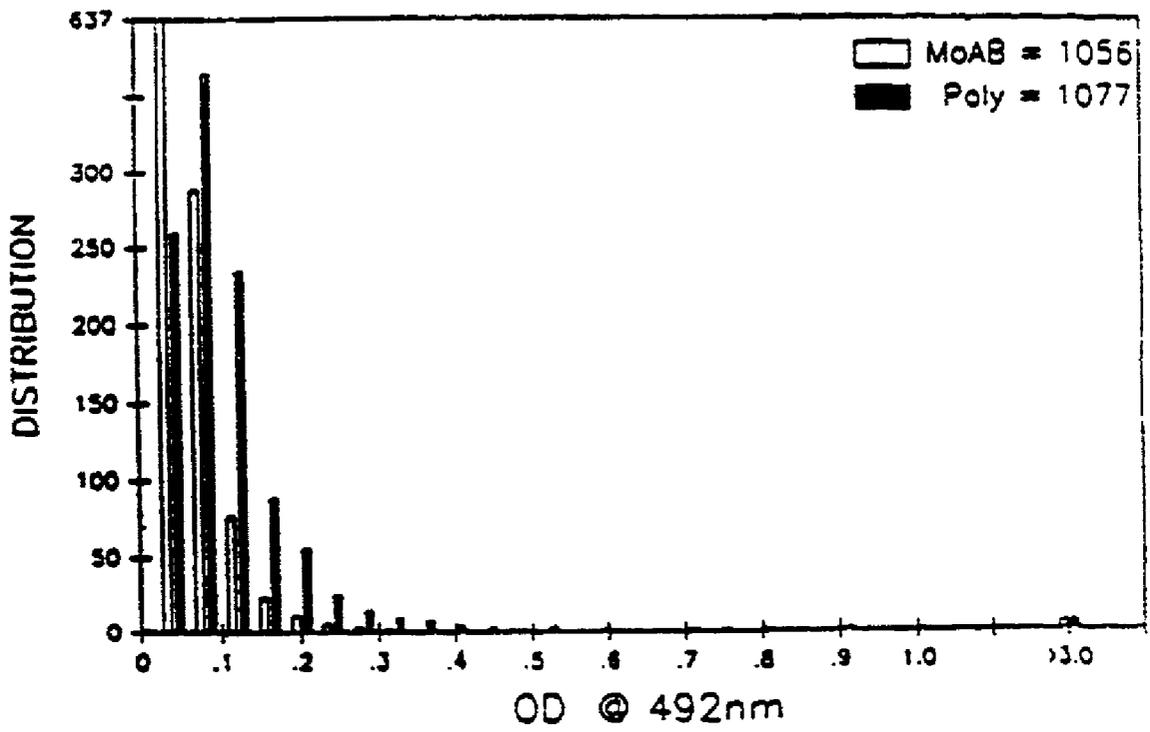
FIG. 45

Name	Common Sequence	Variable Sequence
5'-3-1	AAGCTTGATCGAATTC	CGATCTTGC
-2		CGATCCTGC
-3		CGATCATGC
-4		CGATCGTGC
-5		CGAAGTTGC
-6		CGAAGCTGC
-7		AGATCTTGC
-8		AGATCCTGC
-9		AGATCATGC
-10		AGATCGTGC
-11		AGAAGTTGC
-12		AGAAGCTGC
-13		CGATCTTGT
-14		CGATCCTGT
-15		CGATCATGT
-16		CGATCGTGT
-17		CGAAGTTGT
-18		CGAAGCTGT
-19		AGATCTTGT
-20		AGATCCTGT
-21		AGATCATGT
-22		AGATCGTGT
-23		AGAAGTTGT
-24		AGAAGCTGT
-25		CGCTCTTGC
-26		CGCTCCTGC
-27		CGCTCATGC
-28		CGCTCGTGC
-29		CGCAGTTGC
-30		CGCAGCTGC
-31		CGCTCTTGT
-32		CGCTCCTGT
-33		CGCTCATGT
-34		CGCTCGTGT
-35		CGCAGTTGT
-36		CGCAGCTGT

```
        ValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsn
841  GCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGGAAGCGGCTTTGGAGA
     CGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCCTTCGCCGAAACCTCT


        LeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuVal
901  ACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCG
     TGGAGCATTATGAATTA GTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGC


        PhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPhe
961  TGTTCTTCTGCTTTGCATGGTATCTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCT
     ACAAGAAGACGAAACGTACCATAGACTTCCCATTCACCCACGGGCCTCGCCAGATGTGGA


        TyrGlyMetTrpProLeuLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeu
1021  TCTACGGGATGTGGCCTCTCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGCGC
      AGATGCCCTACACCGGAGAGGAGGACGAGGACAACCGCAACGGGGTCGCCCGCATGCGCG


        AspThrGluValAlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThr
1081  TGGACACGGAGGTGGCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTAA
      ACCTGTGCCTCCACCGGCGCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGATT


        LeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeu
1141  CTCTGTCACCATATTACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTC
      GAGACAGTGGTATAATGTTCGCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAG


        ThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArg
1201  TGACCAGAGTGGAAGCGCAACTGCACGTGTGGATTCCCCCCCTCAACGTCCGAGGGGGGC
      ACTGGTCTCACCTTCGCGTTGACGTGCACACCTAAGGGGGGGAGTTGCAGGCTCCCCCCG


        AspAlaValIleLeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLys
1261  GCGACGCTGTCATCTTACTCATGTGTGCTGTACACCCGACTCTGGTATTTGACATCACCA
      CGCTGCGACAGTAGAATGAGTACACACGACATGTGGGCTGAGACCATAAACTGTAGTGGT


        LeuLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAla
1321  AATTGCTGCTGGCCGTCTTCGGACCCCTTTGGATTCTTCAAGCCAG
      TTAACGACGACCGGCAGAAGCCTGGGGAAACCTAAGAAGTTCGGTC
```

FIG. 46-2

FIG. 47-1 COMBINED ORF OF DNAs K9-1 through 15e

```
         GlyCysProGluArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGly
    1    CAGGCTGTCCTGAGAGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGG
         GTCCGACAGGACTCTCCGATCGGTCGACGGCTGGGGAATGGCTAAAACTGGTCCCGACCC

         ProIleSerTyrAlaAsnGlySerGlyProAspGlnArgProTyrCysTrpHisTyrPro
   61    GCCCTATCAGTTATGCCAACGGAAGCGGCCCCGACCAGCGCCCCTACTGCTGGCACTACC
         CGGGATAGTCAATACGGTTGCCTTCGCCGGGGCTGGTCGCGGGGATGACGACCGTGATGG

         ProLysProCysGlyIleValProAlaLysSerValCysGlyProValTyrCysPheThr
  121    CCCCAAAACCTTGCGGTATTGTGCCCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCA
         GGGGTTTTGGAACGCCATAACACGGGCGCTTCTCACACACACCAGGCCATATAACGAAGT

         ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly
  181    CTCCCAGCCCCGTGGTGGTGGGAACGACCGACAGGTCGGGCGCGCCCACCTACAGCTGGG
         GAGGGTCGGGGCACCACCACCCTTGCTGGCTGTCCAGCCCGCGCGGGTGGATGTCGACCC

         GluAsnAspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPhe
  241    GTGAAAATGATACGGACGTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGT
         CACTTTTACTATGCCTGCAGAAGCAGGAATTGTTATGGTCCGGTGGCGACCCGTTAACCA

         GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal
  301    TCGGTTGTACCTGGATGAACTCAACTGGATTCACCAAAGTGTGCGGAGCGCCTCCTTGTG
         AGCCAACATGGACCTACTTGAGTTGACCTAAGTGGTTTCACACGCCTCGCGGAGGAACAC

         IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro
  361    TCATCGGAGGGGCGGGCAACAACACCCTGCACTGCCCCACTGATTGCTTCCGCAAGCATC
         AGTAGCCTCCCCGCCCGTTGTTGTGGGACGTGACGGGGTGACTAACGAAGGCGTTCGTAG

         AspAlaThrTyrSerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAsp
  421    CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCCTGGTCG
         GCCTGCGGTGTATGAGAGCCACGCCGAGGCCAGGGACCTAGTGTGGGTCCACGGACCAGC

         TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg
  481    ACTACCCGTATAGGCTTTGGCATTATCCTTGTACCATCAACTACACCATATTTAAAATCA
         TGATGGGCATATCCGAAACCGTAATAGGAACATGGTAGTTGATGTGGTATAAATTTTAGT

         MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu
  541    GGATGTACGTGGGAGGGGTCGAACACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCG
         CCTACATGCACCCTCCCCAGCTTGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCCGC

         ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr
  601    AACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTA
         TTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGAT

         GlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeuIle
  661    CACAGTGGCAGGTCCTCCCGTGTTCCTTCACAACCCTACCAGCCTTGTCCACCGGCCTCA
         GTGTCACCGTCCAGGAGGGCACAAGGAAGTGTTGGGATGGTCGGAACAGGTGGCCGGAGT

         HisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAla
  721    TCCACCTCCACCAGAACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCG
         AGGTGGAGGTGGTCTTGTAACACCTGCACGTCATGAACATGCCCCACCCCAGTTCGTAGC

         SerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeuLeuAlaAspAlaArg
  781    CGTCCTGGGCCATTAAGTGGGAGTACGTCGTTCTCCTGTTCCTTCTGCTTGCAGACGCGC
         GCAGGACCCGGTAATTCACCCTCATGCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCG

         ValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsn
  841    GCGTCTGCTCCTGCTTGTGGATGATGCTACTCATATCCCAAGCGGAGGCGGCTTTGGAGA
         CGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCCTCCGCCGAAACCTCT

         LeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuVal
  901    ACCTCGTAATACTTAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCG
         TGGAGCATTATGAATTACGTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGC
```

```
        PhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPhe
961     TGTTCTTCTGCTTTGCATGGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCT
        ACAAGAAGACGAAACGTACCATAAACTTCCCATTCACCCACGGGCCTCGCCAGATGTGGA

        TyrGlyMetTrpProLeuLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeu
1021    TCTACGGGATGTGGCCTCTCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGCGC
        AGATGCCCTACACCGGAGAGGAGGACGAGGACAACCGCAACGGGGTCGCCCGCATGCGCG

        AspThrGluValAlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThr
1081    TGGACACGGAGGTGGCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTGA
        ACCTGTGCCTCCACCGGCGCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGACT

        LeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeu
1141    CTCTGTCACCATATTACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTC
        GAGACAGTGGTATAATGTTCGCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAG

        ThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArg
1201    TGACCAGAGTGGAAGCGCAACTGCACGTGTGGATTCCCCCCCTCAACGTCCGAGGGGGGC
        ACTGGTCTCACCTTCGCGTTGACGTGCACACCTAAGGGGGGGAGTTGCAGGCTCCCCCCG

        AspAlaValIleLeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLys
1261    GCGACGCCGTCATCTTACTCATGTGTGCTGTACACCCGACTCTGGTATTTGACATCACCA
        CGCTGCGGCAGTAGAATGAGTACACACGACATGTGGGCTGAGACCATAAACTGTAGTGGT .

        LeuLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAlaSerLeuLeuLysValPro
1321    AATTGCTGCTGGCCGTCTTCGGACCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTAC
        TTAACGACGACCGGCAGAAGCCTGGGGAAACCTAAGAAGTTCGGTCAAACGAATTTCATG

        TyrPheValArgValGlnGlyLeuLeuArgPheCysAlaLeuAlaArgLysMetIleGly
1381    CCTACTTTGTGCGCGTCCAAGGCCTTCTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCG
        GGATGAAACACGCGCAGGTTCCGGAAGAGGCCAAGACGCGCAATCGCGCCTTCTACTAGC

        GlyHisTyrValGlnMetValIleIleLysLeuGlyAlaLeuThrGlyThrTyrValTyr
1441    GAGGCCATTACGTGCAAATGGTCATCATTAAGTTAGGGGCGCTTACTGGCACCTATGTTT
        CTCCGGTAATGCACGTTTACCAGTAGTAATTCAATCCCCGCGAATGACCGTGGATACAAA

        AsnHisLeuThrProLeuArgAspTrpAlaHisAsnGlyLeuArgAspLeuAlaValAla
1501    ATAACCATCTCACTCCTCTTCGGGACTGGGCGCACAACGGCTTGCGAGATCTGGCCGTGG
        TATTGGTAGAGTGAGGAGAAGCCCTGACCCGCGTGTTGCCGAACGCTCTAGACCGGCACC

        ValGluProValValPheSerGlnMetGluThrLysLeuIleThrTrpGlyAlaAspThr
1561    CTGTAGAGCCAGTCGTCTTCTCCCAAATGGAGACCAAGCTCATCACGTGGGGGGCAGATA
        GACATCTCGGTCAGCAGAAGAGGGTTTACCTCTGGTTCGAGTAGTGCACCCCCCGTCTAT

        AlaAlaCysGlyAspIleIleAsnGlyLeuProValSerAlaArgArgGlyArgGluIle
1621    CCGCCGCGTGCGGTGACATCATCAACGGCTTGCCTGTTTCCGCCCGCAGGGGCCGGGAGA
        GGCGGCGCACGCCACTGTAGTAGTTGCCGAACGGACAAAGGCGGGCGTCCCCGGCCCTCT

        LeuLeuGlyProAlaAspGlyMetValSerLysGlyTrpArgLeuLeuAlaProIleThr
1681    TACTGCTCGGGCCAGCCGATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCA
        ATGACGAGCCCGGTCGGCTACCTTACCAGAGGTTCCCCACCTCCAACGACCGCGGGTAGT

        AlaTyrAlaGlnGlnThrArgGlyLeuLeuGlyCysIleIleThrSerLeuThrGlyArg
1741    CGGCGTACGCCCAGCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCC
        GCCGCATGCGGGTCGTCTGTTCCCCGGAGGATCCCACGTATTAGTGGTCGGATTGACCGG

        AspLysAsnGlnValGluGlyGluValGlnIleValSerThrAlaAlaGlnThrPheLeu
1801    GGGACAAAAACCAAGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCC
        CCCTGTTTTTGGTTCACCTCCCACTCCAGGTCTAACACAGTTGACGACGGGTTTGGAAGG

        AlaThrCysIleAsnGlyValCysTrpThrValTyrHisGlyAlaGlyThrArgThrIle
1861    TGGCAACGTGCATCAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGACGACCA
        ACCGTTGCACGTAGTTACCCCACACGACCTGACAGATGGTGCCCCGGCCTTGCTCCTGGT

        AlaSerProLysGlyProValIleGlnMetTyrThrAsnValAspGlnAspLeuValGly
```

FIG. 47-2

```
1921   TCGCGTCACCCAAGGGTCCTCTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGG
       AGCGCAGTGGGTTCCCAGGACAGTAGGTCTACATATGGTTACATCTGGTTCTGGAACACC


         TrpProAlaProGlnGlySerArgSerLeuThrProCysThrCysGlySerSerAspLeu
1981   GCTGGCCCGCTCCGCAAGGTAGCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACC
       CGACCGGGCGAGGCGTTCCATCGGCGAGTAACTGTGGGACGTGAACGCCGAGGAGCCTGG


         TyrLeuValThrArgHisAlaAspValIleProValArgArgArgGlyAspSerArgGly
2041   TTTACCTGGTCACGAGGCACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGG
       AAATGGACCAGTGCTCCGTGCGGCTACAGTAAGGGCACGCGGCCGCCCCACTATCGTCCC


         SerLeuLeuSerProArgProIleSerTyrLeuLysGlySerSerGlyGlyProLeuLeu
2101   GCAGCCTGCTGTCGCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGT
       CGTCGGACGACAGCGGGGCCGGGTAAAGGATGAACTTTCCGAGGAGCCCCCCAGGCGACA


         CysProAlaGlyHisAlaValGlyIlePheArgAlaAlaValCysThrArgGlyValAla
2161   TGTGCCCCGCGGGGCACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGCGGAGTGG
       ACACGGGGCGCCCCGTGCGGCACCCGTATAAATCCCGGCGCCACACGTGGGCACCTCACC


         LysAlaValAspPheIleProValGluAsnLeuGluThrThrMetArgSerProValPhe
2221   CTAAGGCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGT
       GATTCCGCCACCTGAAATAGGGACACCTCTTGGATCTCTGTTGGTACTCCAGGGGCCACA


         ThrAspAsnSerSerProProValValProGlnSerPheGlnValAlaHisLeuHisAla
2281   TCACGGATAACTCCTCTCCACCAGTAGTGCCCCAGAGCTTCCAGGTGGCTCACCTCCATG
       AGTGCCTATTGAGGAGAGGTGGTCATCACGGGGTCTCGAAGGTCCACCGAGTGGAGGTAC


         ProThrGlySerGlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLys
2341   CTCCCACAGGCAGCGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATA
       GAGGGTGTCCGTCGCCGTTTTCGTGGTTCCAGGGCCGACGTATACGTCGAGTCCCGATAT


         ValLeuValLeuAsnProSerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLys
2401   AGGTGCTAGTACTCAACCCCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCA
       TCCACGATCATGAGTTGGGGAGACAACGACGTTGTGACCCGAAACCACGAATGTACAGGT


         AlaHisGlyIleAspProAsnIleArgThrGlyValArgThrIleThrThrGlySerPro
2461   AGGCTCATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCC
       TCCGAGTACCCTAGCTAGGATTGTAGTCCTGGCCCCACTCTTGTTAATGGTGACCGTCGG


         IleThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyr
2521   CCATCACGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGCGCTT
       GGTAGTGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCCGCCCACGAGCCCCCCGCGAA


         AspIleIleIleCysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGly
2581   ATGACATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCG
       TACTGTATTATTAAACACTGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTAGC


         ThrValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThr
2641   GCACTGTCCTTGACCAAGCAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCA
       CGTGACAGGAACTGGTTCGTCTCTGACGCCCCCGCTCTGACCAACACGAGCGGTGGCGGT


         ProProGlySerValThrValProHisProAsnIleGluGluValAlaLeuSerThrThr
2701   CCCCTCCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCA
       GGGGAGGCCCGAGGCAGTGACACGGGGTAGGGTTGTAGCTCCTCCAACGAGACAGGTGGT


         GlyGluIleProPheTyrGlyLysAlaIleProLeuGluValIleLysGlyGlyArgHis
2761   CCGGAGAGATCCCTTTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGGAGAC
       GGCCTCTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCCCTCTG


         LeuIlePheCysHisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeu
2821   ATCTCATCTTCTGTCATTCAAAGAAGAAGTGCGACGAACTCGCCGCAAAGCTGGTCGCAT
       TAGAGTAGAAGACAGTAAGTTTCTTCTTCACGCTGCTTGAGCGGCGTTTCGACCAGCGTA


         GlyIleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGly
2881   TGGGCATCAATGCCGTGGCCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCG
       ACCCGTAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTCGC
```

<div align="center">FIG. 47-3</div>

```
             AspValValValValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSer
2941  GCGATGTTGTCGTCGTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACT
      CGCTACAACAGCAGCACCGTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGA

             ValIleAspCysAsnThrCysValThrGlnThrValAspPheSerLeuAspProThrPhe
3001  CGGTGATAGACTGCAATACGTGTGTCACCCAGACAGTCGATTTCAGCCTTGACCCTACCT
      GCCACTATCTGACGTTATGCACACAGTGGGTCTGTCAGCTAAAGTCGGAACTGGGATGGA

             ThrIleGluThrIleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArg
3061  TCACCATTGAGACAATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCA
      AGTGGTAACTCTGTTAGTGCGAGGGGGTCCTACGACAGAGGGCGTGAGTTGCAGCCCCGT

             ThrGlyArgGlyLysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGly
3121  GGACTGGCAGGGGGAAGCCAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCG
      CCTGACCGTCCCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGC

             MetPheAspSerSerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeu
3181  GCATGTTCGACTCGTCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGC
      CGTACAAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCG

             ThrProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProVal
3241  TCACGCCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCG
      AGTGCGGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGC

             CysGlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAla
3301  TGTGCCAGGACCATCTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATG
      ACACGGTCCTGGTAGAACTTAAAACCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTAC

             HisPheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGln
3361  CCCACTTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACC
      GGGTGAAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGG

             AlaThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCys
3421  AAGCCACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGT
      TTCGGTGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCA

             LeuIleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAla
3481  GTTTGATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCG
      CAAACTAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGC

             ValGlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSer
3541  CTGTTCAGAATGAAATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGT
      GACAAGTCTTACTTTAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACGTACA

             AlaAspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeu
3601  CGGCCGACCTGGAGGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTT
      GCCGGCTGGACCTCCAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGACGAA

             AlaAlaTyrCysLeuSerThrGlyCysValValIleValGlyArgValValLeuSerGly
3661  TGGCCGCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCG
      ACCGGCGCATAACGGACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGC

             LysProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGlu
3721  GGAAGCCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAG
      CCTTCGGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTC

             CysSerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGln
3781  AGTGCTCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGC
      TCACGAGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCG

             LysAlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaVal
3841  AGAAGGCCCTCGGCCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTG
      TCTTCCGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGAC

             GlnThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSer
```

FIG. 47-4

```
3901  TCCAGACCAACTGGCAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCA
      AGGTCTGGTTGACCGTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGT

          GlyIleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeu
3961  GTGGGATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACCCGCCATTGCTTCAT
      CACCCTATGTTATGAACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACGAAGTA

          MetAlaPheThrAlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsn
4021  TGATGGCTTTTACAGCTGCTGTCACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCA
      ACTACCGAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGT

          IleLeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheVal
4081  ACATATTGGGGGGGTGGGTGGCTGCCCAGCTCGCCGCCCCGGTGCCGCTACTGCCTTTG
      TGTATAACCCCCCCACCCACCGACGGGTCGAGCGGCGGGGGCCACGGCGATGACGGAAAC

          GlyAlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAsp
4141  TGGGCGCTGGCTTAGCTGGCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAG
      ACCCGCGACCGAATCGACCGCGGCGGTAGCCGTCACAACCTGACCCCTTCCAGGAGTATC

          IleLeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSer
4201  ACATCCTTGCAGGGTATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGA
      TGTAGGAACGTCCCATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACT

          GlyGluValProSerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGly
4261  GCGGTGAGGTCCCCTCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCG
      CGCCACTCCAGGGGAGGTGCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGC

          AlaLeuValValGlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGlu
4321  GAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCG
      CTCGGGAGCATCAGCCGCACCAGACACGTCGTTATGACGCGGCCGTGCAACCGGGCCCGC

          GlyAlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSer
4381  AGGGGGCAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTT
      TCCCCCGTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAA

          ProThrHisTyrValProGluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSer
4441  CCCCCACGCACTACGTGCCGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCA
      GGGGGTGCGTGATGCACGGCCTCTCGCTACGTCGACGGGCGCAGTGACGGTATGAGTCGT

          LeuThrValThrGlnLeuLeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThr
4501  GCCTCACTGTAACCCAGCTCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGTGTACCA
      CGGAGTGACATTGGGTCGAGGACTCCGCTGACGTGGTCACCTATTCGAGCCTCACATGGT

          ProCysSerGlySerTrpLeuArgAspIleTrpAspTrpIleCysGluValLeuSerAsp
4561  CTCCATGCTCCGGTTCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTTGAGCG
      GAGGTACGAGGCCAAGGACCGATTCCCTGTAGACCCTGACCTATACGCTCCACAACTCGC

          PheLysThrTrpLeuLysAlaLysLeuMetProGlnLeuProGlyIleProPheValSer
4621  ACTTTAAGACCTGGCTAAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCTTTGTGT
      TGAAATTCTGGACCGATTTTCGATTCGAGTACGGTGTCGACGGACCCTAGGGGAAACACA

          CysGlnArgGlyTyrLysGlyValTrpArgValAspGlyIleMetHisThrArgCysHis
4681  CCTGCCAGCGCGGGTATAAGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCC
      GGACGGTCGCGCCCATATTCCCCCAGACCGCTCACCTGCCGTAGTACGTGTGAGCGACGG

          CysGlyAlaGluIleThrGlyHisValLysAsnGlyThrMetArgIleValGlyProArg
4741  ACTGTGGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTA
      TGACACCTCGACTCTAGTGACCTGTACAGTTTTTGCCCTGCTACTCCTAGCAGCCAGGAT

          ThrCysArgAsnMetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGlyProCys
4801  GGACCTGCAGGAACATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCT
      CCTGGACGTCCTTGTACACCTCACCCTGGAAGGGGTAATTACGGATGTGGTGCCCGGGGA

          ThrProLeuProAlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyr
4861  GTACCCCCCTTCCTGCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAAT
      CATGGGGGGAAGGACGCGGCTTGATGTGCAAGCGCGATACCTCCCACAGACGTCTCCTTA
```

<p style="text-align:center">FIG. 47-5</p>

```
            ValGluIleArgGlnValGlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeu
4921  ATGTGGAGATAAGGCAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATC
      TACACCTCTATTCCGTCCACCCCCTGAAGGTGATGCACTGCCCATACTGATGACTGTTAG

            LysCysProCysGlnValProSerProGluPhePheThrGluLeuAspGlyValArgLeu
4981  TCAAATGCCCGTGCCAGGTCCCATCGCCCGAATTTTTCACAGAATTGGACGGGGTGCGCC
      AGTTTACGGGCACGGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTAACCTGCCCCACGCGG

            HisArgPheAlaProProCysLysProLeuLeuArgGluGluValSerPheArgValGly
5041  TACATAGGTTTGCGCCCCCCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTCAGAGTAG
      ATGTATCCAAACGCGGGGGGACGTTCGGGAACGACGCCCTCCTCCATAGTAAGTCTCATC

            LeuHisGluTyrProValGlySerGlnLeuProCysGluProGluProAspValAlaVal
5101  GACTCCACGAATACCCGGTAGGGTCGCAATTACCTTGCGAGCCCGAACCGGACGTGGCCG
      CTGAGGTGCTTATGGGCCATCCCAGCGTTAATGGAACGCTCGGGCTTGGCCTGCACCGGC

            LeuThrSerMetLeuThrAspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeu
5161  TGTTGACGTCCATGCTCACTGATCCCTCCCATATAACAGCAGAGGCGGCCGGGCGAAGGT
      ACAACTGCAGGTACGAGTGACTAGGGAGGGTATATTGTCGTCTCCGCCGGCCCGCTTCCA

            AlaArgGlySerProProSerValAlaSerSerSerAlaSerGlnLeuSerAlaProSer
5221  TGGCGAGGGGATCACCCCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCAT
      ACCGCTCCCCTAGTGGGGGGAGACACCGGTCGAGGAGCCGATCGGTCGATAGGCGAGGTA

            LeuLysAlaThrCysThrAlaAsnHisAspSerProAspAlaGluLeuIleGluAlaAsn
5281  CTCTCAAGGCAACTTGCACCGCTAACCATGACTCCCCTGATGCTGAGCTCATAGAGGCCA
      GAGAGTTCCGTTGAACGTGGCGATTGGTACTGAGGGGACTACGACTCGAGTATCTCCGGT

            LeuLeuTrpArgGlnGluMetGlyGlyAsnIleThrArgValGluSerGluAsnLysVal
5341  ACCTCCTATGGAGGCAGGAGATGGGCGGCAACATCACCAGGGTTGAGTCAGAAAACAAAG
      TGGAGGATACCTCCGTCCTCTACCCGCCGTTGTAGTGGTCCCAACTCAGTCTTTTGTTTC

            ValIleLeuAspSerPheAspProLeuValAlaGluGluAspGluArgGluIleSerVal
5401  TGGTGATTCTGGACTCCTTCGATCCGCTTGTGGCGGAGGAGGACGAGCGGGAGATCTCCG
      ACCACTAAGACCTGAGGAAGCTAGGCGAACACCGCCTCCTCCTGCTCGCCCTCTAGAGGC

            ProAlaGluIleLeuArgLysSerArgArgPheAlaGlnAlaLeuProValTrpAlaArg
5461  TACCCGCAGAAATCCTGCGGAAGTCTCGGAGATTCGCCCAGGCCCTGCCCGTTTGGGCGC
      ATGGGCGTCTTTAGGACGCCTTCAGAGCCTCTAAGCGGGTCCGGGACGGGCAAACCCGCG

            ProAspTyrAsnProProLeuValGluThrTrpLysLysProAspTyrGluProProVal
5521  GGCCGGACTATAACCCCCCGCTAGTGGAGACGTGGAAAAAGCCCGACTACGAACCACCTG
      CCGGCCTGATATTGGGGGGCGATCACCTCTGCACCTTTTTCGGGCTGATGCTTGGTGGAC

            ValHisGlyCysProLeuProProProLysSerProProValProProProArgLysLys
5581  TGGTCCATGGCTGTCCGCTTCCACCTCCAAAGTCCCCTCCTGTGCCTCCGCCTCGGAAGA
      ACCAGGTACCGACAGGCGAAGGTGGAGGTTTCAGGGGAGGACACGGAGGCGGAGCCTTCT

            ArgThrValValLeuThrGluSerThrLeuSerThrAlaLeuAlaGluLeuAlaThrArg
5641  AGCGGACGGTGGTCCTCACTGAATCAACCCTATCTACTGCCTTGGCCGAGCTCGCCACCA
      TCGCCTGCCACCAGGAGTGACTTAGTTGGGATAGATGACGGAACCGGCTCGAGCGGTGGT

            SerPheGlySerSerSerThrSerGlyIleThrGlyAspAsnThrThrThrSerSerGlu
5701  GAAGCTTTGGCAGCTCCTCAACTTCCGGCATTACGGGCGACAATACGACAACATCCTCTG
      CTTCGAAACCGTCGAGGAGTTGAAGGCCGTAATGCCCGCTGTTATGCTGTTGTAGGAGAC

            ProAlaProSerGlyCysProProAspSerAspAlaGluSerTyrSerSerMetProPro
5761  AGCCCGCCCCTTCTGGCTGCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCCATGCCCC
      TCGGGCGGGGAAGACCGACGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGG

            LeuGluGlyGluProGlyAspProAspLeuSerAspGlySerTrpSerThrValSerSer
5821  CCCTGGAGGGGGAGCCTGGGGATCCGGATCTTAGCGACGGGTCATGGTCAACGGTCAGTA
      GGGACCTCCCCCTCGGACCCCTAGGCCTAGAATCGCTGCCCAGTACCAGTTGCCAGTCAT

      GluAlaAsnAlaGluAspValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeu
```

FIG. 47-6

```
5881  GTGAGGCCAACGCGGAGGATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCAC
      CACTCCGGTTGCGCCTCCTACAGCACACGACGAGTTACAGAATGAGAACCTGTCCGCGTG

          ValThrProCysAlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeu
5941  TGGTCACCCCGTGCGCCGCGGAAGAACAGAAACTGCCCATCAATGCACTAAGCAACTCGT
      AGCAGTGGGGCACGCGGCGCCTTCTTGTCTTTGACGGGTAGTTACGTGATTCGTTGAGCA

          LeuArgHisHisAsnLeuValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLys
6001  TGCTACGTCACCACAATTTGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGA
      ACGATGCAGTGGTGTTAAACCACATAAGGTGGTGGAGTGCGTCACGAACGGTTTCCGTCT

          LysValThrPheAspArgLeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGlu
6061  AGAAAGTCACATTTGACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGG
      TCTTTCAGTGTAAACTGTCTGACGTTCAAGACCTGTCGGTAATGGTCCTGCATGAGTTCC

          ValLysAlaAlaAlaSerLysValLysAlaAsnLeuLeuSerValGluGluAlaCysSer
6121  AGGTTAAAGCAGCGGCCGTCAAAAGTGAAGGCTAACTTGCTATCCGTAGAGGAAGCTTGCA
      TCCAATTTCGTCGCCGGCAGTTTTCACTTCCGATTGAACGATAGGCATCTCCTTCGAACGT

          LeuThrProProHisSerAlaLysSerLysPheGlyTyrGlyAlaLysAspValArgCys
6181  GCCTGACGCCCCCACACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAAGACGTCCGTT
      CGGACTGCGGGGGTGTGAGTCGGTTTAGGTTCAAACCAATACCCCGTTTTCTGCAGGCAA

          HisAlaArgLysAlaValThrHisIleAsnSerValTrpLysAspLeuLeuGluAspAsn
6241  GCCATGCCAGAAAGGCCGTAACCCACATCAACTCCGTGTGGAAAGACCTTCTGGAAGACA
      CGGTACGGTCTTTCCGGCATTGGGTGTAGTTGAGGCACACCTTTCTGGAAGACCTTCTGT

          ValThrProIleAspThrThrIleMetAlaLysAsnGluValPheCysValGlnProGlu
6301  ATGTAACACCAATAGACACTACCATCATGGCTAAGAACGAGGTTTTCTGCGTTCAGCCTG
      TACATTGTGGTTATCTGTGATGGTAGTACCGATTCTTGCTCCAAAAGACGCAAGTCGGAC

          LysGlyGlyArgLysProAlaArgLeuIleValPheProAspLeuGlyValArgValCys
6361  AGAAGGGGGGTCGTAAGCCAGCTCGTCTCATCGTGTTCCCCGATCTGGGCGTGCGCGTGT
      TCTTCCCCCCAGCATTCGGTCGAGCAGAGTAGCACAAGGGGCTAGACCCGCACGCGCACA

          GluLysMetAlaLeuTyrAspValValThrLysLeuProLeuAlaValMetGlySerSer
6421  GCGAAAAGATGGCTTTGTACGACGTGGTTACAAAGCTCCCCTTGGCCGTGATGGGAAGCT
      CGCTTTTCTACCGAAACATGCTGCACCAATGTTTCGAGGGGAACCGGCACTACCCTTCGA

          TyrGlyPheGlnTyrSerProGlyGlnArgValGluPheLeuValGlnAlaTrpLysSer
6481  CCTACGGATTCCAATACTCACCAGGACAGCGGGTTGAATTCCTCGTGCAAGCGTGGAAGT
      GGATGCCTAAGGTTATGAGTGGTCCTGTCGCCCAACTTAAGGAGCACGTTCGCACCTTCA

          LysLysThrProMetGlyPheSerTyrAspThrArgCysPheAspSerThrValThrGlu
6541  CCAAGAAAACCCCAATGGGGTTCTCGTATGATACCCGCTGCTTTGACTCCACAGTCACTG
      GGTTCTTTTGGGGTTACCCCAAGAGCATACTATGGGCGACGAAACTGAGGTGTCAGTGAC

          SerAspIleArgThrGluGluAlaIleTyrGlnCysCysAspLeuAspProGlnAlaArg
6601  AGAGCGACATCCGTACGGAGGAGGCAATCTACCAATGTTGTGACCTCGACCCCCAAGCCC
      TCTCGCTGTAGGCATGCCTCCTCCGTTAGATGGTTACAACACTGGAGCTGGGGGTTCGGG

          ValAlaIleLysSerLeuThrGluArgLeuTyrValGlyGlyProLeuThrAsnSerArg
6661  GCGTGGCCATCAAGTCCCTCACCGAGAGGCTTTATGTTGGGGGCCCTCTTACCAATTCAA
      CGCACCGGTAGTTCAGGGAGTGGCTCTCCGAAATACAACCCCCGGGAGAATGGTTAAGTT

          GlyGluAsnCysGlyTyrArgArgCysArgAlaSerGlyValLeuThrThrSerCysGly
6721  GGGGGGAGAACTGCGGCTATCGCAGGTGCCGCGCGAGCGGCGTACTGACAACTAGCTGTG
      CCCCCCTCTTGACGCCGATAGCGTCCACGGCGCGCTCGCCGCATGACTGTTGATCGACAC

          AsnThrLeuThrCysTyrIleLysAlaArgAlaAlaCysArgAlaAlaGlyLeuGlnAsp
6781  GTAACACCCTCACTTGCTACATCAAGGCCCGGGCAGCCTGTCGAGCCGCAGGGCTCCAGG
      CATTGTGGGAGTGAACGATGTAGTTCCGGGCCCGTCGGACAGCTCGGCGTCCCGAGGTCC

          CysThrMetLeuValCysGlyAspAspLeuValValIleCysGluSerAlaGlyValGln
6841  ACTGCACCATGCTCGTGTGTGGCGACGACTTAGTCGTTATCTGTGAAAGCGCGGGGTCC
      TGACGTGGTACGAGCACACACCGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCCAGG
```

<div align="center">FIG. 47-7</div>

```
        GluAspAlaAlaSerLeuArgAlaPheThrGluAlaMetThrArgTyrSerAlaProPro
6901    AGGAGGACGCGGCGAGCCTGAGAGCCTTCACGGAGGCTATGACCAGGTACTCCGCCCCCC
        TCCTCCTGCGCCGCTCGGACTCTCGGAAGTGCCTCCGATACTGGTCCATGAGGCGGGGGG

        GlyAspProProGlnProGluTyrAspLeuGluLeuIleThrSerCysSerSerAsnVal
6961    CTGGGGACCCCCCACAACCAGAATACGACTTGGAGCTCATAACATCATGCTCCTCCAACG .
        GACCCCTGGGGGGTGTTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGAGGTTGC

        SerValAlaHisAspGlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThr
7021    TGTCAGTCGCCCACGACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACAA
        ACAGTCAGCGGGTGCTGCCGCGACCTTTCTCCCAGATGATGGAGTGGGCACTGGGATGTT

        ProLeuAlaArgAlaAlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGly
7081    CCCCCCTCGCGAGAGCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAG
        GGGGGGAGCGCTCTCGACGCACCCTCTGTCGTTCTGTGTGAGGTCAGTTAAGGACCGATC

        AsnIleIleMetPheAlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePhe
7141    GCAACATAATCATGTTTGCCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTTCT
        CGTTGTATTAGTACAAACGGGGGTGTGACACCCGCTCCTACTATGACTACTGGGTAAAGA

        SerValLeuIleAlaArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAla
7201    TTAGCGTCCTTATAGCCAGGGACCAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGG
        AATCGCAGGAATATCGGTCCCTGGTCGAACTTGTCCGGGAGCTAACGCTCTAGATGCCCC

        CysTyrSerIleGluProLeuAspLeuProProIleIleGlnArgLeu
7251    CCTGCTACTCCATAGAACCACTTGATCTACCTCCAATCATTCAAAGACTC
        GGACGATGAGGTATCTTGGTGAACTAGATGGAGGTTAGTAAGTTTCTGAG
```

FIG. 47-8

Translation of DNA 131

```
        ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly
  1     CTCCCAGCCCCGTGGTGGTGGGAACGACCGACAGGTCGGGCGCGCCTACCTACAGCTGGG
        GAGGGTCGGGGCACCACCACCCTTGCTGGCTGTCCAGCCCGCGCGGATGGATGTCGACCC

        GluAsnAspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPhe
 61     GTGAAAATGATACGGACGTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGT
        CACTTTTACTATGCCTGCAGAAGCAGGAATTGTTATGGTCCGGTGGCGACCCGTTAACCA

        GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal
121     TCGGTTGTACCTGGATGAACTCAACTGGATTCACCAAAGTGTGCGGAGCGCCTCCTTGTG
        AGCCAACATGGACCTACTTGAGTTGACCTAAGTGGTTTCACACGCCTCGCGGAGGAACAC

        IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro
181     TCATCGGAGGGGCGGGCAACAACACCCTGCACTGCCCCACTGATTGCTTCCGCAAGCATC
        AGTAGCCTCCCCGCCCGTTGTTGTGGGACGTGACGGGGTGACTAACGAAGGCGTTCGTAG

        AspAlaThrTyrSerArgCysGlySerGlyProTrpLeuThrProArgCysLeuValAsp
241     CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGCTCACACCCAGGTGCCTGGTCG
        GCCTGCGGTGTATGAGAGCCACGCCGAGGCCAGGGACCGAGTGTGGGTCCACGGACCAGC

                                                ---------------------
        TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg
301     ACTACCCGTATAGGCTTTGGCATTATCCTTGTACCATCAACTACACCATATTTAAAATCA
        TGATGGGCATATCCGAAACCGTAATAGGAACATGGTAGTTGATGTGGTATAAATTTTAGT

        ------------------------------------------------------------
        MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu
361     GGATGTACGTGGGAGGGGTCGAGCACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCG
        CCTACATGCACCCTCCCCAGCTCGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCCGC

        ----------------Overlap with 12f------------------------------
        ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr
421     AACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTA
        TTGCAACGCTAGACCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGAT

        -----------------------------------------------------------
        GlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeu
481     CACAGTGGCAGGTCCTCCCGTGTTCCTTCACAACCCTGCCAGCCTTGTCCACCGGCCTCA
        GTGTCACCGTCCAGGAGGGCACAAGGAAGTGTTGGGACGGTCGGAACAGGTGGCCGGAGT
```

FIGURE 48

Translation of DNA 26j

```
        LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
    1   GCTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGAGAGGCTAGCCAGCTGCCG
        CGAAAAGATAGTGGTGTTCAAGTTGAGAAGTCCGACAGGACTCTCCGATCGGTCGACGGC


        ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
   61   ACCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTATGCCAACGGAAGCGGCCC
        TGGGGAATGGCTAAAACTGGTCCCGACCCCGGGATAGTCAATACGGTTGCCTTCGCCGGG


        AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
  121   CGACCAGCGCCCCTACTGCTGGCACTACCCCCCAAAACCTTGCGGTATTGTGCCCGCGAA
        GCTGGTCGCGGGGATGACGACCGTGATGGGGGGTTTTGGAACGCCATAACACGGGCGCTT


                                        ---Overlap with 131---
        SerValCysGlyProValTyrCysPheThrProSerProValValVal
  181   GAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGTGGTGGTGGG
        CTCACACACACCAGGCCATATAACGAAGTGAGGGTCGGGGCACCACCACCC
```

FIGURE 49

Translation of DNA CA59a

```
     LeuValMetAlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIleAlaGlyAla
  1  TTGGTAATGGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATCGCTGGTGCT
     AACCATTACCGAGTCGACGAGGCCTAGGGTGTTCGGTAGAACCTGTACTAGCGACCACGA


     HisTrpGlyValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrpAlaLysVal
 61  CACTGGGGAGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGGGCGAAGGTC
     GTGACCCCTCAGGACCGCCCGTATCGCATAAAGAGGTACCACCCCTTGACCCGCTTCCAG


     LeuValValLeuLeuLeuPheAlaGlyValAspAlaGluThrHisValThrGlyGlySer
121  CTGGTAGTGCTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGAAGT
     GACCATCACGACGACGATAAACGGCCGCAGCTGCGCCTTTGGGTGCAGTGGCCCCCTTCA


     AlaGlyHisThrValSerGlyPheValSerLeuLeuAlaProGlyAlaLysGlnAsnVal
181  GCCGGCCACACTGTGTCTGGATTTGTTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTC
     CGGCCGGTGTGACACAGACCTAAACAATCGGAGGAGCGTGGTCCGCGGTTCGTCTTGCAG


     GlnLeuIleAsnThrAsnGlySerTrpHisLeuAsnSerThrAlaLeuAsnCysAsnAsp
241  CAGCTGATCAACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAACTGCAATGAT
     GTCGACTAGTTGTGGTTGCCGTCAACCGTGGAGTTATCGTGCCGGGACTTGACGTTACTA
                                                               -----


     SerLeuAsnThrGlyTrpLeuAlaGlyLeuPheTyrHisHisLysPheAsnSerSerGly
301  AGCCTCAACACCGGCTGGTTGGCAGGGCTTTTCTATCACCACAAGTTCAACTCTTCAGGC
     TCGGAGTTGTGGCCGACCAACCGTCCCGAAAAGATAGTGGTGTTCAAGTTGAGAAGTCCG
                              -------Overlap with 26j-------------


     -------Overlap with K9-1---------
     CysProGluArgLeuAlaSerCysArgPro
361  TGTCCTGAGAGGCTAGCCAGCTGCCGACCCC
     ACAGGACTCTCCGATCGGTCGACGGCTGGGG
     ----------------------------------
```

FIGURE 50

Translation of DNA CA84a

```
      GlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrpAsp
  1   CGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACGGGTCACCGCATGGCATGGG
      GCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCCAGTGGCGTACCGTACCC
                                          -----------------------------

      MetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIlePro
 61   ATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATCC
      TATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCTAGG


      --------------------------------------------------------------
      GlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAlaTyr
121   CACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGGAGTCCTGGCGGGCATAGCGT
      GTGTTCGGTAGAACCTGTACTAGCGACCACGAGTGACCCCTCAGGACCGCCCGTATCGCA

      -----------Overlap with CA59a--------------------------------
      PheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuLeuPheAlaGlyVal
181   ATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGTGCTGCTGCTATTTGCCGGCG
      TAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCACGACGACGATAAACGGCCGC


      -----------------------------------------
      AspAlaGluThrHisValThrGly
241   TCGACGCGGAAACCCACGTCACCGGGG
      AGCTGCGCCTTTGGGTGCAGTGGCCCC
```

FIGURE 51

Translation of DNA CA156e

```
       CysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGln
   1   GTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCCCGCGACGCA
       CACAACCCACCGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGCGCTGCGT

       LeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrVal
  61   GCTTCGACGTCACATCGATCTGCTTGTCGGGAGCGCCACCCTCTGTTCGGCCCTCTACGT
       CGAAGCTGCAGTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGGAGATGCA

       GlyAspLeuCysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArg
 121   GGGGGACCTATGCGGGTCTGTCTTTCTTGTCGGCCAACTGTTCACCTTCTCTCCCAGGCG
       CCCCCTGGATACGCCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAAGAGAGGGTCCGC

       _____

       HisTrpThrThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArg
 181   CCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACGGGTCACCG
       GGTGACCTGCTGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCCAGTGGC

       _____Overlap with CA84a_____
       MetAlaTrpAspMetMetMetAsnTrpSerProThrThrAlaLeuValValAlaGlnLeu
 241   CATGGCATGGGATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAGTGGCTCAGCT
       GTACCGTACCCTATACTACTACTTGACCAGGGGATGCTGCCGCAACCATCACCGAGTCGA

       _____

       LeuArgIleProGlnAla
 301   GCTCCGGATCCCACAAGCC
       CGAGGCCTAGGGTGTTCGG
```

FIGURE 52

Translation of DNA CA167b

```
        SerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAla
  1     CTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGC
        GAGGTGCCCCGAAATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACATGCTCCG


        AlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSer
  61    GGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTGAGGGCAACGCCTC
        CCGGCTACGGTAGGACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTTGCGGAG


        ------------------------------------------------------------
        ArgCysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThr
  121   GAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCCCGCGAC
        CTCCACAACCCACCGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGCGCTG


        ----------------Overlap with CA156e-------------------------
        GlnLeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyr
  181   GCAGCTTCGACGTCACATCGATCTCCTTGTCGGGAGCGCTACCCTCTGTTCGGCCCTCTA
        CGTCGAAGCTGCAGTGTAGCTAGACGAACAGCCCTCGCGATGGGAGACAAGCCGGGAGAT


        ------------------------------------------------
        ValGlyAspLeuCysGlySerValPheLeu
  241   CGTGGGGGACTTGTGCGGGTCTGTCTTTCTTG
        GCACCCCCTGAACACGCCCAGACAGAAAGAAC
```

FIGURE 53

COMBINED ORF OF DNAs pi14a THROUGH 15e

(pi14a/CA167b/CA156e/CA84a/CA59a/K9-1/12f/14i/11b/7f/7e/

8h/33c/40b/37b/35/36/8l/32/33b/25c/14c/8f/33f/33g/39c/

35f/19g/26g & 15e)


```
          ArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGlyPheAlaAspLeuMet
     1    AGGTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATG
          TCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCACGCCGAAGCGGCTGGAGTAC

          GlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGly
    61    GGGTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGGC
          CCCATGTATGGCGAGCAGCCGCGGGGAGAACCTCCGCGACGGTCCCGGGACCGCGTACCG

          ValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPhe
   121    GTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTTC
          CAGGCCCAAGACCTTCTGCCGCACTTGATACGTTGTCCCTTGGAAGGACCAACGAGAAAG

          SerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnVal
   181    TCTATCTTCCTTCTGGCCCTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGTG
          AGATAGAAGGAAGACCGGGACGAGAGAACGAACTGACACGGGCGAAGCCGGATGGTTCAC

          ArgAsnSerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIleValTyr
   241    CGCAACTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTAC
          GCGTTGAGGTGCCCCGAAATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACATG

          GluAlaAlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGluGlyAsn
   301    GAGGCGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTGAGGGCAAC
          CTCCGCCGGCTACGGTAGGACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTTG

          AlaSerArgCysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuPro
   361    GCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCCC
          CGGAGCTCCACAACCCACCGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGG

          AlaThrGlnLeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAla
   421    GCGACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGCGCCACCCTCTGTTCGGCC
          CGCTGCGTCGAAGCTGCAGTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGG

          LeuTyrValGlyAspLeuCysGlySerValPheLeuValGlyGlnLeuPheThrPheSer
   481    CTCTACGTGGGGGACCTATGCGGGTCTGTCTTTCTTGTCGGCCAACTGTTCACCTTCTCT
          GAGATGCACCCCCTGGATACGCCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAAGAGA

          ProArgArgHisTrpThrThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThr
   541    CCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACG
          GGGTCCGCGGTGACCTGCTGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGC

          GlyHisArgMetAlaTrpAspMetMetMetAsnTrpSerProThrThrAlaLeuValMet
   601    GGTCACCGCATGGCATGGGATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAATG
          CCAGTGGCGTACCGTACCCTATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTAC

          AlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGly
   661    GCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGGA
          CGAGTCGACGAGGCCTAGGGTGTTCGGTAGAACCTGTACTAGCGACCACGAGTGACCCCT

          ValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrpAlaLysValLeuValVal
   721    GTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGTG
          CAGGACCGCCCGTATCGCATAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCAC

          LeuLeuLeuPheAlaGlyValAspAlaGluThrHisValThrGlyGlySerAlaGlyHis
   781    CTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGAAGTGCCGGCCAC
          GACGACGATAAACGGCCGCAGCTGCGCCTTTGGGTGCAGTGGCCCCCTTCACGGCCGGTG
```

FIGURE 54-1

```
          ThrValSerGlyPheValSerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIle
     841  ACTGTGTCTGGATTTGTTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGATC
          TGACACAGACCTAAACAATCGGAGGAGCGTGGTCCGCGGTTCGTCTTGCAGGTCGACTAG

          AsnThrAsnGlySerTrpHisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsn
     901  AACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCTCAAC
          TTGTGGTTGCCGTCAACCGTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTG

          ThrGlyTrpLeuAlaGlyLeuPheTyrHisHisLysPheAsnSerSerGlyCysProGlu
     961  ACCGGCTGGTTGGCAGGGCTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGAG
          TGGCCGACCAACCGTCCCGAAAAGATAGTGGTGTTCAAGTTGAGAAGTCCGACAGGACTC

          ArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyr
    1021  AGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTAT
          TCCGATCGGTCGACGGCTGGGGAATGGCTAAAACTGGTCCCGACCCCGGGATAGTCAATA

          AlaAsnGlySerGlyProAspGlnArgProTyrCysTrpHisTyrProProLysProCys
    1081  GCCAACGGAAGCGGCCCCGACCAGCGCCCCTACTGCTGGCACTACCCCCCAAAACCTTGC
          CGGTTGCCTTCGCCGGGGCTGGTCGCGGGGATGACGACCGTGATGGGGGGTTTTGGAACG

          GlyIleValProAlaLysSerValCysGlyProValTyrCysPheThrProSerProVal
    1141  GGTATTGTGCCCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGTG
          CCATAACACGGGCGCTTCTCACACACACCAGGCCATATAACGAAGTGAGGGTCGGGGCAC

          ValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThr
    1201  GTGGTGGGAACGACCGACAGGTCGGGCGCGCCCACCTACAGCTGGGGTGAAAATGATACG
          CACCACCCTTGCTGGCTGTCCAGCCCGCGCGGGTGGATGTCGACCCCACTTTTACTATGC

          AspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrp
    1261  GACGTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGTTCGGTTGTACCTGG
          CTGCAGAAGCAGGAATTGTTATGGTCCGGTGGCGACCCGTTAACCAAGCCAACATGGACC

          MetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysValIleGlyGlyAla
    1321  ATGAACTCAACTGGATTCACCAAAGTGTGCGGAGCGCCTCCTTGTGTCATCGGAGGGGCG
          TACTTGAGTTGACCTAAGTGGTTTCACACGCCTCGCGGAGGAACACAGTAGCCTCCCCGC

          GlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisProAspAlaThrTyr
    1381  GGCAACAACACCCTGCACTGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATAC
          CCGTTGTTGTGGGACGTGACGGGGTGACTAACGAAGGCGTTCGTAGGCCTGCGGTGTATG

          SerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAspTyrProTyrArg
    1441  TCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCCTGGTCGACTACCCGTATAGG
          AGAGCCACGCCGAGGCCAGGGACCTAGTGTGGGTCCACGGACCAGCTGATGGGCATATCC

          LeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArgMetTyrValGly
    1501  CTTTGGCATTATCCTTGTACCATCAACTACACCATATTTAAAATCAGGATGTACGTGGGA
          GAAACCGTAATAGGAACATGGTAGTTGATGTGGTATAAATTTTAGTCCTACATGCACCCT

          GlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeu
    1561  GGGGTCGAACACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCTG
          CCCCAGCTTGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCCGCTTGCAACGCTAGAC

          GluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnVal
    1621  GAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGTC
          CTTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGATGTGTCACCGTCCAG

          LeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGln
    1681  CTCCCGTGTTCCTTCACAACCCTACCAGCCTTGTCCACCGGCCTCATCCACCTCCACCAG
          GAGGGCACAAGGAAGTGTTGGGATGTTCGGAACAGGTGGCCGGAGTAGGTGGAGGTGGTC

          AsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIle
    1741  AACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCGCGTCCTGGGCCATT
          TTGTAACACCTGCACGTCATGAACATGCCCCACCCCAGTTCGTAGCGCAGGACCCGGTAA

          LysTrpGluTyrValValLeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCys
    1801  AACTGGGAGTACGTGGTGCTGCTGTTCCTGCTGCTGGCAGATGCCCGCGTCTGCTCCTGC
```

FIGURE 54-2

```
                TTCACCCTCATGCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACG

          LeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeu
    1861  TTGTGGATGATGCTACTCATATCCCAAGCGGAGGCGGCTTTGGAGAACCTCGTAATACTT
          AACACCTACTACGATGAGTATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGAA

          AsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePheCysPhe
    1921  AATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCGTGTTCTTCTGCTTT
          TTACGTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAA

          AlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrp
    1981  GCATGGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGG
          CGTACCATAAACTTCCCATTCACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACACC

          ProLeuLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluVal
    2041  CCTCTCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGTG
          GGAGAGGAGGACGAGGACAACCGCAACGGGGTCGCCCGCATGCGCGACCTGTGCCTCCAC

          AlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyr
    2101  GCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATAT
          CGGCGCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGACTGAGACAGTGGTATA

          TyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGlu
    2161  TACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGAA
          ATGTTCGCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTT

          AlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArgAspAlaValIle
    2221  GCGCAACTGCACGTGTGGATTCCCCCCCTCAACGTCCGAGGGGGGCGCGACGCCGTCATC
          CGCGTTGACGTGCACACCTAAGGGGGGGAGTTGCAGGCTCCCCCCGCGCTGCGGCAGTAG

          LeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLysLeuLeuLeuAla
    2281  TTACTCATGTGTGCTGTACACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCC
          AATGAGTACACACGACATGTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGG

          ValPheGlyProLeuTrpIleLeuGlnAlaSerLeuLeuLysValProTyrPheValArg
    2341  GTCTTCGGACCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTTGTGCGC
          CAGAAGCCTGGGGAAACCTAAGAAGTTCGGTCAAACGAATTTCATGGGATGAAACACGCG

          ValGlnGlyLeuLeuArgPheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrVal
    2401  GTCCAAGGCCTTCTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTG
          CAGGTTCCGGAAGAGGCCAAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCAC

          GlnMetValIleIleLysLeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThr
    2461  CAAATGGTCATCATTAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACT
          GTTTACCAGTAGTAATTCAATCCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGA

          ProLeuArgAspTrpAlaHisAsnGlyLeuArgAspLeuAlaValAlaValGluProVal
    2521  CCTCTTCGGGACTGGGCGCACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTC
          GGAGAAGCCCTGACCCGCGTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCAG

          ValPheSerGlnMetGluThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGly
    2581  GTCTTCTCCCAAATGGAGACCAAGCTCATCACGTGGGGGGGCAGATACCGCCGCGTGCGGT
          CAGAAGAGGGTTTACCTCTGGTTCGAGTAGTGCACCCCCCGTCTATGGCGGCGCACGCCA

          AspIleIleAsnGlyLeuProValSerAlaArgArgGlyArgGluIleLeuLeuGlyPro
    2641  GACATCATCAACGGCTTGCCTGTTTCCGCCCGCAGGGGCCGGGAGATACTGCTCGGGCCA
          CTGTAGTAGTTGCCGAACGGACAAAGGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGGT

          AlaAspGlyMetValSerLysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGln
    2701  GCCGATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCAG
          CGGCTACCTTACCAGAGGTTCCCCACCTCCAACGACCGCGGGTAGTGCCGCATGCGGGTC

          GlnThrArgGlyLeuLeuGlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGln
    2761  CAGACAAGGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAA
          GTCTGTTCCCCGGAGGATCCCACGTATTAGTGGTCGGATTGACCGGCCCTGTTTTTGGTT
```

FIGURE 54-3

```
           ValGluGlyGluValGlnIleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIle
 2821  GTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATC
        CACCTCCCACTCCAGGTCTAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAG

           AsnGlyValCysTrpThrValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLys
 2881  AATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAG
        TTACCCCACACGACCTGACAGATGGTGCCCCGGCCTTGCTCCTGGTAGCGCAGTGGGTTC

           GlyProValIleGlnMetTyrThrAsnValAspGlnAspLeuValGlyTrpProAlaPro
 2941  GGTCCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCG
        CCAGGACAGTAGGTCTACATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGC

           GlnGlySerArgSerLeuThrProCysThrCysGlySerSerAspLeuTyrLeuValThr
 3001  CAAGGTAGCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACG
        GTTCCATCGGCGAGTAACTGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAGTGC

           ArgHisAlaAspValIleProValArgArgArgGlyAspSerArgGlySerLeuLeuSer
 3061  AGGCACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCG
        TCCGTGCGGCTACAGTAAGGGCACGCGGCCGCCCCACTATCGTCCCCGTCGGACGACAGC

           ProArgProIleSerTyrLeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGly
 3121  CCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGG
        GGGGCCGGGTAAAGGATGAACTTTCCGAGGAGCCCCCCAGGCGACAACACGGGGCGCCCC

           HisAlaValGlyIlePheArgAlaAlaValCysThrArgGlyValAlaLysAlaValAsp
 3181  CACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGAC
        GTGCGGCACCCGTATAAATCCCGGCGCCACACGTGGGCACCTCACCGATTCCGCCACCTG

           PheIleProValGluAsnLeuGluThrThrMetArgSerProValPheThrAspAsnSer
 3241  TTTATCCCTGTGGAGAACCTAGAGACAACAATGAGGTCCCCGGTGTTCACGGATAACTCC
        AAATAGGGACACCTCTTGGATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGG   FIGURE 54-4

           SerProProValValProGlnSerPheGlnValAlaHisLeuHisAlaProThrGlySer
 3301  TCTCCACCAGTAGTGCCCCAGAGCTTCCAGGTGGCTCACCTCCATGCTCCCACAGGCAGC
        AGAGGTGGTCATCACGGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTCG

           GlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeu
 3361  GGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTC
        CCGTTTTCGTGGTTCCAGGGCCGACGTATACGTCGAGTCCCGATATTCCACGATCATGAG

           AsnProSerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIle
 3421  AACCCCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATC
        TTGGGGAGACAACGACGTTGTGACCCGAAACCACGAATGTACAGGTTCCGAGTACCCTAG

           AspProAsnIleArgThrGlyValArgThrIleThrThrGlySerProIleThrTyrSer
 3481  GATCCTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTCC
        CTAGGATTGTAGTCCTGGCCCCACTCTTGTTAATGGTGACCGTCGGGGTAGTGCATGAGG

           ThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIle
 3541  ACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGCGCTTATGACATAATAATT
        TGGATGCCGTTCAAGGAACGGCTGCCGCCCACGAGCCCCCGCGAATACTGTATTATTAA

           CysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAsp
 3601  TGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCGGCACTGTCCTTGAC
        ACACTGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAACTG

           GlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProProGlySer
 3661  CAAGCAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTCC
        GTTCGTCTCTGACGCCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCCGAGG

           ValThrValProHisProAsnIleGluGluValAlaLeuSerThrThrGlyGluIlePro
 3721  GTCACTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCCT
        CAGTGACACGGGGTAGGGTTGTAGCTCCTCCAACGAGACAGGTGGTGGCCTCTCTAGGGA

           PheTyrGlyLysAlaIleProLeuGluGlnValIleThrValGlyLysArgHisGlnIlePheCys
```

```
           AAAATGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCCCTCTGTAGAGTAGAAGACA

           HisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAla
    3841   CATTCAAAGAAGAAGTGCGACGAACTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGCC
           GTAAGTTTCTTCTTCACGCTGCTTGAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACGG

           ValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAspValValVal
    3901   GTGGCCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGTC
           CACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAG

           ValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerValIleAspCys
    3961   GTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTGC
           CACCGTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACG

           AsnThrCysValThrGlnThrValAspPheSerLeuAspProThrPheThrIleGluThr
    4021   AATACGTGTGTCACCCAGACAGTCGATTTCAGCCTTGACCCTACCTTCACCATTGAGACA
           TTATGCACACAGTGGGTCTGTCAGCTAAAGTCGGAACTGGGATGGAAGTGGTAACTCTGT

           IleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArgThrGlyArgGly
    4081   ATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGG
           TAGTGCGAGGGGGTCCTACGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCC

           LysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGlyMetPheAspSer
    4141   AAGCCAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCGGCATGTTCGACTCG
           TTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGC

           SerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGlu
    4201   TCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCCGCCGAG
           AGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAGTGCGGGCGGCTC

           ThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProValCysGlnAspHis
    4261   ACTACAGTTAGGCTACGAGCGTACATGAACACCCGGGGCTTCCCGTGTGCCAGGACCAT      FIGURE 54-5
           TGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCACACGGTCCTGGTA

           LeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSer
    4321   CTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCCCACTTTCTATCC
           GAACTTAAAACCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGG

           GlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCys
    4381   CAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGC
           GTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACG

           AlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeu
    4441   GCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGTTTGATTCGCCTC
           CGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCACAAACTAAGCGGAG

           LysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGlu
    4501   AAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGAA
           TTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGCGACAAGTCTTACTT

           IleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSerAlaAspLeuGlu
    4561   ATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGAG
           TAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTC

           ValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCys
    4621   GTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTGC
           CAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGACGAAACCGGCGCATAACG

           LeuSerThrGlyCysValValIleValGlyArgValValLeuSerGlyLysProAlaIle
    4681   CTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAATC
           GACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTAG

           IleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHis
    4741   ATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCAC
           TATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCGTG
```

```
            LeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGly
      4801  TTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGC
            AATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCCG

            LeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrp
      4861  CTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTGG
            GAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACC

            GlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyr
      4921  CAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATAC
            GTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTATG

            LeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThr
      4981  TTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACA
            AACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAAATGT

            AlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGly
      5041  GCTGCTGTCACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGGG
            CGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCC

            TrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeu
      5101  TGGGTGGCTGCCCAGCTCGCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTTA
            ACCCACCGACGGGTCGAGCGGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAAT

            AlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGly
      5161  GCTGGCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGGG
            CGACCGCGGCGGTAGCCGTCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCC

            TyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGluValPro
      5221  TATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCC
            ATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGG

            SerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValVal
      5281  TCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGTC
            AGGTGCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAG

            GlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAlaValGln
      5341  GGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCAG
            CCGCACCAGACACGTCGTTATGACGCGGCCGTGCAACCGGGCCCGCTCCCCCGTCACGTC

            TrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProThrHisTyr
      5401  TGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCCCACGCACTAC
            ACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCGTGATG

            ValProGluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSerLeuThrValThr
      5461  GTGCCGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACC
            CACGGCCTCTCGCTACGTCGACGGGCGCAGTGACGGTATGAGTCGTCGGAGTGACATTGG

            GlnLeuLeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThrProCysSerGly
      5521  CAGCTCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGGT
            GTCGAGGACTCCGCTGACGTGGTCACCTATTCGAGCCTCACATGGTGAGGTACGAGGCCA

            SerTrpLeuArgAspIleTrpAspTrpIleCysGluValLeuSerAspPheLysThrTrp
      5581  TCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTGG
            AGGACCGATTCCCTGTAGACCCTGACCTATACGCTCCACAACTCGCTGAAATTCTGGACC

            LeuLysAlaLysLeuMetProGlnLeuProGlyIleProPheValSerCysGlnArgGly
      5641  CTAAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCTTTGTGTCCTGCCAGCGCGGG
            GATTTTCGATTCGAGTACGGTGTCGACGGACCCTAGGGGAAACACAGGACGGTCGCGCCC

            TyrLysGlyValTrpArgValAspGlyIleMetHisThrArgCysHisCysGlyAlaGlu
      5701  TATAAGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAG
            ATATTCCCCCAGACCGCTCACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTC

            IleThrGlyHisValLysAsnGlyThrMetArgIleValGlyProArgThrCysArgAsn
      5761  ATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAAC
            TAGTGACCTGTACAGTTTTTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTG
```

FIGURE 54-6

```
        TAGTGACCTGTACAGTTTTTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTG

             MetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGlyProCysThrProLeuPro
  5821   ATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCTGTACCCCCCTTCCT
         TACACCTCACCCTGGAAGGGGTAATTACGGATGTGGTGCCCGGGGACATGGGGGGAAGGA

             AlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyrValGluIleArg
  5881   GCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGG
         CGCGGCTTGATGTGCAAGCGCGATACCTCCCACAGACGTCTCCTTATACACCTCTATTCC

             GlnValGlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeuLysCysProCys
  5941   CAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTGC
         GTCCACCCCCTGAAGGTGATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGCACG

             GlnValProSerProGluPhePheThrGluLeuAspGlyValArgLeuHisArgPheAla
  6001   CAGGTCCCATCGCCCGAATTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGCG
         GTCCAGGGTAGCGGGCTTAAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACGC

             ProProCysLysProLeuLeuArgGluGluValSerPheArgValGlyLeuHisGluTyr
  6061   CCCCCCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATAC
         GGGGGGACGTTCGGGAACGACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTATG

             ProValGlySerGlnLeuProCysGluProGluProAspValAlaValLeuThrSerMet
  6121   CCGGTAGGGTCGCAATTACCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCATG
         GGCCATCCCAGCGTTAATGGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTAC

             LeuThrAspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySer
  6181   CTCACTGATCCCTCCCATATAACAGCAGAGGCGGCCGGGCGAAGGTTGGCGAGGGGATCA
         GAGTGACTAGGGAGGGTATATTGTCGTCTCCGCCGGCCCGCTTCCAACCGCTCCCCTAGT

             ProProSerValAlaSerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThr
  6241   CCCCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACT
         GGGGGGAGACACCGGTCGAGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTGA

             CysThrAlaAsnHisAspSerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArg    FIGURE 54-7
  6301   TGCACCGCTAACCATGACTCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGG
         ACGTGGCGATTGGTACTGAGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCC

             GlnGluMetGlyGlyAsnIleThrArgValGluSerGluAsnLysValValIleLeuAsp
  6361   CAGGAGATGGGCGGCAACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGAC
         GTCCTCTACCCGCCGTTGTAGTGGTCCCAACTCAGTCTTTTGTTTCACCACTAAGACCTG

             SerPheAspProLeuValAlaGluGluAspGluArgGluIleSerValProAlaGluIle
  6421   TCCTTCGATCCGCTTGTGGCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAATC
         AGGAAGCTAGGCGAACACCGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAG

             LeuArgLysSerArgArgPheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsn
  6481   CTGCGGAAGTCTCGGAGATTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAAC
         GACGCCTTCAGAGCCTCTAAGCGGGTCCGGGACGGGCAAACCCGCGCCGGCCTGATATTG

             ProProLeuValGluThrTrpLysLysProAspTyrGluProProValValHisGlyCys
  6541   CCCCCGCTAGTGGAGACGTGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTGT
         GGGGGCGATCACCTCTGCACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACA

             ProLeuProProProLysSerProProValProProProArgLysLysArgThrValVal
  6601   CCGCTTCCACCTCCAAAGTCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGTC
         GGCGAAGGTGGAGGTTTCAGGGGAGGACACGGAGGCGGAGCCTTCTTCGCCTGCCACCAG

             LeuThrGluSerThrLeuSerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySer
  6661   CTCACTGAATCAACCCTATCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGC
         GAGTGACTTAGTTGGGATAGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCG

             SerSerThrSerGlyIleThrGlyAspAsnThrThrThrSerSerGluProAlaProSer
  6721   TCCTCAACTTCCGGCATTACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCCTTCT
         AGGAGTTGAAGGCCGTAATGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGA
```

```
        GlyCysProProAspSerAspAlaGluSerTyrSerSerMetProProLeuGluGlyGlu
6781    GGCTGCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCCATGCCCCCCCTGGAGGGGGAG
        CCGACGGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTC

        ProGlyAspProAspLeuSerAspGlySerTrpSerThrValSerSerGluAlaAsnAla
6841    CCTGGGGATCCGGATCTTAGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGCG
        GGACCCCTAGGCCTAGAATCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCGC

        GluAspValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeuValThrProCys
6901    GAGGATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGC
        CTCCTACAGCACACGACGAGTTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCACG

        AlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHis
6961    GCCGCGGAAGAACAGAAACTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCAC
        CGGCGCCTTCTTGTCTTTGACGGGTAGTTACGTGATTCGTTGAGCAACGATGCAGTGGTG

        AsnLeuValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLysLysValThrPhe
7021    AATTTGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATTT
        TTAAACCACATAAGGTGGTGGAGTGCGTCACGAACGGTTTCCGTCTTCTTTCAGTGTAAA

        AspArgLeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGluValLysAlaAla
7081    GACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCG
        CTGTCTGACGTTCAAGACCTGTCGGTAATGGTCCTGCATGAGTTCCTCCAATTTCGTCGC

        AlaSerLysValLysAlaAsnLeuLeuSerValGluGluAlaCysSerLeuThrProPro
7141    GCGTCAAAAGTGAAGGCTAACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCCA
        CGCAGTTTTCACTTCCGATTGAACGATAGGCATCTCCTTCGAACGTCGGACTGCGGGGGT

        HisSerAlaLysSerLysPheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLys
7201    CACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAAGACGTCCGTTGCCATGCCAGAAAG
        GTGAGTCGGTTTAGGTTCAAACCAATACCCCGTTTTCTGCAGGCAACGGTACGGTCTTTC

        AlaValThrHisIleAsnSerValTrpLysAspLeuLeuGluAspAsnValThrProIle
7261    GCCGTAAGCCACATCAACTCCGTGTGGAAAGACCTTCTGGAAGACAATGTAACACCAATA
        CGGCATTGGGTGTAGTTGAGGCACACCTTTCTGGAAGACCTTCTGTTACATTGTGGTTAT

        AspThrThrIleMetAlaLysAsnGluValPheCysValGlnProGluLysGlyGlyArg
7321    GACACTACCATCATGGCTAAGAACGAGGTTTTCTGCGTTCAGCCTGAGAAGGGGGGTCGT
        CTGTGATGGTAGTACCGATTCTTGCTCCAAAAGACGCAAGTCGGACTCTTCCCCCCAGCA

        LysProAlaArgLeuIleValPheProAspLeuGlyValArgValCysGluLysMetAla
7381    AAGCCAGCTCGTCTCATCGTGTTCCCCGATCTGGGCGTGCGCGTGTGCGAAAAGATGGCT
        TTCGGTCGAGCAGAGTAGCACAAGGGGCTAGACCCGCACGCGCACACGCTTTTCTACCGA

        LeuTyrAspValValThrLysLeuProLeuAlaValMetGlySerSerTyrGlyPheGln
7441    TTGTACGACGTGGTTACAAAGCTCCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCAA
        AACATGCTGCACCAATGTTTCGAGGGGAACCGGCACTACCCTTCGAGGATGCCTAAGGTT

        TyrSerProGlyGlnArgValGluPheLeuValGlnAlaTrpLysSerLysLysThrPro
7501    TACTCACCAGGACAGCGGGTTGAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCCA
        ATGAGTGGTCCTGTCGCCCAACTTAAGGAGCACGTTCGCACCTTCAGGTTCTTTTGGGGT

        MetGlyPheSerTyrAspThrArgCysPheAspSerThrValThrGluSerAspIleArg
7561    ATGGGGTTCTCGTATGATACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGT
        TACCCCAAGAGCATACTATGGGCGACGAAACTGAGGTGTCAGTGACTCTCGCTGTAGGCA

        ThrGluGluAlaIleTyrGlnCysCysAspLeuAspProGlnAlaArgValAlaIleLys
7621    ACGGAGGAGGCCAATCTACCAATGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAAG
        TGCCTCCTCCGTTAGATGGTTACAACACTGGAGCTGGGGGTTCGGGCGCACCGGTAGTTC

        SerLeuThrGluArgLeuTyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCys
7681    TCCCTCACCGAGAGGCTTTATGTTGGGGGCCCTCTTACCAATTCAAGGGGGGAGAACTGC
        AGGGAGTGGCTCTCCGAAATACAACCCCCGGGAGAATGGTTAAGTTCCCTCCTCTTGACG

        GlyTyrArgArgCysArgAlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThr
7741    GGCTATCGCAGGTGCCGCGCGAGCGGCGTACTGACAACTAGCTGTGGTAACACCCTCACT
```

                                                        FIGURE 54-8

```
          CCGATAGCGTCCACGGCGCGCTCGCCGCATGACTGTTGATCGACACCATTGTGGGAGTGA

          CysTyrIleLysAlaArgAlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeu
   7801   TGCTACATCAAGGCCCGGGCAGCCTGTCGAGCCGCAGGGCTCCAGGACTGCACCATGCTC
          ACGATGTAGTTCCGGGCCCGTCGGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGAG

          ValCysGlyAspAspLeuValValIleCysGluSerAlaGlyValGlnGluAspAlaAla
   7861   GTGTGTGGCGACGACTTAGTCGTTATCTGTGAAAGCGCGGGGGTCCAGGAGGACGCGGCG
          CACACACCGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCCAGGTCCTCCTGCGCCGC

          SerLeuArgAlaPheThrGluAlaMetThrArgTyrSerAlaProProGlyAspProPro
   7921   AGCCTGAGAGCCTTCACGGAGGCTATGACCAGGTACTCCGCCCCCCCTGGGGACCCCCCA
          TCGGACTCTCGGAAGTGCCTCCGATACTGGTCCATGAGGCGGGGGGGACCCCTGGGGGGT

          GlnProGluTyrAspLeuGluLeuIleThrSerCysSerSerAsnValSerValAlaHis
   7981   CAACCAGAATACGACTTGGAGCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCAC
          GTTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGTG

          AspGlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThrProLeuAlaArg
   8041   GACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAGA
          CTGCCGCGACCTTTCTCCCAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCT

          AlaAlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMet
   8101   GCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATG
          CGACGCACCCTCTGTCGTTCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTAC

          PheAlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePheSerValLeuIle
   8161   TTTGCCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTATA
          AAACGGGGGTGTGACACCCGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATAT

          AlaArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIle
   8221   GCCAGGGACCAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATA
          CGGTCCCTGGTCGAACTTGTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTAT

          GluProLeuAspLeuProProIleIleGlnArgLeu
   8281   GAACCACTTGATCTACCTCCAATCATTCAAAGACTC
          CTTGGTGAACTAGATGGAGGTTAGTAAGTTTCTGAG
```

FIGURE 54-
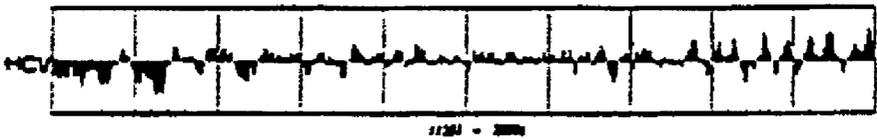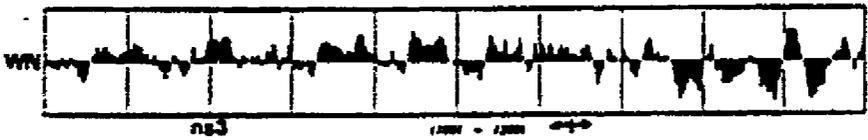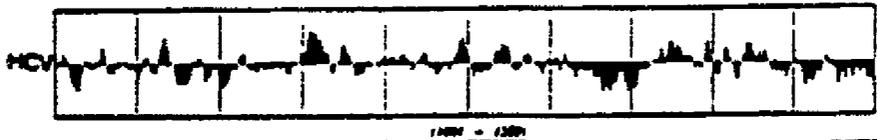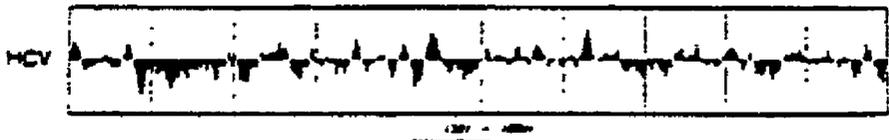
Translation of DNA CA216a

```
       ArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGlyPheAlaAsp
  1    CCCGCCGTAGGTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCG
       GGGCCGCATCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCACGCCGAAGCGGC

       LeuMetGlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAla
  61   ACCTCATGGGGTACATACCGCTCGTCGGCCCCCCTCTTGGAGGCGCTGCCAGGGCCCTGG
       TGGAGTACCCCATGTATGGCGAGCAGCCGCGGGGAGAACCTCCGCGACGGTCCCGGGACC

       HisGlyValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsnLeuProGlyCys
  121  CGCATGGCGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAACCTTCCTGGTT
       GCGTACCGCAGGCCCAAGACCTTCTGCCGCACTTGATACGTTGTCCCTTGGAAGGACCAA

       SerPheSerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValProAlaSerAlaTyr
  181  GCTCTTTCTCTATCTTCCTTCTGGCCCTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCT
       CGAGAAAGAGATAGAAGGAAGACCGGGACGAGAGAACGAACTGACACGGGCGAAGCCGGA
```

```
       GlnValArgAsnSerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIle
  241  ACCAAGTGCGCAACTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTA
       TGGTTCACGCGTTGAGGTGCCCCGAAATGGTGCAGTGGTTACTAACGGGATTGAGCTCAT
```

---------------------------Overlap with CA167b------------------------

```
       ValTyrGluAlaAlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGlu
  301  TTGTCTACGAAGCCGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTG
       AACACATGCTTCGCCGGCTACGGTAGGACGTGTGAGGCCCCACGCAGGGAACGCAAGCAC
```

```
       GlyAsnAlaSerArgCysTrpValAlaMetThrProThrValAla
  361  AGGGCAACGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCC
       TCCCGTTGCGGAGCTCCACAACCCACCGCTACTGGGGATGCCACCGG
```

FIGURE 56

Translation of DNA CA290a

```
        LysLysAsnLysArgAsnThrAsnArgArgProGlnAspValLysPheProGlyGlyGly
  1  AAAAAAAAAACAAACGTAACACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCG
     TTTTTTTTTTGTTTGCATTGTGGTTGGCAGCGGGTGTCCTGCAGTTCAAGGGCCCACCGC

        GlnIleValGlyGlyValTyrLeuLeuProArgArgGlyProArgLeuGlyValArgAla
 61  GTCAGATCGTTGGTGGAGTTTACTTGTTGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCG
     CAGTCTAGCAACCACCTCAAATGAACAACGGCGCGTCCCCGGGATCTAACCCACACGCGC

        ThrArgLysThrSerGluArgSerGlnProArgGlyArgArgGlnProIleProLysAla
121  CCACGAGAAAGACTTCCCAGCCGTCGCAACCTCGAGGTAGACGCCAGCCTATCCCCAAGC
     GGTGCTCTTTCTGAAGGGTCGGCAGCGTTGGAGCTCCATCTGCGGTCGGATAGGGGTTCC

        ArgArgProGluGlyArgThrTrpAlaGlnProGlyTyrProTrpProLeuTyrGlyAsn
181  CTCGTCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGGTACCCTTGGCCCCTCTATGGCA
     GAGCAGCCGGGCTCCCGTCCTGGACCCGAGTCGGGCCCATGGGAACCGGGGAGATACCGT

        GluGlyCysGlyTrpAlaGlyTrpLeuLeuSerProArgGlySerArgProSerTrpGly
241  ATGAGGGCCGCGGGTGGGCGGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGG
     TACTCCCGGCGCCCACCCGCCCTACCGAGGACAGAGGGGCACCGAGAGCCGGATCGACCC

        _____

        ProThrAspProArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCys
301  GCCCCACAGACCCCCGGCGTAGGTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGT
     CGGGGTGTCTGGGGGCCGCATCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCA

        _____

        GlyPheAlaAspLeuMetGlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAla
361  CGGGCCTTTGCCGACCTCATGGGCTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCGCTG
     CGCCCGGAAACGGCTGGAGTACCCCATGTATGGCGAGCAGCCGCGGGGAGAACCTCCGCGAC

        _____Overlap with CA316a_____
        ArgAlaLeuAlaHisGlyValArgValLeuGluAspGlyValAlaAsnTyrAlaThrGlyAsn
421  CCAGGGCCCTGGCGCATCGGCGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGA
     GGTCCCGGGACCGCGTAGCCGCAGGCCCAAGACCTTCTGCCGCACTTGATACGTTGTCCCT

        LeuProGlyCysSerPheSerThrPhe
481  ACCTCCTGGTTGCTCTTTCTCTACCTTC
     TGGAGGACCAACGAGAAAGAGATGGAAG
```

FIGURE 57

Translation of DNA ag30a

FIG. 58-1

```
                                          #MetSerValValGlnProProGlyProProLeu

                              #MetAlaLeuValGP
   1  CGCAGAAAGCGTCTAGCCATGGCCTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCC
      GCGTCTTTCGCAGATCGGTACCGCAATCATACTCACAGCACGTCGGAGGTCCTGGGGGGG


         ProGlyGluProAM

  61  TCCCCGGGAGAGCCATAGTGGTCTGCGGGAACCGGTGAGTACACCGGAATTGCCAGGACGAC
      AGGGCCCTCTCGGTATCACCAGACGCCTTGGCCACTCATGTGGCCTTAACGGTCCTGCTG


                                      #MetProGlyAspLeuGlyValProProGlnAsp

 121  CGGGTCCTTTCTTGGATCAACCCGCTCAATGCCTGGAGATTTGGGCCGTGCCCCCGCAAGA
      GCCCAGGAAAGAACCTAGTTGGGCGAGTTACGGACCTCTAAACCCGCACGGGGGCGTTCT


                                                OP AM GlyAlaCys
         CysAM                                                 *

 181  CTGCTAGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTT
      GACGATCGGCTCATCACAACCCAGCGCTTTCCGGAACACCATGACGGACTATCCCACGAA

                                         |
         GluCysProGlyArgSerArgArgProCysThrMetSerThrAsnProLysProGlnLys
                                                                      -
 241  GCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAA
      CGCTCACGGGGCCCTCCAGAGCATCTGGCACCTGGTACTCGTGCTTAGGATTTGGAGTTT


         LysAsnLysArgAsnThrAsnArgArgProGlnAspValLysPheProGlyGlyGlyGln

 301  AAAAAAACAAACGTAACACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCGGTC
      TTTTTTTGTTTGCATTGTGGTTGGCAGCGGGTGTCCTGCAGTTCAAGGGCCCACCGCCAG


         IleValGlyGlyValTyrLeuLeuProArgArgGlyProArgLeuGlyValArgAlaThr

 361  AGATCGTTGGTGGAGTTTACTTGTTGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGA
      TCTAGCAACCACCTCAAATGAACAACGGCGCGTCCCCGGGATCTAACCCACACGCGCGCT


         ArgLysThrSerGluArgSerGlnProArgGlyArgArgGlnProIleProLysAlaArg

 421  CGAGAAAGACTTCCGAGCGGTCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCTC
      GCTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCATCTGCAGTCGGATAGGGGTTCCGAG


         ArgProGluGlyArgThrTrpAlaGlnProGlyTyrProTrpProLeuTyrGlyAsnGlu

         ----------------------Overlap with CA390a---------------------
 481  GTCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGGTACCCTTGGCCCCTCTATGGCAATG
      CAGCCGGGCTCCCGTCCTGGACCCGAGTCGGGCCCATGGGAACCGGGGAGATACCGTTAC
```

FIG. 58-2

```
        GlyCysGlyTrpAlaGlyTrpLeuLeuSerProArgGlySerArgProSerTrpGlyPro
        ------------------------------------------------------------
541     AGGGCTGCGGGTGGGCGGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTCGGGCC
        TCCCGACGCCCACCCGCCCTACCGAGGACAGAGGGGCACCGAGAGCCGGATCGACCCCGG


        ThrAspProArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGly
        ------------------------------------------------------------
601     CCACAGACCCCCGGCGTAGGTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCG
        GGTGTCTGGGGGCCGCATCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCACGC


        Phe
        -----
661     GCTTC
        CGAAG


            * = Start of long HCV ORF
            | = Putative first amino acid of large HCV polyprotein
            # = Putative small encoded peptides( that may play a
                translational regulatory role)
```

Translation of DNA CA205a

```
      ValLeuGlyArgGluArgProCysGlyThrAlaOP AM GlyAlaCysGluCysProGly
  1   GTCTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGG
      CAGAACCCAGCGCTTTCCGGAACACCATGACGGACTATCCCACGAACGCTCACGGGGCCC
                            *              ------------

      ArgSerArgArgProCysThrMetSerThrAsnProLysProGlnArgLysThrLysArg
 61   AGGTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAACGT
      TCCAGAGCATCTGGCACGTGGTACTCGTGCTTAGGATTTGGAGTTTCTTTTTGGTTTGCA
      --------------------------------------

      AsnThrAsnArgArgProGlnAspValLysPheProGlyGlyGlyGlnIleValGlyGly
121   AACACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGA
      TTGTGGTTGGCAGCGGGTGTCCTGCAGTTCAAGGGCCCACCGCCAGTCTAGCAACCACCT
      -----------------------------------------------

      ValTyrLeuLeuProArgArgGlyProArgLeuGlyValArgAlaThrArgLysThrSer
181   GTTTACTTGTTGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGAAACACTTCC
      CAAATGAACAACGGCGCGTCCCCGGGATCTAACCCACACGCGCGCTGCTCTTTCTGAAGG
      --------------------Overlap with CA290a------------------

      GluArgSerGlnProArgGlyArgArgGlnProIleProLysAlaArgArgProGluGly
241   GAGCGGTCGCAACCTCGACCTACACGTCAGCCTATCCCCAAGGCTCGTCGCCCCGAGGGC
      CTCGCCAGCGTTGGAGCTCCATCTGCAGTCGGATAGGGGTTCCGAGCAGCCGGGCTCCCG

      ArgThrTrpAlaGlnProGlyTyrProTrpProLeuTyrGlyAsnGluGlyCys
301   AGGACCTGGGCTCAGCCCGGCTACCCTTGGCCCCTCTATGGCAATGAGGGCTGCC
      TCCTGGACCCGAGTCGGGCCGATGGGAACCGGGGAGATACCGTTACTCCCGACGC
```

* = putative initiator methionine codon

FIGURE 59

Translation of DNA 18g

```
#ProProOP
#SerThrMetAsnHisSerProValArgAsnTyrCysLeuHisAlaGluSerValAM
#LeuHisHisGluSerLeuProCysGluGluLeuLeuSerSerArgArgLysArgLeuAla
  1  CTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAGCC
     GAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAGTGCGTCTTTCGCAGATCGG


                        #MetSerValValGlnProProGlyProProLeuProGlyGluProAM

     MetAlaLeuValCP
 61  ATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATAGT
     TACCGCAATCATACTCACAGCACGTCGGAGGTCCTGGGGGGAGGGCCCTCTCGGTATCA




121  GGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATC
     CCAGACGCCTTGGCCACTCATGTGGCCTTAACGGTCCTGCTGGCCCAGGAAAGAACCTAG



                    ----------Overlap with ag30a----------------------

                #MetProGlyAspLeuGlyValProProGlnAspCysAM

181  AACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGTGT
     TTGGGCGAGTTACGGACCTCTAAACCCGCACGGGGGCGTTCTGACGATCGGCTCATCACA




                                   OP AM GlyAlaCysGluCysProGlyArgSer
                                      •

241  TGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAGGT
     ACCCAGCGCTTTCCGGAACACCATGACGGACTATCCCACGAACGCTCACGGGGCCCTCCA




         ArgArg

301  CTCGTAGA
     GAGCATCT
```

         * = Start of long HCV ORF
         # = Putative small encoded peptides(that may play
             a translational regulatory role).

FIG. 60

Translation of DNA 16jh

```
      ------Overlap with 15e -------------------------------------
      GlyAlaCysTyrSerIleGluProLeuAspLeuProProIleIleGlnArgLeuHisGly
   1  GGGGCCTGCTACTCCATAGAACCACTGGATCTACCTCCAATCATTCAAAGACTCCATGGC
      CCCCGGACGATGAGGTATCTTGGTGACCTAGATGGAGGTTAGTAAGTTTCTGAGGTACCG


      LeuSerAlaPheSerLeuHisSerTyrSerProGlyGluIleAsnArgValAlaAlaCys
  61  CTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGTGAAATTAATAGGGTGGCCGCATGC
      GAGTCGCGTAAAAGTGAGGTGTCAATGAGAGGTCCACTTTAATTATCCCACCGGCGTACG


                                      Gly*
                                      G
      LeuArgLysLeuGlyValProProLeuArgAlaTrpArgHisArgAlaArgSerValArg
 121  CTCAGAAAACTTGGGGTACCGCCCTTGCGAGCTTGGAGACACCGGGCCCGGAGCGTCCGC
      GAGTCTTTTGAACCCCATGGCGGGAACGCTCGAACCTCTGTGGCCCGGGCCTCGCAGGCG


      AlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIleCysGlyLysTyrLeuPheAsnTrp
 181  GCTAGGCTTCTGGCCAGAGGAGGCAGGGCTGCCATATGTGGCAAGTACCTCTTCAACTGG
      CGATCCGAAGACCGGTCTCCTCCGTCCCGACGGTATACACCGTTCATGGAGAAGTTGACC


      AlaValArgThrLysLeuLys
 241  GCAGTAAGAACAAAGCTCAAAC
      CGTCATTCTTGTTTCGAGTTTG
```

* = nucleotide heterogeneity

FIGURE 61

SEQUENCE OF THE HCV cDNA SENSE STRAND

(AS DEDUCED FROM OVERLAPPING CLONES b114a/18g/eq30a/CA205a/
CA290a/CA216a, b114a/CA167b/CA156a/CA84a/CA59a/K9-1/26j/131/
12f/141/11b/7f/7e/8h/33c/40b/37b/35/36/81/32/33b/25c/14c/8f/
33f/33g/39c/35f/19g/26g/15e/b5a/16jh)

```
CACTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAG
CCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATA
GTGGTCTGCGGAACCGGTGAGTACACCCGAATTGCCAGGACGACCGGGTCCTTTCTTGGA
TCAACCCGCTCAATGCCTGGGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGT
GTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTCCGAGTGCCCCGGGAG-300
```

(Putative initiator methionine codon)

```
GTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAAAAAAAACAAACGTAA
CACCAACCGTGCCCACAGGACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTCGTGGAGT
TTACTTGTTGCCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCCGA
GCGGTCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCCTGTCGGCCCCGAGGGCAC
GACCTCGGCTCAGCCCGCGTACCCTTGGCCCCTCTATGGCAATGAGGGCTGCGGGTGGGC-600
GGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCCCGCCG
TACCTCGCGCAATTTCGGTAAGGTCATCGATACCCTTACGTGCGGCTTCCGGACCTCAT
GGGGTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGG
CGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGCAACCTTCCTGGTTGCTCTTT
CTCTATCTTCCTTCTGGCCCTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGT-900
GCGCAACTCCACGGGGGCTTTACCACGTCACCAATGATTGCCCCTAACTCCGACTATTGTGTA
CCAAGGCGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTGAGGGCAA
CGCCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCACGGATGGCAAACTCCC
CGCGACGGCACCTTCGACGTCACATCGATCTGCTTGTCGGGAGCGCCACCCTCTGTTCGGC
CCTCTACGTGGGGGACCTATGCGGGTCTGTCTTTCTTGTCGGCCAACTGTTCACCTTCTC-1200
TCCCAGGCGCCACTGGACGACGCCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAAC
GGGTCACCGCATGGCATGGGATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAAT
GGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATCATCGCTGGTGCTCACTGGGG
AGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGT
GCTGCTGCTATTTGCCGGCGTCGATGCGGAAACCCACGTCACGGCGGCAAGTGCCGCCCCA-1500
CACTGTGTCTGGATTTGTTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGAT
CAACACCAACGGCAGTTGGCACCTTAATAGCACGGCCCCTGAACTGCAATCATAGCCTCAA
CACCGCCTGGTTGGCAGGGCTTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGA
GAGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTA
TGCCAACGGAAGCGGCCCCCGACCAGCGCCCCTACTGCTGGCACTACCCCCCAAAACCTTG-1800
CGGTATTGTGCCCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGT
GGTGGTGGGAACGACCGACAGGTCGGGCGCGCCCACCTACAGCTGGGCTGAAAATGATAC
GGACGTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGTTCGGTTGTACCTG
GATGAACTCAACTGGATTCACCAAAGTGTGCGGAGCGCCTCCTTGTGTCATCGGAGCGGC
GGGCAACAACACCCTGCACTCCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATA-2100
CTCTCGGTGCGGCTCCGGTCCCTGGATCACACACCCAGGTGCCTGGTCGACTACCCGTATAG
GCTTTGGCATTATCCTTGTACCATCAACTACACCATATTTAAAATCATGATGTACGTGGG
AGGGTCGAACACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCCAACGTTGCGATCT
GGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTCACCACTACACAGTGGCCAGGT
CCTCCGTGTTCCTTCACAACCCTACCAGCCTTGTCCACCGGCCTCATCCACCTCCACCA-2400
GAACATTGTGGACGTGCAGTACTTGTACGGGCTGGCGGTCAAGCATCCCGTCCTGGGCCAT
TAACTGGCACTACGTCGTTCTCCTCGTTCCTTCTGCTTGCAGACGCGCGCGTCTGCTCCTG
CTTGTGGATGATGCTACTCCATATCCCAAGCGGAGGCGGCCTTTGGAGAACCTCGTAAATACT
TAATGCAGCCATCCCTGGCCCCGGCACGCACCGCTCTTCTATCCCTCCTCGTCTTCTTCTGCTT
TGCATGGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTG-2700
GCCTCTCCTCCTGCCGCCTGTTGACGTTGCCCCAGCGGCGCGTACGCGCTGGACACGCACGT
GGCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGGTTGATGGCGCTGACTCTGTCACCATA
TTACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGA
AGCCCAACTCCACGTCTGCGATTCCCCCCCTCAACGTCCGAGGGGGCGCGACGCCGTCAT
CTTACTCATGTGTGCTGTACACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGC-3000
CGTCTTCGGACCCCTTTGGATTCTTCAAGCCACGTTTGCTTAAAGTACCCTACTTTGTGCG
CGTCCAAGGCCTTCTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGT
CCAAATGGTCATCATTAAGTTAGGGCGCGCTTACTGCCACCTATGTTTATAACCATCTCAC
TCCTCTTCGGGACTGGGCGCACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGT
CGTCTTCTCCCAAATGGAGACCAAGCTCATCACGTGCCGCGCCCAGATACCGCCGCGTGCGG-3300
```

FIGURE 62

```
TGACATCATCAACGGCTTGCCTGCTTCCGCCCGCAGGGGCCGGGAGATACTGCTCGGGCC
AGCCGATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCA
GCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCA
AGTGGAGGGTGACGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCAT
CAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGAACGAGGACCATCGCGTCACCCAA-3600
CGGTCCTGCTCATCCAGATCTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCGCTCC
GCAAGGTAGCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCAC
GAGGCACGCCGATCTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTC
GCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGTCCGCTCTTGTGCCCCGCGGGG
GCACGCCGTGGGCATATTTAGGGCCGCGGTCTGCCACCCGTGGAGTGGCTAAGGCGGTGGA-3900
CTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCACCGATAACTC
CTCTCCACCAGTAGTGCCCCAGAGCCTTCCAGGTGCCTCACCTCCATGCTCCCACAGGCAG
CGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACT
CAACCCCTGTTGCTGCAACACTGGCCTTTGGTGCTTACATGTCCAAGGCTCATGGGAT
CGATCCTAACATCAGGACCGGGGTGAGAACCAATTACCACTGGCGATAGTCACACGTACTC-4200
CACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGCCCGCTTATGCACATAATAAT
TTGTGACCACTGCCACTCCACGGATGCCACATCCATCTTGGGCATGGCACTGTCCTTGA
CCAAGCAGAGACTGCGGGGGCCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGCGCCTC
CGTCACTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCC
TTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGGAGACATCTCATCTTCTC-4500
TCATTCAAACAAGAAGTCCGACGAACTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGC
CGTGGCCTACTACCGCGGGTCTTGACGTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGT
CGTGGCAACCGATGCCCTCATCACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTG
CAATACGTGTGTCACCCAGACAGTCGGATTTCAGCCTTGACCCTACCTTCACCATTGAGAC
AATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCCGCGGCAGGCACTGGCAGGGG-4800
CAAGCCCAGCCATCTACACATTTGTGGCACCGGGGGAGCGCCCCTCCGGCATGTTCGACTC
GTCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGACCTCACCGCCGCCGA
GACTACAGTTAGGCCTACGAGCGTACATCAACACCCCGGGCTTCCCGTGTGCCAGGACCA
TCTTGAATTTTGGGAGGGGCGTCTTTACAGGCCTCACTCATAIAGATGCCCACTTTCTATC
CCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTG-5100
CCCTAGCGGCTCAAGCCCCTCCCCATCGTGGGACCCAGATGTGGAAGTGTTTGATTCGCCT
CAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTCGGCGCTGTTCAGAATCA
AATCACCCCTGACCCACCCAGTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGA
GGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTC
CCTGTCAACAGGCTGCGTGCCTCATAGTGGGCAGCCTCCTCTTGTCGGGAAGCCGGCAAT-5400
CATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGACTGGAAGAGTGCTCTCAGCA
CTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAACGCAGAGCCCCTCCG
CCTCCTGCAGAACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCGCTGTCCAGACCAACTG
GCAAAAACTCGAGACCCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATA
CTTGGCGGCCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTAC-5700
AGCTGCTGTCACCAGCCCACTAACCCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGG
GTGGGTGGCTGCCCAGCTCGCCGCCCCCGGTGCCGCCTACTCCCTTTGTGGGCGCTGGCTT
AGCTGGCGCCGCCATCGGCAGTGTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGG
GTATGGCGCGGGCGTGGCCGGGACCTCTTGTGGCATTCAAGATCATCAGCGGTGAGGTCCC
CTCCACGGAGGACCTGGTCAATCTACTGCCGGCCATCCTCTCGCCGGGAGCCCTCGTAGT-6000
CGGCGTGGCTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGCAGCGGGCAGTGCA
GTCCATGAACCGCGCTCATAGCCTTCGCCTCCCGGGGGGAACCATGTTTCCCCCACGCCACTA
CGTTGCCGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAAC
CCAGCTCCTGAGCCGACTCCACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGG
TTCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTG-6300
GCTAAAAGCTAAGCTCATGCCACAGCTGCCTCGGGATCCCCTTTGTGTCCTGCCAGCGCGG
GTATAAGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCCCACTGTTGCAGCTGA
GATCACTGGACATGTCAAAAACGGGACCATCAGGATCGTCGCTCCTAGGACCTGCAGCAA
CATGTGGAGTGGGACCTTCCCCATTAATGCCTACAGCACGGGCCCCTGTACCCCCCTTCC
TGCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAG-6600
GCCAGCTGGGGCACTTCCACTACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTG
CCAGGTCCCATCGCCCGAATTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGC
GCCCCCCTGCAATCCCTTGCTGCGCCAGGAGGTATCATTCAGAGTAGGACTCCACGAATA
CCTTGTAGGGTGTCAATTACCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCAT
GCTCACTGCATCCCTCCCATATAACAGCAGAGGCGGCCGGCCGAAGGTTGGCGAGGGGATC-6900
ACCCCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAAC
TTGCACCGCTAACCATGACTCCCTCATGCTGAGCCTCATAGAGGCCAACCTCCTATGGAG
GCAGGAGATGGGCCGGCAACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGA
CTCCTTCGATCCGCTTGTGGCGGAGGAGGACGAGCGGGACATCTCCTACCCGCAGAAAT
CCTGCGGAAGTCTCGGAGATTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAA-7200
CCCCCCGCTAGTCGAGACGTGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTG
```

FIGURE 62.1

```
TCCGCTTCCACCTCCAAAGTCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGT
CCTCACTGAATCAACCCTATCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAG
CTCCTCAACTTCCGGCATTACGGGCGACAATACCACAACATCCTCTCAGCCCGGCCCCTTC
TGGCTGCCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCCATGCCCCCCCTGGAGGGGGA-7500
GCCTGGGGATCCGGATCTTAGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGC
GGAGGATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTG
CGCCGCGGAAGAACAGAAACTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCA
CAACTTGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATT
TGACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGC-7800
GGCGTCAAAAGTGAAGGCTAACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCGCCCC
ACACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAAGACGTCCGTTGCCATGCCAGAAA
GGCCGTAACCCACATCAACTCCGTGTGGAAAGACCTTCTGCAAGACAATGTAACACCAAT
AGACACTACCATCATGGCTAAGAACGAGGTTTTCTGCGTTCAGCCTGAGAAGGGGGGTCG
TAAGCCAGCTCGTCTCATCGTGTTCCCCGATCTGGGCGTGCGCGTCTGCGCAAAAGATGGC-8100
TTTCTACGACCTGGTTACAAAGCTCCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCA
ATACTCACCAGGACAGCGGGTTGAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCC
AATGCCCTTCTCGTATGATACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCG
TACGGAGGAGGCAATCTACCAATGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAA
GTCCCTCACCGACAGGCTTTATGTTGGCGGCCCCTCTTACCAATTCAAGGGGGGAGAACTG-8400
CGGCTATCGCAGGGTGCCGCGCGAGCGGCGTACTGACAACTAGCTGTGGTAACACCCTCAC
TTGCTACATCAAGGCCCGGGCAGCCCTGTCCAGCCGCACGGCCTCCAGGACTGCACCATGCT
CCTGTGTGGCGACGACTTAGTCGTTATCTGTGAAAGCGCGGGGGGTCCAGGAGGACGCGGC
GAGCCTGAGAGCCTTCACGGAGGCTATGACCACCTACTCCGCCCCCCCCTCGGGACCCCCC
ACAACCAGAATACGACTTGGAGCCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCA-8700
CGACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAC
AGCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCAT
GTTTGCCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTAT
AGCCAGGGACCAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCAT
AGAACCACTTGATCTACCTCCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACT-9000
CCACAGTTACTCTCCAGGTGAAATTAATAGGGTGGCCGCATGCCTCAGAAAACTTGGGGT
ACCGCCCTTGCGAGCTTGGAGACACCGGGCCCGGAGCGTCCGCGCTAGGCTTCTGGCCAG
AGGAGGCAGGGCTGCCATATGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCT
CAAAC
```

FIGURE 62.2

```
   1  CACTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAG
      GTGAGGTGGTACTTAGTGAGGGGACACTCCTTGATGACAGAAGTGCGTCTTTCGCAGATC

  61  CCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATA
      GGTACCGCAATCATACTCACAGCACGTCGGAGGTCCTGGGGGGGAGGGCCCTCTCGGTAT

 121  GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGA
      CACCAGACGCCTTGGCCACTCATGTGGCCTTAACGGTCCTGCTGGCCCAGGAAAGAACCT

 181  TCAACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGT
      AGTTGGGCGAGTTACGGACCTCTAAACCCGCACGGGGGCGTTCTGACGATCGGCTCATCA

 241  GTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAG
      CAACCCAGCGCTTTCCGGAACACCATGACGGACTATCCCACGAACGCTCACGGGGCCCTC

 301  GTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAAAAAAAACAAACGTAA
      CAGAGCATCTGGCACGTGGTACTCGTGCTTAGGATTTGGAGTTTTTTTTTTTGTTTGCATT

 361  CACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGT
      GTGGTTGGCAGCGGGTGTCCTGCAGTTCAAGGGCCCACCGCCAGTCTAGCAACCACCTCA

 421  TTACTTGTTGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCCGA
      AATGAACAACGGCGCGTCCCCGGGATCTAACCCACACGCGCGCTGCTCTTTCTGAAGGCT

 481  GCGGTCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCTCGTCGGCCCGAGGGCAG
      CGCCAGCGTTGGAGCTCCATCTGCAGTCGGATAGGGGTTCCGAGCAGCCGGGCTCCCGTC

 541  GACCTGGGCTCAGCCCGGGTACCCTTGGCCCCTCTATGGCAATGAGGGCTGCGGGTGGGC
      CTGGACCCGAGTCGGGCCCATGGGAACCGGGGAGATACCGTTACTCCCGACGCCCACCCG

 601  GGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCCCGGCG
      CCCTACCGAGGACAGAGGGGCACCGAGAGCCGGATCGACCCCGGGGTGTCTGGGGGCCGC

 661  TAGGTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCAT
      ATCCAGCGCGTTAAACCCATTCCAGTAGCTATGGGAATGCACGCCGAAGCGGCTGGAGTA

 721  GGGGTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGG
      CCCCATGTATGGCGAGCAGCCGCGGGGAGAACCTCCGCGACGGTCCCGGGACCGCGTACC

 781  CGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTT
      GCAGGCCCAAGACCTTCTGCCGCACTTGATACGTTGTCCCTTGGAAGGACCAACGAGAAA

 841  CTCTATCTTCCTTCTGGCCCTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGT
      GAGATAGAAGGAAGACCGGGACGAGAGAACGAACTGACACGGGCGAAGCCGGATGGTTCA

 901  GCGCAACTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTA
      CGCGTTGAGGTGCCCCGAAATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACAT

 961  CGAGGCGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTGAGGGCAA
      GCTCCGCCGGCTACGGTAGGACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTT

1021  CGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCC
      GCGGAGCTCCACAACCCACCGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGG

1081  CGCGACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGCGCCACCCTCTGTTCGGC
      GCGCTGCGTCGAAGCTGCAGTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCG

1141  CCTCTACGTGGGGGACCTATGCGGGTCTGTCTTTCTTGTCGGCCAACTGTTCACCTTCTC
      GGAGATGCACCCCCTGGATACGCCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAAGAG

1201  TCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAAC
      AGGGTCCGCGGTGACCTGCTGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTG

1261  GGGTCACCGCATGGCATGGGATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAAT
      CCCAGTGGCGTACCGTACCCTATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTA
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FIGURE 62A-1

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1321   GGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGG
       CCGAGTCGACGAGGCCTAGGGTGTTCGGTAGAACCTGTACTAGCGACCACGAGTGACCCC

1381   AGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGT
       TCAGGACCGCCCGTATCGCATAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCA

1441   GCTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGAAGTGCCGGCCA
       CGACGACGATAAACGGCCGCAGCTGCGCCTTTGGGTGCAGTGGCCCCCTTCACGGCCGGT

1501   CACTGTGTCTGGATTTGTTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGAT
       GTGACACAGACCTAAACAATCGGAGGAGCGTGGTCCGCGGTTCGTCTTGCAGGTCGACTA

1561   CAACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCTCAA
       GTTGTGGTTGCCGTCAACCGTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTT

1621   CACCGGCTGGTTGGCAGGGCTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGA
       GTGGCCGACCAACCGTCCCGAAAAGATAGTGGTGTTCAAGTTGAGAAGTCCGACAGGACT ˙

1681   GAGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTA
       CTCCGATCGGTCGACGGCTGGGGAATGGCTAAAACTGGTCCCGACCCCGGGATAGTCAAT

1741   TGCCAACGGAAGCGGCCCCGACCAGCGCCCCTACTGCTGGCACTACCCCCCAAAACCTTG   FIGURE 62A-2
       ACGGTTGCCTTCGCCGGGGCTGGTCGCGGGGATGACGACCGTGATGGGGGGTTTTGGAAC

1801   CGGTATTGTGCCCGCGAAGAGTGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGT
       GCCATAACACGGGCGCTTCTCACACACACCAGGCCATATAACGAAGTGAGGGTCGGGGCA

1861   GGTGGTGGGAACGACCGACAGGTCGGGCGCGCCCACCTACAGCTGGGGTGAAAATGATAC
       CCACCACCCTTGCTGGCTGTCCAGCCCGCGCGGGTGGATGTCGACCCCACTTTTACTATG

1921   GGACGTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGTTCGGTTGTACCTG
       CCTGCAGAAGCAGGAATTGTTATGGTCCGGTGGCGACCCGTTAACCAAGCCAACATGGAC

1981   GATGAACTCAACTGGATTCACCAAAGTGTGCGGAGCGCCTCCTTGTGTCATCGGAGGGGC
       CTACTTGAGTTGACCTAAGTGGTTTCACACGCCTCGCGGAGGAACACAGTAGCCTCCCCG

2041   GGGCAACAACACCCTGCACTGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATA
       CCCGTTGTTGTGGGACGTGACGGGGTGACTAACGAAGGCGTTCGTAGGCCTGCGGTGTAT

2101   CTCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCCTGGTCGACTACCCGTATAG
       GAGAGCCACGCCGAGGCCAGGGACCTAGTGTGGGTCCACGGACCAGCTGATGGGCATATC

2161   GCTTTGGCATTATCCTTGTACCATCAACTACACCATATTTAAAATCAGGATGTACGTGGG
       CGAAACCGTAATAGGAACATGGTAGTTGATGTGGTATAAATTTTAGTCCTACATGCACCC

2221   AGGGGTCGAACACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCT
       TCCCCAGCTTGTGTCCGACCTTCGACGGACGTTGACCTGCGCCCCGCTTGCAACGCTAGA

2281   GGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGT
       CCTTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGATGTGTCACCGTCCA

2341   CCTCCCGTGTTCCTTCACAACCCTACCAGCCTTGTCCACCGGCCTCATCCACCTCCACCA
       GGAGGGCACAAGGAAGTGTTGGGATGGTCGGAACAGGTGGCCGGAGTAGGTGGAGGTGGT

2401   GAACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCGCGTCCTGGGCCAT
       CTTGTAACACCTGCACGTCATGAACATGCCCCACCCCAGTTCGTAGCGCAGGACCCGGTA

2461   TAAGTGGGAGTACGTCGTTCTCCTGTTCCTTCTGCTTGCAGACGCGCGCGTCTGCTCCTG
       ATTCACCCTCATGCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGAC

2521   CTTGTGGATGATGCTACTCATATCCCAAGCGGAGGCGGCTTTGGAGAACCTCGTAATACT
       GAACACCTACTACGATGAGTATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGA˙

2581   TAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCGTGTTCTTCTGCTT
       ATTACGTCGTAGGGACCGGCCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAA
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2641　TGCATGGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTG
　　　　ACGTACCATAAACTTCCCATTCACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACAC

2701　GCCTCTCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGT
　　　　CGGAGAGGAGGACGAGGACAACCGCAACGGGGTCGCCCGCATGCGCGACCTGTGCCTCCA

2761　GGCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATA
　　　　CCGGCGCAGCACACCGCCACAACAAGAGCAGCCCAACTACCGCGACTGAGACAGTGGTAT

2821　TTACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGA
　　　　AATGTTCGCGATATAGTCGACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCT

2881　AGCGCAACTGCACGTGTGGATTCCCCCCCTCAACGTCCGAGGGGGGCGCGACGCCGTCAT
　　　　TCGCGTTGACGTGCACACCTAAGGGGGGGGAGTTGCAGGCTCCCCCCGCGCTGCGGCAGTA

2941　CTTACTCATGTGTGCTGTACACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGC
　　　　GAATGAGTACACACGACATGTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCG

3001　CGTCTTCGGACCCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTTGTGCG
　　　　GCAGAAGCCTGGGGAAACCTAAGAAGTTCGGTCAAACGAATTTCATGGGATGAAACACGC

3061　CGTCCAAGGCCTTCTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGT　FIGURE 62A-3
　　　　GCAGGTTCCGGAAGAGGCCAAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCA

3121　GCAAATGGTCATCATTAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCAC
　　　　CGTTTACCAGTAGTAATTCAATCCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTG

3181　TCCTCTTCGGGACTGGGCGCACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGT
　　　　AGGAGAAGCCCTGACCCGCGTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCA

3241　CGTCTTCTCCCAAATGGAGACCAAGCTCATCACGTGGGGGGGCAGATACCGCCGCGTGCGG
　　　　GCAGAAGAGGGTTTACCTCTGGTTCGAGTAGTGCACCCCCCGTCTATGGCGGCGCACGCC

3301　TGACATCATCAACGGCTTGCCTGTTTCCGCCCGCAGGGGCCGGGAGATACTGCTCGGGCC
　　　　ACTGTAGTAGTTGCCGAACGGACAAAGGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGG

3361　AGCCGATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCA
　　　　TCGGCTACCTTACCAGAGGTTCCCCACCTCCAACGACCGCGGGTAGTGCCGCATGCGGGT

3421　GCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCA
　　　　CGTCTGTTCCCCGGAGGATCCCACGTATTAGTGGTCGGATTGACCGGCCCTGTTTTTGGT

3481　AGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCAT
　　　　TCACCTCCCACTCCAGGTCTAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTA

3541　CAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAA
　　　　GTTACCCCACACGACCTGACAGATGGTGCCCCGGCCTTGCTCCTGGTAGCGCAGTGGGTT

3601　GGGTCCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCC
　　　　CCCAGGACAGTAGGTCTACATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGG

3661　GCAAGGTAGCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCAC
　　　　CGTTCCATCGGCGAGTAACTGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAGTG

3721　GAGGCACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTC
　　　　CTCCGTGCGGCTACAGTAAGGGCACGCGGCCGCCCCACTATCGTCCCCGTCGGACGACAG

3781　GCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGG
　　　　CGGGGCCGGGTAAAGGATGAACTTTCCGAGGAGCCCCCCAGGCGACAACACGGGGCGCCC

3841　GCACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGA
　　　　CGTGCGGCACCCGTATAAATCCCGGCGCCACACGTGGGCACCTCACCGATTCCGCCACCT

3901　CTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTC
　　　　GAAATAGGGACACCTCTTGGATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAG

3961   CTCTCCACCAGTAGTGCCCCAGAGCTTCCAGGTGGCTCACCTCCATGCTCCCACAGGCAG
       GAGAGGTGGTCATCACGGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTC

4021   CGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACT
       GCCGTTTTCGTGGTTCCAGGGCCGACGTATACGTCGAGTCCCGATATTCCACGATCATGA

4081   CAACCCCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGAT
       GTTGGGGAGACAACGACGTTGTGACCCGAAACCACGAATGTACAGGTTCCGAGTACCCTA

4141   CGATCCTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTC
       GCTAGGATTGTAGTCCTGGCCCCACTCTTGTTAATGGTGACCGTCGGGGTAGTGCATGAG

4201   CACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGCGCTTATGACATAATAAT
       GTGGATGCCGTTCAAGGAACGGCTGCCGCCCACGAGCCCCCCGCGAATACTGTATTATTA

4261   TTGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCGGCACTGTCCTTGA
       AACACTGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAACT ᵀ

4321   CCAAGCAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTC   FIGURE 62A-4
       GGTTCGTCTCTGACGCCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCCGAG

4381   CGTCACTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCC
       GCAGTGACACGGGGTAGGGTTGTAGCTCCTCCAACGAGACAGGTGGTGGCCTCTCTAGGG

4441   TTTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGGGAGACATCTCATCTTCTG
       AAAAATGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCᴦCCCCTCTGTAGAGTAGAAGAC

4501   TCATTCAAAGAAGAAGTGCGACGAACTCGCCGCAᴬAGCTGGTCGCATTGGGCATCAATGC
       AGTAAGTTTCTTCTTCACGCTGCTTGAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACG

4561   CGTGGCCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGT
       GCACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCA

4621   CGTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTG
       GCACCGTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGAC

4681   CAATACGTGTGTCACCCAGACAGTCGATTTCAGCCTTGACCCTACCTTCACCATTGAGAC
       GTTATGCACACAGTGGGTCTGTCAGCTAAAGTCGGAACTGGGATGGAAGTGGTAACTCTG

4741   AATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGG
       TTAGTGCGAGGGGGTCCTACGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCC

4801   GAAGCCAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCGGCATGTTCGACTC
       CTTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAG

4861   GTCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCCGCCGA
       CAGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAGTGCGGGCGGCT

4921   GACTACAGTTAGGCTACGAGCGTACATGAACACCCGGGGGCTTCCCGTGTGCCAGGACCA
       CTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCACACGGTCCTGGT

4981   TCTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCCCACTTTCTATC
       AGAACTTAAAACCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAG

5041   CCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTG
       GGTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACAC

5101   CGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGTTTGATTCGCCT
       GCGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCACAAACTAAGCGGA

5161   CAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGA
       GTTCGGGTGGGAGGTACCCGGTTGTGGGACGATATGTCTGACCCGCGACAAGTCTTACT

5221   AATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGA
       TTAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCT

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5281   GGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTG
       CCAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGACGAAACCGGCGCATAAC

5341   CCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAAT
       GGACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTA

5401   CATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCA
       GTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCGT

5461   CTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGG
       GAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCC

5521   CCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTG
       GGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGAC

5581   GCAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATA
       CGTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTAT

5641   CTTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTAC   FIGURE 62A-5
       GAACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAAATG

5701   AGCTGCTGTCACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGG
       TCGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCC

5761   GTGGGTGGCTGCCCAGCTCGCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTT
       CACCCACCGACGGGTCGAGCGGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAA

5821   AGCTGGCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGG
       TCGACCGCGGCGGTAGCCGTCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCC

5881   GTATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCC
       CATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGG

5941   CTCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGT
       GAGGTGCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCA

6001   CGGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCA
       GCCGCACCAGACACGTCGTTATGACGCGGCCGTGCAACCGGGCCCGCTCCCCCGTCACGT

6061   GTGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGGAACCATGTTTCCCCCACGCACTA
       CACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCGTGAT

6121   CGTGCCGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAAC
       GCACGGCCTCTCGCTACGTCGACGGGCGCAGTGACGGTATGAGTCGTCGGAGTGACATTG

6181   CCAGCTCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGG
       GGTCGAGGACTCCGCTGACGTGGTCACCTATTCGAGCCTCACATGGTGAGGTACGAGGCC

6241   TTCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTG
       AAGGACCGATTCCCTGTAGACCCTGACCTATACGCTCCACAACTCGCTGAAATTCTGGAC

6301   GCTAAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCTTTGTGTCCTGCCAGCGCGG
       CGATTTTCGATTCGAGTACGGTGTCGACGGACCCTAGGGGAAACACAGGACGGTCGCGCC

6361   GTATAAGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGA
       CATATTCCCCCAGACCGCTCACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACT

6421   GATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAA
       CTAGTGACCTGTACAGTTTTTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTT

6481   CATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCTGTACCCCCCTTCC
       GTACACCTCACCCTGGAAGGGGTAATTACGGATGTGGTGCCCGGGGACATGGGGGGAAGG

6541   TGCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAG
       ACGCGGCTTGATGTGCAAGCGCGATACCTCCCACAGACGTCTCCTTATACACCTCTATTC
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6601   GCAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTG
       CGTCCACCCCCTGAAGGTGATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGCAC

6661   CCAGGTCCCATCGCCCGAATTTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGC
       GGTCCAGGGTAGCGGGCTTAAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACG

6721   GCCCCCCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATA
       CGGGGGGACGTTCGGGAACGACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTAT

6781   CCCGGTAGGGTCGCAATTACCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCAT
       GGGCCATCCCAGCGTTAATGGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTA

6841   GCTCACTGATCCCTCCCATATAACAGCAGAGGCGGCCGGGCGAAGGTTGGCGAGGGGATC
       CGAGTGACTAGGGAGGGTATATTGTCGTCTCCGCCGGCCCGCTTCCAACCGCTCCCCTAG

6901   ACCCCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAAC
       TGGGGGGAGACACCGGTCGAGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTG

6961   TTGCACCGCTAACCATGACTCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAG   FIGURE 62A-6
       AACGTGGCGATTGGTACTGAGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTC

7021   GCAGGAGATGGGCGGCAACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGA
       CGTCCTCTACCCGCCGTTGTAGTGGTCCCAACTCAGTCTTTTGTTTCACCACTAAGACCT

7081   CTCCTTCGATCCGCTTGTGGCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAAT
       GAGGAAGCTAGGCGAACACCGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTA

7141   CCTGCGGAAGTCTCGGAGATTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAA
       GGACGCCTTCAGAGCCTCTAAGCGGGTCCGGGACGGGCAAACCCGCGCCGGCCTGATATT

7201   CCCCCCGCTAGTGGAGACGTGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTG
       GGGGGGCGATCACCTCTGCACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGAC

7261   TCCGCTTCCACCTCCAAAGTCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGT
       AGGCGAAGGTGGAGGTTTCAGGGGAGGACACGGAGGCGGAGCCTTCTTCGCCTGCCACCA

7321   CCTCACTGAATCAACCCTATCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAG
       GGAGTGACTTAGTTGGGATAGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTC

7381   CTCCTCAACTTCCGGCATTACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCCTTC
       GAGGAGTTGAAGGCCGTAATGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAG

7441   TGGCTGCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCCATGCCCCCCCTGGAGGGGGA
       ACCGACGGGGGGGCTGAGGCTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCT

7501   GCCTGGGGATCCGGATCTTAGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGC
       CGGACCCCTAGGCCTAGAATCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCG

7561   GGAGGATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTG
       CCTCCTACAGCACACGACGAGTTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCAC

7621   CGCCGCGGAAGAACAGAAACTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCA
       GCGGCGCCTTCTTGTCTTTGACGGGTAGTTACGTGATTCGTTGAGCAACGATGCAGTGGT

7681   CAATTTGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATT
       GTTAAACCACATAAGGTGGTGGAGTGCGTCACGAACGGTTTCCGTCTTCTTTCAGTGTAA

7741   TGACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGC
       ACTGTCTGACGTTCAAGACCTGTCGGTAATGGTCCTGCATGAGTTCCTCCAATTTCGTCG

7801   GGCGTCAAAAGTGAAGGCTAACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCC
       CCGCAGTTTTCACTTCCGATTGAACGATAGGCATCTCCTTCGAACGTCGGACTGCGGGGG

7861   ACACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAAGACGTCCGTTGCCATGCCAGAAA
       TGTGAGTCGGTTTAGGTTCAAACCAATACCCCGTTTTCTGCAGGCAACGGTACGGTCTTT
```

7921 GGCCGTAACCCACATCAACTCCGTGTGGAAAGACCTTCTGGAAGACAATGTAACACCAAT
      CCGGCATTGGGTGTAGTTGAGGCACACCTTTCTGGAAGACCTTCTGTTACATTGTGGTTA

7981 AGACACTACCATCATGGCTAAGAACGAGGTTTTCTGCGTTCAGCCTGAGAAGGGGGGTCG
      TCTGTGATGGTAGTACCGATTCTTGCTCCAAAAGACGCAAGTCGGACTCTTCCCCCCAGC

8041 TAAGCCAGCTCGTCTCATCGTGTTCCCCGATCTGGGCGTGCGCGTGTGCGAAAAGATGGC
      ATTCGGTCGAGCAGAGTAGCACAAGGGGCTAGACCCGCACGCGCACACGCTTTTCTACCG

8101 TTTGTACGACGTGGTTACAAAGCTCCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCA
      AAACATGCTGCACCAATGTTTCGAGGGGAACCGGCACTACCCTTCGAGGATGCCTAAGGT

8161 ATACTCACCAGGACAGCGGGTTGAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCC
      TATGAGTGGTCCTGTCGCCCAACTTAAGGAGCACGTTCGCACCTTCAGGTTCTTTTGGGG

8221 AATGGGGTTCTCGTATGATACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCG
      TTACCCCAAGAGCATACTATGGGCGACGAAACTGAGGTGTCAGTGACTCTCGCTGTAGGC

8281 TACGGAGGAGGCAATCTACCAATGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAA
      ATGCCTCCTCCGTTAGATGGTTACAACACTGGAGCTGGGGGTTCGGGCGCACCGGTAGTT

8341 GTCCCTCACCGAGAGGCTTTATGTTGGGGGCCCTCTTACCAATTCAAGGGGGGGAGAACTG
      CAGGGAGTGGCTCTCCGAAATACAACCCCCGGGAGAATGGTTAAGTTCCCCCCTCTTGAC   FIGURE 62A-7

8401 CGGCTATCGCAGGTGCCGCGCGAGCGGCGTACTGACAACTAGCTGTGGTAACACCCTCAC
      GCCGATAGCGTCCACGGCGCGCTCGCCGCATGACTGTTGATCGACACCATTGTGGGAGTG

8461 TTGCTACATCAAGGCCCGGGCAGCCTGTCGAGCCGCAGGGCTCCAGGACTGCACCATGCT
      AACGATGTAGTTCCGGGCCCGTCGGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGA

8521 CGTGTGTGGCGACGACTTAGTCGTTATCTGTGAAAGCGCGGGGGTCCAGGAGGACGCGGC
      GCACACACCGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCCAGGTCCTCCTGCGCCG

8581 GAGCCTGAGAGCCTTCACGGAGGCTATGACCAGGTACTCCGCCCCCCCTGGGGACCCCCC
      CTCGGACTCTCGGAAGTGCCTCCGATACTGGTCCATGAGGCGGGGGGGACCCCTGGGGGG

8641 ACAACCAGAATACGACTTGGAGCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCA
      TGTTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGT

8701 CGACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAG
      GCTGCCGCGACCTTTCTCCCAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTC

8761 AGCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCAT
      TCGACGCACCCTCTGTCGTTCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTA

8821 GTTTGCCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTAT
      CAAACGGGGGTGTGACACCCGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATA

8881 AGCCAGGGACCAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCAT
      TCGGTCCCTGGTCGAACTTGTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTA

8941 AGAACCACTTGATCTACCTCCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACT
      TCTTGGTGAACTAGATGGAGGTTAGTAAGTTTCTGAGGTACCGGAGTCGCGTAAAAGTGA

9001 CCACAGTTACTCTCCAGGTGAAATTAATAGGGTGGCCGCATGCCTCAGAAAACTTGGGGT
      GGTGTCAATGAGAGGTCCACTTTAATTATCCCACCGGCGTACGGAGTCTTTTGAACCCCA

9061 ACCGCCCTTGCGAGCTTGGAGACACCGGGCCCGGAGCGTCCGCGCTAGGCTTCTGGCCAG
      TGGCGGGAACGCTCGAACCTCTGTGGCCCGGGCCTCGCAGGCGCGATCCGAAGACCGGTC

9121 AGGAGGCAGGGCTGCCATATGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCT
      TCCTCCGTCCCGACGGTATACACCGTTCATGGAGAAGTTGACCCGTCATTCTTGTTTCGA

9181 CAAAC
      GTTTG

FIGURE 63

HCV ANTIGENIC MAPPING

IMMUNOLOGICAL SCREENING IN BACTERIA

FIGURE 64

FIGURE 65

Structure of the HCV polyprotein

(From the putative initiator methionine)

       R T
MSTNPKPQKKNKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATR
KTSERSQPRGRRQPIPKARRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSP-100
RGSRPSWGPTDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPLGGAARA

           T   \
LAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSTGL-200
YHVTNDCPNSSIVYEAADAILHTPGCVPCVREGNASRCWVAMTPTVATRD
GKLPATQLRRHIDLLVGSATLCSALYVGDLCGSVFLVGQLFTFSPRRHWT-300

                V
TQGCNCSIYPGHITGHRMAWDMMMNWSPTTALVMAQLLRIPQAILDMIAG
AHWGVLAGIAYFSMVGNWAKVLVVLLLFAGVDAETHVTGGSAGHTVSGFV-400
SLLAPGAKQNVQLINTNGSWHLNSTALNCNDSLNTGWLAGLFYHHKFNSS
GCPERLASCRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAR-500
SVCGPVYCFTPSPVVVGTTDRSGAPTYSWGENDTDVFVLNNTRPPLGNWF
GCTWMNSTGFTKVCGAPPCVIGGAGNNTLHCPTDCFRKHPDATYSRCGSG-600

     I
PWLTPRCLVDYPYRLWHYPCTINYTIFKIRMYVGGVEHRLEAACNWTRGE
RCDLEDRDRSELSPLLLTTTQWQVLPCSFTTLPALSTGLIHLHQNIVDVQ-700
YLYGVGSSIASWAIKWEYVVLFPLLLADARVCSCLWMMLLISQAEAALEN
LVILHAASLAGTHGLVSFLVFFCPAWYLKGRMVPGAVYTFYGMWPLLLLL-800

                   (N)
LALPQRAYALDTEVAASCGGVVLVGLMALTLSPYYKRYISWCLWWLQYFL
TRVEAQLHVWIPPLNVRGGRDAVILLMCAVHPTLVFDITKLLLAVFGPLW-900
ILQASLLKVPYFVRVQGLLRPCALARKMIGGHYVQMVIIKLGALTGTYVY
NHLTPLRDWAHNCLRDLAVAVEPVVFSQMETKLITWGADTAACGDIINGL-1000
PVSARRGREILLGPADGMVSKGWRLLAPITAYAQQTRGLLGCIITSLTGR
DKNQVEGEVQIVSTAAQTFLATCINGVCWTVYHGAGTRTIASPKGPVIQM-1100
YTNVDQDLVGWPAPQGSRSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRG
SLLSPRPISYLKGSSGGPLLCPAGHAVGIFRAAVCTRGVAKAVDFIPVEN-1200
LETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYK

             L
VLVLNPSVAATLGFGAYMSKAHGIDPNIRTCVRTITTGSPITYSTYGKFL-1300

ADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATAT
PPGSVTVPHPNIEEVALSTTGEIPFYGKAIPLEVIKGGRHLIFCHSKKKC-1400
DELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVATDALMTGYTGDFDS

    Y   (S)
VIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRTQRRGRTGRGRPGIYR-1500
FVAPGERPSGHFDSSVLCECYDAGCAWYELTPAETTVRLRAYMNTPGLPV
CQDHLEFWEGVFTGLTHIDAHFLSQTKQSGENLPYLVAYQATVCARAQAP-1600
PPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEITLTHPVTKYIMTCMS
ADLEVVTSTWVLVGGVLAALAAYCLSTGCVVIVGRVVLSGKPAIIPDREV-1700
LYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAV
QTNWQKLETFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTAAVTSP-1800
LTTSGTLLFNILGGWVAAQLAAPGAATAFVGAGLAGAAIGSVGLGKVLID

           (G)
ILAGYGAGVAGALVAFKIMSGEVPSTEDLVNLLPAILSPGALVVGVVCAA-1900

             (HC)
ILRRHVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAAARVTAILSS
LTVTQLLRRLHQWISSECTTPCSGSWLRDIWDWICEVLSDFKTWLKAKLM-2000

FIGURE 66

```
                          (V)
POLPCIPFVSCCRCYECVWRGDGDGHTRCHCGAEITGHVKNGTMRIVGPR
TCRNWGGTFPINAYTTGPCTPLPAPNYTPALMKVSAEEYVEIRQVGDFH-2100
YVTGMTTINLKCPCQVPSPSPSFTELDGVRLHRPAPPCKPLLREEVSFRVG
LHEYPVGSQLPCEPSPDVAVLTRRLTDPSHITAEAAGRRLARGSPPSVAS-2200
SSASQLSAPSLKATCTANHDSPDAELIEANLLWRQEMGGNITRVESEDKV
VTLDSFDPLVAEEDEREISVPAEILRKSRRFAQALFVWARPDYNPPLVET-2300


                                                 S
WKKPDYEPPVVHGCPLPPPKSPFVPPPRKKRTVVLTESILSTALAELATR

                          (FA)
SFGSSSTSGITGDNTTTSSEPAPSGCPPDSDAFSYSSMPPLEGEPGDPDL-2400
SDGSWGTVSSEANAEDVVCCSMBYSWTGALVTPCAAEEQKLPINALSNSL
LRHHNLVYSTTSRSACQRQKKVTFDRLQVLDSHYQDVLKEVKAAASKVKA-2500

(F)
NJLSVEEACSLTPPHSAKSKFGYGAKDVRCHARKAVTHINSVWKDILEDN
VTPIDTTIMAKNEVFCVQPEKGGKKPARLIVFPDLGVHVCEKMALYDVVT-2600
KLPLAVMGSSYGFQYSPCQRVEFLVQAWKSKKTPMGFSYDTRCFDSTVTE

                          (C)
SDIRTEEAIYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENCGYRRCR-2700
ASGVLTTSCGNTLTCYIKARAACEAAGLQDCTMLVCGDDLVVICESAGVQ
EDAASLRAPTEAMTRYSAPPGDPPQPEYDLELITSCSSNVSVAHDGAGKR-2800
VYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMFAPTLWARNILMTHFF
SVLIARDQLEQALDCEIYGACYSIEPLDLPPIIQRLEGLSAPSLHSYSPG-2900

                          G
EINRVAACLRKLGVPPLRAMRHRARSYRARLLARGGRAAICGKYLFMKAV
RTKLK---------------(Stop codon not yet reached)
```

FIGURE 66.1

```
{ } = Heterogeneity due to possible 5' or 3'
      terminal cloning artefacts.
```

FIG.66A

```
                    R T
MSTNPKPQKKNKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATR
KTSERSQPRGRRQPIPKARRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSP-100
RGSRPSWGPTDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPLGGAARA

                     T
LAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSTGL-200
YHVTNDCPNSSIVYEAADAILHTPGCVPCVREGNASRCWVAMTPTVATRD
GKLPATQLRRHIDLLVGSATLCSALYVGDLCGSVFLVGQLFTFSPRRHWT-300

                             V
TGGCNCSIYPGHITGHRMAWDWWWNWSPTTALVMAQLLRIPQAILDMIAG
AHWGVLAGIAYFSWVGNWAKVLVVLLLLFAGVDAETHVTGGSAGHTVSGFV-400
SLLAPGAKQNVQLINTNGSWHLNSTALNCNDSLNTGWLAGLFYHHKFNSS
GCPERLASCRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAK-500
SVCGPVYCFTPSPVVVGTTDRSGAPTYSWGENDTDVFVLNNTRPPLGMWF
GCTWWNSTGFTKVCGAPPCVIGGAGNNTLHCPTDCFRKHPDATYSRCGSG-600

     I
PWLTPRCLVDYPYRLWHYPCTINYTIFKIRMYVGGVEHRLEAACNWTRGE
RCDLEDRDRSELSPLLLTTTQWQVLPCSFTTLPALSTGLIHLHQNIVDVQ-700
YLYGVGSSIASWAIKWEYVVLLFLLLADARVCSCLWWWLLISQAEAALEN
LVILNAASLAGTHGLVSFLVFFCFAWYLKGKWVPGAVYTFYGMWPLLLLL-800

                               (N)
LALPQRAYALDTEVAASCGGVVLVGLMALTLSPYYKRYISWCLWWLQYFL
TRVEAQLHVWIPPLNVRGGRDAVILLMCAVHPTLVFDITKLLLAVFGPLN-900
ILQASLLKVPYFVRVQGLLRFCALARKMIGGHYVQMVIIKLGALTGTYVY
NHLTPLRDWAHNGLRDLAVAVEFVVFSQMETKLITWGADTAACGDIINGL-1000
PVSARRGREILLGPADGMVSKGWRLLAPITAYAQQTRGLLGCIITSLTGR
DKNQVEGEVQIVSTAAQTTFATCINGVCWTVYHGAGTRTIASPKGPVIQM-1100
YTNVDQDLVGWPAPQGSRSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRG
SLLSPRPISYLKGSSGGPLLCPAGHAVGIFRAAVCTRGVAKAVDFIPVEN-1200
LETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYK

                   L
VLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFL-1300

ADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATAT
PPGSVTVPHPNIEEVALSTTGEIPFYGKAIPLEVIKGGRHLIFCHSKKKC-1400
DELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVATDALMTGYTGDFDS

     Y              (S)
YIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRTQRRGRTGRGKPGIYR-1500
FVAPGERPSGMFDSSVLCECYDAGCAWYELTPAETTVRLRAYMNTPGLPV
CQDHLEFWEGVFTGLTHIDAHFLSQTKQSGENLPYLVAYQATVCARAQAP-1600
PPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEITLTHPVTKYIMTCMS
ADLEVVTSTWVLVGGVLAALAAYCLSTGCVVIVGRVVLSGKPAIIPDREV-1700
LYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAV
QTNWQKLETFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTAAVTSP-1800
LTTSQTLLFNILGGWVAAQLAAPGAATAFVGAGLAGAAIGSVGLGKVLID
```

FIG.66B

```
                              (G)
    ILAGYGAGVAGALVAFKIMSGEVPSTEDLVNLLPAILSPGALVVGVVCAA-1900


                                              (HC)
    ILRRHVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAAARVTAILSS
    LTVTQLLRRLHQWISSECTTPCSGSWLRDIWDWICEVLSDFKTWLKAKLM-2000


                      (V)
    PQLPGIPFVSCQRGYKGVWRGDGIMHTRCHCGAEITGHVKNGTMRIVGPR
    TCRMMWSGTFPINAYTTGPCTPLPAPNYTFALWRVSAEEYVEIRQVGDFH-2100
    YVTGMTTDNLKCPCQVPSPEFFTELDGVRLHRFAPPCKPLLREEVSFRVG
    LHEYPVGSQLPCEPEPDVAVLTSMLTDPSHITAEAAGRRLARGSPPSVAS-2200
    SSASQLSAPSLKATCTANHDSPDAELIEANLLWRQEMGGNITRVESENKV
    VILDSFDPLVAEEDEREISVPAEILRKSRRFAQALPVWARPDYNPPLVET-2300


                                                     S
    WKKPDYEPPVVHGCPLPPPKSPPVPPPRKKRTVVLTESTLSTALAELATR


                                  (FA)
    SFGSSSTSGITGDNTTTSSEPAPSGCPPDSDAESYSSMPPLEGEPGDPDL-2400
    SDGSWSTVSSEANAEDVVCCSMSYSWTGALVTPCAAEEQKLPINALSNSL
    LRHHNLVYSTTSRSACQRQKKVTFDRLQVLDSHYQDVLKEVKAAASKVKA-2500

    (F)
    NLLSVEEACSLTPPHSAKSKFGYGAKDVRCHARKAVTHINSVWKDLLEDN
    VTPIDTTIMAKNEVFCVQPEKGGRKPARLIVFPDLGVRVCEKMALYDVVT-2600
    KLPLAVMGSSYGFQYSPGQRVEFLVQAWKSKKTPMGFSYDTRCFDSTVTE


                                   (G)
    SDIRTEEAIYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENCGYRRCR-2700
    ASGVLTTSCGNTLTCYIKARAACRAAGLQDCTMLVCGDDLVVICESAGVQ
    EDAASLRAFTEAMTRYSAPPGDPPQPEYDLELITSCSSNVSVAHDGAGKR-2800  -
    VYYLTRDPTTPLARAAWETARHTFVNSWLGNIIMFAPTLWARMILMTHFF
    SVLIARDQLEQALDCEIYGACYSIEPLDLPPIIQRLHGLSAFSLHSYSPG-2900


                   G
    EINRVAACLRKLGVPPLRAWRHRARSVRARLLARGGRAAICGKYLFNWAV
    RTKLK--------------(Stop codon not yet reached)


             ( ) = Heterogeneity due to possible 5' or 3'
                   terminal cloning artefacts.
```

FIGURE 67-1

HCV

FIGURE 67-2

(2001 — 2500)

(2501 — 3000)

(3001 — 3011)

HCV

Some conserved co-linear peptides in HCV & flaviviruses

	NS3 region		NS5 Highly-conserved Polymerase region
Flaviviruses (Yellow Fever, West Nile,Dengue)	TATPPG----------SAAQRRGRIGRNP----	------------GDDCVV	
	******	* ***** **	*** **
HCV	TATPPG----------SRTQRRGRTGRGK----	------------GDDLVV	
	#1348	#1483	#2737

FIGURE 68

FIGURE 69

Hind III 9400

pUC8 vector

Bst EII 960

Pvu II 1350

Xho I 1900

pAc373

Eco RI 7100

Sal I 2870

Pvu II 6300

Sal I 3180

Hind III 6080
Sal I 6050

Hind III 5630
Poly A 4910

Polyhedrin

Eco RV 3810
Bam HI 4000
Hind III 4050

Kpn I 4460

pAc373

-8

GT CAAATCTCTATCTC
Ava IIBol Sst2BamI

•177   •752

TTTC ... GTTATT

pVL985

+3   •14

CCTATAAAT ATG CCG GAT TAT TCA TAC CGT CCC ACC ATC GGG CCG GAT CC
Sal I BspHII   BamHI

•177

TTTC ... GTTATT

yhedrin

+1

       met pro asp tyr ser tyr arg pro thr ile gly
CCTATAAAT ATG CCG GAT TAT TCA TAC CGT CCC ACC ATC GGG

Figure 7.   AcNPV transfer vectors for high level expression of nonfused foreign proteins. The numbers shown refer to positions within the native gene, where the A of the ATG codon is +1.

FIGURE 70

Translation of DNA 6k

```
                    -------------------------Overlap with 16jh--------------------
     GlyArgAlaAlaIleCysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLys
  1  GGCAGGGCTGCCATATGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCTCAAA
     CCGTCCCGACGGTATACACCGTTCATGGAGAAGTTGACCCGTCATTCTTGTTTCGAGTTT
     -

     LeuThrProIleAlaAlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyr
 61  CTCACTCCAATAGCGGCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTCACGGCTGGCTAC
     GAGTGAGGTTATCGCCGGCGACCGGTCGACCTGAACAGGCCGACCAAGTGCCGACCGATG

     SerGlyGlyAspIleTyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCys
121  AGCGGGGGAGACATTTATCACAGCGTGTCTCATGCCCGGCCCCGCTGGATCTGGTTTTGC
     TCGCCCCCTCTGTAAATAGTGTCGCACAGAGTACGGGCCGGGGCGACCTAGACCAAAACG

181  CC
     GG
```

FIGURE 71

FIGURE 72-1

```
        MetSerThrAsnProLysProGlnArgLysThrLysArgAsnThrAsnArgArgProGln
    1   ATGAGCACGAATCCTAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAG
        TACTCGTGCTTAGGATTTGGAGTTTCTTTTTGGTTTGCATTGTGGTTGGCAGCGGGTGTC

        AspValLysPheProGlyGlyGlyGlnIleValGlyGlyValTyrLeuLeuProArgArg
   61   GACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGTTGCCGCGCAGG
        CTGCAGTTCAAGGGCCCACCGCCAGTCTAGCAACCACCTCAAATGAACAACGGCGCGTCC

        GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly
  121   GGCCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCCGAGCGGTCGCAACCTCGAGGT
        CCGGGATCTAACCCACACGCGCGCTGCTCTTTCTGAAGGCTCGCCAGCGTTGGAGCTCCA

        ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly
  181   AGACGTCAGCCTATCCCCAAGGCTCGTCGGCCCGAGGGCAGGACCTGGGCTCAGCCCGGG
        TCTGCAGTCGGATAGGGGTTCCGAGCAGCCGGGCTCCCGTCCTGGACCCGAGTCGGGCCC.

        TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro
  241   TACCCTTGGCCCCTCTATGGCAATGAGGGCTGCGGGTGGGCGGGATGGCTCCTGTCTCCC
        ATGGGAACCGGGGAGATACCGTTACTCCCGACGCCCACCCGCCCTACCGAGGACAGAGGG

        ArgGlySerArgProSerTrpGlyProThrAspProArgArgArgSerArgAsnLeuGly
  301   CGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCCCGGCGTAGGTCGCGCAATTTGGGT
        GCACCGAGAGCCGGATCGACCCCGGGGTGTCTGGGGGCCGCATCCAGCGCGTTAAACCCA

        LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal
  361   AAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTC
        TTCCAGTAGCTATGGGAATGCACGCCGAAGCGGCTGGAGTACCCCATGTATGGCGAGCAG

        GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp
  421   GGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGAC
        CCGCGGGGAGAACCTCCGCGACGGTCCCGGGACCGCGTACCGCAGGCCCAAGACCTTCTG

        GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla
  481   GGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTCTATCTTCCTTCTGGCC
        CCGCACTTGATACGTTGTCCCTTGGAAGGACCAACGAGAAAGAGATAGAAGGAAGACCGG

        LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu
  541   CTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGTGCGCAACTCCACGGGGCTT
        GACGAGAGAACGAACTGACACGGGCGAAGCCGGATGGTTCACGCGTTGAGGTGCCCCGAA

        TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle
  601   TACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGCGGCCGATGCCATC
        ATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACATGCTCCGCCGGCTACGGTAG

        LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal
  661   CTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTGAGGGCAACGCCTCGAGGTGTTGGGTG
        GACGTGTGAGGCCCCACGCAGGGAACGCAAGCACTCCCGTTGCGGAGCTCCACAACCCAC

        AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg
  721   GCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCCCGCGACGCAGCTTCGACGT
        CGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTTGAGGGGCGCTGCGTCGAAGCTGCA

        HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu
  781   CACATCGATCTGCTTGTCGGGAGCGCCACCCTCTGTTCGGCCCTCTACGTGGGGGACCTG
        GTGTAGCTAGACGAACAGCCCTCGCGGTGGGAGACAAGCCGGGAGATGCACCCCCTGGAC

        CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr
  841   TGCGGGTCTGTCTTTCTTGTCGGCCAACTGTTCACCTTCTCTCCCAGGCGCCACTGGACG
        ACGCCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAAGAGAGGGTCCGCGGTGACCTGC

        ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp
  901   ACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACGGGTCACCGCATGGCATGG
        TGCGTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCCAGTGGCGTACCGTACC
```

FIGURE 72-2

```
       AspMetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIle
  961  GATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATC
       CTATACTACTACTTGACCAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCTAG

       ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla
 1021  CCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGGAGTCCTGGCGGGCATAGCG
       GGTGTTCGGTAGAACCTGTACTAGCGACCACGAGTGACCCCTCAGGACCGCCCGTATCGC

       TyrPheSerMetValGlyAsnTrpAlaLysValLeuValValLeuLeuLeuPheAlaGly
 1081  TATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGTGCTGCTGCTATTTGCCGGC
       ATAAAGAGGTACCACCCCTTGACCCGCTTCCAGGACCATCACGACGACGATAAACGGCCG

       ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal
 1141  GTCGACGCGGAAACCCACGTCACCGGGGGGAAGTGCCGGCCACACTGTGTCTGGATTTGTT
       CAGCTGCGCCTTTGGGTGCAGTGGCCCCCTTCACGGCCGGTGTGACACAGACCTAAACAA

       SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp
 1201  AGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGATCAACACCAACGGCAGTTGG
       TCGGAGGAGCGTGGTCCGCGGTTCGTCTTGCAGGTCGACTAGTTGTGGTTGCCGTCAACC

       HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly
 1261  CACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCTCAACACCGGCTGGTTGGCAGGG
       GTGGAGTTATCGTGCCGGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACCGTCCC

       LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
 1321  CTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGAGAGGCTAGCCAGCTGCCGA
       GAAAAGATAGTGGTGTTCAAGTTGAGAAGTCCGACAGGACTCTCCGATCGGTCGACGGCT

       ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
 1381  CCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTATGCCAACGGAAGCGGCCCC
       GGGGAATGGCTAAAACTGGTCCCGACCCCGGGATAGTCAATACGGTTGCCTTCGCCGGGG

       AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
 1441  GACCAGCGCCCCTACTGCTGGCACTACCCCCCAAAACCTTGCGGTATTGTGCCCGCGAAG
       CTGGTCGCGGGGATGACGACCGTGATGGGGGGTTTTGGAACGCCATAACACGGGCGCTTC

       SerValCysGlyProValTyrCysPheThrProSerProValValValGlyThrThrAsp
 1501  AGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGTGGTGGTGGGAACGACCGAC
       TCACACACACCAGGCCATATAACGAAGTGAGGGTCGGGGCACCACCACCCTTGCTGGCTG

       ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn
 1561  AGGTCGGGCGCGCCCACCTACAGCTGGGGTGAAAATGATACGGACGTCTTCGTCCTTAAC
       TCCAGCCCGCGCGGGTGGATGTCGACCCCACTTTTACTATGCCTGCAGAAGCAGGAATTG

       AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe
 1621  AATACCAGGCCACCGCTGGGCAATTGGTTCGGTTGTACCTGGATGAACTCAACTGGATTC
       TTATGGTCCGGTGGCGACCCGTTAACCAAGCCAACATGGACCTACTTGAGTTGACCTAAG

       ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis
 1681  ACCAAAGTGTGCGGAGCGCCTCCTTGTGTCATCGGAGGGGCGGGCAACAACACCCTGCAC
       TGGTTTCACACGCCTCGCGGAGGAACACAGTAGCCTCCCCGCCCGTTGTTGTGGGACGTG

       CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly
 1741  TGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATACTCTCGGTGCGGCTCCGGT
       ACGGGGTGACTAACGAAGGCGTTCGTAGGCCTGCGGTGTATGAGAGCCACGCCGAGGCCA

       ProTrpIleThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys
 1801  CCCTGGATCACACCCAGGTGCCTGGTCGACTACCCGTATAGGCTTTGGCATTATCCTTGT
       GGGACCTAGTGTGGGTCCACGGACCAGCTGATGGGCATATCCGAAACCGTAATAGGAACA

       ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu
 1861  ACCATCAACTACACCATATTTAAAATCAGGATGTACGTGGGAGGGGTCGAACACAGGCTG
       TGGTAGTTGATGTGGTATAAATTTTAGTCCTACATGCACCCTCCCCAGCTTGTGTCCGAC

       GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer
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FIGURE 72-3

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              CTTCGACGGACGTTGACCTGCGCCCCGCTTGCAACGCTAGACCTTCTGTCCCTGTCCAGG

              GluLeuSerProLeuLeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThr
      1981    GAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGTCCTCCCGTGTTCCTTCACA
              CTCGAGTCGGGCAATGACGACTGGTGATGTGTCACCGTCCAGGAGGGCACAAGGAAGTGT

              ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln
      2041    ACCCTACCAGCCTTGTCCACCGGCCTCATCCACCTCCACCAGAACATTGTGGACGTGCAG
              TGGGATGGTCGGAACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAACACCTGCACGTC

              TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal
      2101    TACTTGTACGGGGTGGGGTCAAGCATCGCGTCCTGGGCCATTAAGTGGGAGTACGTCGTT
              ATGAACATGCCCCACCCCAGTTCGTAGCGCAGGACCCGGTAATTCACCCTCATGCAGCAA

              LeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu
      2161    CTCCTGTTCCTTCTGCTTGCAGACGCGCGCGTCTGCTCCTGCTTGTGGATGATGCTACTC
              GAGGACAAGGAAGACGAACGTCTGCGCGCGCAGACGAGGACGAACACCTACTACGATGAG.

              IleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla
      2221    ATATCCCAAGCGGAGGCGGCTTTGGAGAACCTCGTAATACTTAATGCAGCATCCCTGGCC
              TATAGGGTTCGCCTCCGCCGAAACCTCTTGGAGCATTATGAATTACGTCGTAGGGACCGG

              GlyThrHisGlyLeuValSerPheLeuValPhePheCysPheAlaTrpTyrLeuLysGly
      2281    GGGACGCACGGTCTTGTATCCTTCCTCGTGTTCTTCTGCTTTGCATGGTATTTGAAGGGT
              CCCTGCGTGCCAGAACATAGGAAGGAGCACAAGAAGACGAAACGTACCATAAACTTCCCA

              LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeuLeu
      2341    AAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTGGCCTCTCCTCCTGCTCCTG
              TTCACCCACGGGCCTCGCCAGATGTGGAAGATGCCCTACACCGGAGAGGAGGACGAGGAC

              LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly
      2401    TTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGTGGCCGCGTCGTGTGGCGGT
              AACCGCAACGGGGTCGCCCGCATGCGCGACCTGTGCCTCCACCGGCGCAGCACACCGCCA

              ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer
      2461    GTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATATTACAAGCGCTATATCAGC
              CAACAAGAGCAGCCCAACTACCGCGACTGAGACAGTGGTATAATGTTCGCGATATAGTCG

              TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp
      2521    TGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGAAGCGCAACTGCACGTGTGG
              ACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTTCGCGTTGACGTGCACACC

              IleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal
      2581    ATTCCCCCCCTCAACGTCCGAGGGGGGCGCGACGCCGTCATCTTACTCATGTGTGCTGTA
              TAAGGGGGGGAGTTGCAGGCTCCCCCCGCGCTGCGGCAGTAGAATGAGTACACACGACAT

              HisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyProLeuTrp
      2641    CACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGCCGTCTTCGGACCCCTTTGG
              GTGGGCTGAGACCATAAACTGTAGTGGTTTAACGACGACCGGCAGAAGCCTGGGGAAACC

              IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg
      2701    ATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTTGTGCGCGTCCAAGGCCTTCTCCGG
              TAAGAAGTTCGGTCAAACGAATTTCATGGGATGAAACACGCGCAGGTTCCGGAAGAGGCC

              PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys
      2761    TTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTGCAAATGGTCATCATTAAG
              AAGACGCGCAATCGCGCCTTCTACTAGCCTCCGGTAATGCACGTTTACCAGTAGTAATTC

              LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla
      2821    TTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCACTCCTCTTCGGGACTGGGCG
              AATCCCCGCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAAGCCCTGACCCGC

              HisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGlnMetGlu
      2881    CACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTTCTCCCAAATGGAG
              GTGTTGCCGAACGCTCTAGACCGGCACCGACATCTCGGTCAGCAGAAGAGGGTTTACCTC
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FIGURE 72-4

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      ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu
2941  ACCAAGCTCATCACGTGGGGGGCAGATACCGCCGCGTGCGGTGACATCATCAACGGCTTG
      TGGTTCGAGTAGTGCACCCCCCGTCTATGGCGGCGCACGCCACTGTAGTAGTTGCCGAAC

      ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer
3001  CCTGTTTCCGCCCGCAGGGGCCGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCC
      GGACAAAGGCGGGCGTCCCCGGCCCTCTATGACGAGCCCGGTCGGCTACCTTACCAGAGG

      LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu
3061  AAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCAGCAGACAAGGGGCCTCCTA
      TTCCCCACCTCCAACGACCGCGGGTAGTGCCGCATGCGGGTCGTCTGTTCCCCGGAGGAT

      GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln
3121  GGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAG
      CCCACGTATTAGTGGTCGGATTGACCGGCCCTGTTTTTGGTTCACCTCCCACTCCAGGTC

      IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr
3181  ATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACT
      TAACACAGTTGACGACGGGTTTGGAAGGACCGTTGCACGTAGTTACCCCACACGACCTGA

      ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet
3241  GTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAAGGGTCCTGTCATCCAGATG
      CAGATGGTGCCCCGGCCTTGCTCCTGGTAGCGCAGTGGGTTCCCAGGACAGTAGGTCTAC

      TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu
3301  TATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCCGCAAGGTAGCCGCTCATTG
      ATATGGTTACATCTGGTTCTGGAACACCCGACCGGGCGAGGCGTTCCATCGGCGAGTAAC

      ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle
3361  ACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCACGAGGCACGCCGATGTCATT
      TGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCAGTGCTCCGTGCGGCTACAGTAA

      ProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr
3421  CCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTTCCTAC
      GGGCACGCGGCCGCCCCACTATCGTCCCCGTCGGACGACAGCGGGGCCGGGTAAAGGATG

      LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe
3481  TTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGGGCACGCCGTGGGCATATTT
      AACTTTCCGAGGAGCCCCCCAGGCGACAACACGGGGCGCCCCGTGCGGCACCCGTATAAA

      ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn
3541  AGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGACTTTATCCCTGTGGAGAAC
      TCCCGGCGCCACACGTGGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTG

      LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro
3601  CTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTCCTCTCCACCAGTAGTGCCC
      GATCTCTGTTGGTACTCCAGGGGCCACAAGTGCCTATTGAGGAGAGGTGGTCATCACGGG

      GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal
3661  CAGAGCTTCCAGGTGGCTCACCTCCATGCTCCCACAGGCAGCGGCAAAAGCACCAAGGTC
      GTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTCGCCGTTTTCGTGGTTCCAG

      ProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla
3721  CCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTTGCTGCA
      GGCCGACGTATACGTCGAGTCCCGATATTCCACGATCATGAGTTGGGGAGACAACGACGT

      ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr
3781  ACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGATCGATCCTAACATCAGGACC
      TGTGACCCGAAACCACGAATGTACAGGTTCCGAGTACCCTAGCTAGGATTGTAGTCCTGG

      GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu
3841  GGGGTGAGACAATTACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCCTT
      CCCCACTCTTGTTAATGGTGACCGTCGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAA

      AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer
3901  GCCGACGGCGGGTGCTCGGGGGGCGCTTATGACATAATAATTTGTGACGAGTGCCACTCC
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FIGURE 72-5

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             CGGCTGCCGCCCACGAGCCCCCGCGAATACTGTATTATTAAACACTGCTCACGGTGAGG

             ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly
      3961   ACGGATGCCACATCCATCTTGGGCATCGGCACTGTCCTTGACCAAGCAGAGACTGCGGGG
             TGCCTACGGTGTAGGTAGAACCCGTAGCCGTGACAGGAACTGGTTCGTCTCTGACGCCCC

             AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro
      4021   GCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTCCGTCACTGTGCCCCATCCC
             CGCTCTGACCAACACGAGCGGTGGCGGTGGGGAGGCCCGAGGCAGTGACACGGGGTAGGG

             AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle
      4081   AACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCCTTTTTACGGCAAGGCTATC
             TTGTAGCTCCTCCAACGAGACAGGTGGTGGCCTCTCTAGGGAAAAATGCCGTTCCGATAG

             ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysLysCys
      4141   CCCCTCGAAGTAATCAAGGGGGGGAGACATCTCATCTTCTGTCATTCAAAGAAGAAGTGC
             GGGGAGCTTCATTAGTTCCCCCCCTCTGTAGAGTAGAAGACAGTAAGTTTCTTCTTCACG

             AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly
      4201   GACGAACTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGCCGTGGCCTACTACCGCGGT
             CTGCTTGAGCGGCGTTTCGACCAGCGTAACCCGTAGTTACGGCACCGGATGATGGCGCCA

             LeuAspValSerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu
      4261   CTTGACGTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGTCGTGGCAACCGATGCCCTC
             GAACTGCACAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAGCACCGTTGGCTACGGGAG

             MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
      4321   ATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTCACCCAG
             TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTC

             ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
      4381   ACAGTCGATTTCAGCCTTGACCCTACCTTCACCATTGAGACAATCACGCTCCCCCAGGAT
             TGTCAGCTAAAGTCGGAACTGGGATGGAAGTGGTAACTCTGTTAGTGCGAGGGGGTCCTA

             AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
      4441   GCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGGAAGCCAGGCATCTACAGA
             CGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCCTTCGGTCCGTAGATGTCT

             PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys
      4501   TTTGTGGCACCGGGGGAGCGCCCCTCCGGCATGTTCGACTCGTCCGTCCTCTGTGAGTGC
             AAACACCGTGGCCCCCTCGCGGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCACG

             TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg
      4561   TATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCCGCCGAGACTACAGTTAGGCTACGA
             ATACTGCGTCCGACACGAACCATACTCGAGTGCGGGCGGCTCTGATGTCAATCCGATGCT

             AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly
      4621   GCGTACATGAACACCCCGGGGCTTCCCGTGTGCCAGGACCATCTTGAATTTTGGGAGGGC
             CGCATGTACTTGTGGGGCCCCGAAGGGCACACGGTCCTGGTAGAACTTAAAACCCTCCCG

             ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly
      4681   GTCTTTACAGGCCTCACTCATATAGATGCCCACTTTCTATCCCAGACAAAGCAGAGTGGG
             CAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTTCGTCTCACCC

             GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro
      4741   GAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCT
             CTCTTGGAAGGAATGGACCATCGCATGGTTCGGTGGCACACGCGATCCCGAGTTCGGGGA

             ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly
      4801   CCCCCATCGTGGGACCAGATGTGGAAGTGTTTGATTCGCCTCAAGCCCACCCTCCATGGG
             GGGGGTAGCACCCTGGTCTACACCTTCACAAACTAAGCGGAGTTCGGGTGGGAGGTACCC

             ProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro
      4861   CCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGAAATCACCCTGACGCACCCA
             GGTTGTGGGGACGATATGTCTGACCCGCGACAAGTCTTACTTTAGTGGGACTGCGTGGGT
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FIGURE 72-6

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           ValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValValThrSerThrTrp
     4921  GTCACCAAATACATCATGACATGCATGTCGGCCGACCTGGAGGTCGTCACGAGCACCTGG
           CAGTGGTTTATGTAGTACTGTACGTACAGCCGGCTGGACCTCCAGCAGTGCTCGTGGACC

           ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysVal
     4981  GTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTGCCTGTCAACAGGCTGCGTG
           CACGAGCAACCGCCGCAGGACCGACGAAACCGGCGCATAACGGACAGTTGTCCGACGCAC

           ValIleValGlyArgValValLeuSerGlyLysProAlaIleIleProAspArgGluVal
     5041  GTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAGTC
           CAGTATCACCCGTCCCAGCAGAACAGGCCCTTCGGCCGTTAGTATGGACTGTCCCTTCAG

           LeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGln
     5101  CTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCACTTACCGTACATCGAGCAA
           GAGATGGCTCTCAAGCTACTCTACCTTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTT

           GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSer
     5161  GGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCTCCTGCAGACCGCGTCC
           CCCTACTACGAGCGGCTCGTCAAGTTCGTCTTCCGGGAGCCGGAGGACGTCTGGCGCAGG

           ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe
     5221  CGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTGGCAAAAACTCGAGACCTTC
           GCAGTCCGTCTCCAATAGCGGGGACGACAGGTCTGGTTGACCGTTTTTGAGCTCTGGAAG

           TrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr
     5281  TGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATACTTGGCGGGCTTGTCAACG
           ACCCGCTTCGTATACACCTTGAAGTAGTCACCCTATGTTATGAACCGCCCGAACAGTTGC

           LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro
     5341  CTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACAGCTGCTGTCACCAGCCCA
           GACGGACCATTGGGGCGGTAACGAAGTAACTACCGAAAATGTCGACGACAGTGGTCGGGT

           LeuThrThrSerGlnThrLeuLeuPheAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu
     5401  CTAACCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGGGTGGGTGGCTGCCCAGCTC
           GATTGGTGATCGGTTTGGGAGGAGAAGTTGTATAACCCCCCCACCCACCGACGGGTCGAG

           AlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaAlaIleGly
     5461  GCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTTAGCTGGCGCCGCCATCGGC
           CGGCGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAATCGACCGCGGCGGTAGCCG

           SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla
     5521  AGTGTTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGGGTATGGCGCGGGCGTGGCG
           TCACAACCTGACCCCTTCCAGGAGTATCTGTAGGAACGTCCCATACCGCGCCCGCACCGC

           GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal
     5581  GGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCCTCCACGGAGGACCTGGTC
           CCTCGAGAACACCGTAAGTTCTAGTACTCGCCACTCCAGGGGAGGTGCCTCCTGGACCAG

           AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla
     5641  AATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGTCGGCGTGGTCTGTGCAGCA
           TTAGATGACGGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGCACCAGACACGTCGT

           IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle
     5701  ATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCAGTGGATGAACCGGCTGATA
           TATGACGCGGCCGTGCAACCGGGCCCGCTCCCCCGTCACGTCACCTACTTGGCCGACTAT

           AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla
     5761  GCCTTCGCCTCCCGGGGGGAACCATGTTTCCCCCACGCACTACGTGCCGGAGAGCGATGCA
           CGGAAGCGGAGGGCCCCCTTGGTACAAAGGGGGTGCGTGATGCACGGCCTCTCGCTACGT

           AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu
     5821  GCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACCCAGCTCCTGAGGCGACTG
           CGACGGGCGCAGTGACGGTATGAGTCGTCGGAGTGACATTGGGTCGAGGACTCCGCTGAC

           HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle
     5881  CACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGGTTCCTGGCTAAGGGACATC
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FIGURE 72-7

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             GTGGTCACCTATTCGAGCCTCACATGGTGAGGTACGAGGCCAAGGACCGATTCCCTGTAG

             TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet
      5941   TGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTGGCTAAAAGCTAAGCTCATG
             ACCCTGACCTATACGCTCCACAACTCGCTGAAATTCTGGACCGATTTTCGATTCGAGTAC

             ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg
      6001   CCACAGCTGCCTGGATCCCCTTTGTGTCCTGCCAGCGCGGGTATAAGGGGGTCTGGCGA
             GGTGTCGACGGACCCTAGGGGAAACACAGGACGGTCGCGCCCATATTCCCCCAGACCGCT

             ValAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys
      6061   GTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGAGATCACTGGACATGTCAAA
             CACCTGCCGTAGTACGTGTGAGCGACGGTGACACCTCGACTCTAGTGACCTGTACAGTTT

             AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe
      6121   AACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTC
             TTGCCCTGCTACTCCTAGCAGCCAGGATCCTGGACGTCCTTGTACACCTCACCCTGGAAG

             ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe
      6181   CCCATTAATGCCTACACCACGGGCCCCTGTACCCCCCTTCCTGCGCCGAACTACACGTTC
             GGGTAATTACGGATGTGGTGCCCGGGGACATGGGGGGAAGGACGCGGCTTGATGTGCAAG

             AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis
      6241   GCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGGACTTCCAC
             CGCGATACCTCCCACAGACGTCTCCTTATACACCTCTATTCCGTCCACCCCCTGAAGGTG

             TyrValThrGlyMetThrThrAspAsnLeuLysCysProCysGlnValProSerProGlu
      6301   TACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTGCCAGGTCCCATCGCCCGAA
             ATGCACTGCCCATACTGATGACTGTTAGAGTTTACGGGCACGGTCCAGGGTAGCGGGCTT

             PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu
      6361   TTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGCGCCCCCCTGCAAGCCCTTG
             AAAAAGTGTCTTAACCTGCCCCACGCGGATGTATCCAAACGCGGGGGGACGTTCGGGAAC

             LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu
      6421   CTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATACCCGGTAGGGTCGCAATTA
             GACGCCCTCCTCCATAGTAAGTCTCATCCTGAGGTGCTTATGGGCCATCCCAGCGTTAAT

             ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis
      6481   CCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCATGCTCACTGATCCCTCCCAT
             GGAACGCTCGGGCTTGGCCTGCACCGGCACAACTGCAGGTACGAGTGACTAGGGAGGGTA

             IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer
      6541   ATAACAGCAGAGGCGGCCGGGCGAAGGTTGGCGAGGGGATCACCCCCCTCTGTGGCCAGC
             TATTGTCGTCTCCGCCGGCCCGCTTCCAACCGCTCCCCTAGTGGGGGGAGACACCGGTCG

             SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp
      6601   TCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAACTTGCACCGCTAACCATGAC
             AGGAGCCGATCGGTCGATAGGCGAGGTAGAGAGTTCCGTTGAACGTGGCGATTGGTACTG

             SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn
      6661   TCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAGGCAGGAGATGGGCGGCAAC
             AGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGTCCTCTACCCGCCGTTG

             IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal
      6721   ATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGACTCCTTCGATCCGCTTGTG
             TAGTGGTCCCAACTCAGTCTTTTGTTTCACCACTAAGACCTGAGGAAGCTAGGCGAACAC

             AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg
      6781   GCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAATCCTGCGGAAGTCTCGGAGA
             CGCCTCCTCCTGCTCGCCCTCTAGAGGCATGGGCGTCTTTAGGACGCCTTCAGAGCCTCT

             PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr
      6841   TTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAACCCCCCGCTAGTGGAGACG
             AAGCGGGTCCGGGACGGGCAAACCCGCGCCGGCCTGATATTGGGGGGCGATCACCTCTGC
```

FIGURE 72-8

```
          TrpLysLysProAspTyrGluProProValValHisGlyCysProLeuProProProLys
 6901     TGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTGTCCGCTTCCACCTCCAAAG
          ACCTTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGCGAAGGTGGAGGTTTC

          SerProProValProProProArgLysLysArgThrValValLeuThrGluSerThrLeu
 6961     TCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGTCCTCACTGAATCAACCCTA
          AGGGGAGGACACGGAGGCGGAGCCTTCTTCGCCTGCCACCAGGAGTGACTTAGTTGGGAT

          SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle
 7021     TCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAGCTCCTCAACTTCCGGCATT
          AGATGACGGAACCGGCTCGAGCGGTGGTCTTCGAAACCGTCGAGGAGTTGAAGGCCGTAA

          ThrGlyAspAsnThrThrThrSerSerGluProAlaProSerGlyCysProProAspSer
 7081     ACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCCTTCTGGCTGCCCCCCCGACTCC
          TGCCCGCTGTTATGCTGTTGTAGGAGACTCGGGCGGGGAAGACCGACGGGGGGGCTGAGG

          AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu
 7141     GACGCTGAGTCCTATTCCTCCATGCCCCCCCTGGAGGGGGAGCCTGGGGATCCGGATCTT -
          CTGCGACTCAGGATAAGGAGGTACGGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAA

          SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys
 7201     AGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGCGGAGGATGTCGTGTGCTGC
          TCGCTGCCCAGTACCAGTTGCCAGTCATCACTCCGGTTGCGCCTCCTACAGCACACGACG

          SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGluGlnLys
 7261     TCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGCGCCGCGGAAGAACAGAAA
          AGTTACAGAATGAGAACCTGTCCGCGTGAGCAGTGGGGCACGCGGCGCCTTCTTGTCTTT

          LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThr
 7321     CTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCACAATTTGGTGTATTCCACC
          GACGGGTAGTTACGTGATTCGTTGAGCAACGATGCAGTGGTGTTAAACCACATAAGGTGG

          ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu
 7381     ACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATTTGACAGACTGCAAGTTCTG
          TGGAGTGCGTCACGAACGGTTTCCGTCTTCTTTCAGTGTAAACTGTCTGACGTTCAAGAC

          AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSerLysValLysAla
 7441     GACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGCGGCGTCAAAAGTGAAGGCT
          CTGTCGGTAATGGTCCTGCATGAGTTCCTCCAATTTCGTCGCCGCAGTTTTCACTTCCGA

          AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys
 7501     AACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCCACACTCAGCCAAATCCAAG
          TTGAACGATAGGCATCTCCTTCGAACGTCGGACTGCGGGGGTGTGAGTCGGTTTAGGTTC

          PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn
 7561     TTTGGTTATGGGGCAAAAGACGTCCGTTGCCATGCCAGAAAGGCCGTAACCCACATCAAC
          AAACCAATACCCCGTTTTCTGCAGGCAACGGTACGGTCTTTCCGGCATTGGGTGTAGTTG

          SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla
 7621     TCCGTGTGGAAAGACCTTCTGGAAGACAATGTAACACCAATAGACACTACCATCATGGCT
          AGGCACACCTTTCTGGAAGACCTTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGA

          LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle
 7681     AAGAACGAGGTTTTCTGCGTTCAGCCTGAGAAGGGGGGTCGTAAGCCAGCTCGTCTCATC
          TTCTTGCTCCAAAAGACGCAAGTCGGACTCTTCCCCCCAGCATTCGGTCGAGCAGAGTAG

          ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr
 7741     GTGTTCCCCGATCTGGGCGTGCGCGTGTGCGAAAAGATGGCTTTGTACGACGTGGTTACA
          CACAAGGGGCTAGACCCGCACGCGCACACGCTTTTCTACCGAAACATGCTGCACCAATGT

          LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg
 7801     AAGCTCCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCAATACTCACCAGGACAGCGG
          TTCGAGGGGAACCGGCACTACCCTTCGAGGATGCCTAAGGTTATGAGTGGTCCTGTCGCC

          ValGluPheLeuValGlnAlaTrpLysSerLysLysThrProMetGlyPheSerTyrAsp
 7861     GTTGAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCCAATGGGGTTCTCGTATGAT
```

FIGURE 72-9

```
              CAACTTAAGGAGCACGTTCGCACCTTCAGGTTCTTTTGGGGTTACCCCAAGAGCATACTA

              ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr
       7921   ACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCGTACGGAGGAGGCAATCTAC
              TGGGCGACGAAACTGAGGTGTCAGTGACTCTCGCTGTAGGCATGCCTCCTCCGTTAGATG

              GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu
       7981   CAATGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAAGTCCCTCACCGAGAGGCTT
              GTTACAACACTGGAGCTGGGGGGTTCGGGCGCACCGGTAGTTCAGGGAGTGGCTCTCCGAA

              TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg
       8041   TATGTTGGGGGGCCCTCTTACCAATTCAAGGGGGGAGAACTGCGGCTATCGCAGGTGCCGC
              ATACAACCCCCGGGAGAATGGTTAAGTTCCCCCCTCTTGACGCCGATAGCGTCCACGGCG

              AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg
       8101   GCGAGCGGCGTACTGACAACTAGCTGTGGTAACACCCTCACTTGCTACATCAAGGCCCGG
              CGCTCGCCGCATGACTGTTGATCGACACCATTGTGGGAGTGAACGATGTAGTTCCGGGCC

              AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu
       8161   GCAGCCTGTCGAGCCGCAGGGCTCCAGGACTGCACCATGCTCGTGTGTGGCGACGACTTA
              CGTCGGACAGCTCGGCGTCCCGAGGTCCTGACGTGGTACGAGCACACACCGCTGCTGAAT

              ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr
       8221   GTCGTTATCTGTGAAAGCGCGGGGGGTCCAGGAGGACGCGGCGAGCCTGAGAGCCTTCACG
              CAGCAATAGACACTTTCGCGCCCCCAGGTCCTCCTGCGCCGCTCGGACTCTCGGAAGTGC

              GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu
       8281   GAGGCTATGACCAGGTACTCCGCCCCCCCTGGGGACCCCCCACAACCAGAATACGACTTG
              CTCCGATACTGGTCCATGAGGCGGGGGGGACCCCTGGGGGGTGTTGGTCTTATGCTGAAC

              GluLeuIleThrSerCysSerSerAsnValSerValAlaHisAspGlyAlaGlyLysArg
       8341   GAGCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCACGACGGCGCTGGAAAGAGG
              CTCGAGTATTGTAGTACGAGGAGGTTGCACAGTCAGCGGGTGCTGCCGCGACCTTTCTCC

              ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla
       8401   GTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAGAGCTGCGTGGGAGACAGCA
              CAGATGATGGAGTGGGCACTGGGATGTTGGGGGGAGCGCTCTCGACGCACCCTCTGTCGT

              ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp
       8461   AGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCATGTTTGCCCCCACACTGTGG
              TCTGTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAAACGGGGGTGTGACACC

              AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu
       8521   GCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTATAGCCAGGGACCAGCTTGAA
              CGCTCCTACTATGACTACTGGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCGAACTT

              GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro
       8581   CAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGAACCACTTGATCTACCT
              GTCCGGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTATCTTGGTGAACTAGATGGA

              ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly
       8641   CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACTCCACAGTTACTCTCCAGGT
              GGTTAGTAAGTTTCTGAGGTACCGGAGTCGCGTAAAAGTGAGGTGTCAATGAGAGGTCCA

              GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp
       8701   GAAATTAATAGGGTGGCCGCATGCCTCAGAAAACTTGGGGTACCGCCCTTGCGAGCTTGG
              CTTTAATTATCCCACCGGCGTACGGAGTCTTTTGAACCCCATGGCGGGAACGCTCGAACC

              ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIle
       8761   AGACACCGGGCCCGGAGCGTCCGCGCTAGGCTTCTGGCCAGAGGAGGCAGGGCTGCCATA
              TCTGTGGCCCGGGCCTCGCAGGCGCGATCCGAAGACCGGTCTCCTCCGTCCCGACGGTAT

              CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLysLeuThrProIleAla
       8821   TGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCTCAAACTCACTCCAATAGCG
              ACACCGTTCATGGAGAAGTTGACCCGTCATTCTTGTTTCGAGTTTGAGTGAGGTTATCGC
```

FIGURE 72-10

```
      AlaAlaGlyGlnLeuAspLeuSerGlyTrpPheThrAlaGlyTyrSerGlyGlyAspIle
8881  GCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTCACGGCTGGCTACAGCGGGGGAGACATT
      CGGCGACCGGTCGACCTGAACAGGCCGACCAAGTGCCGACCGATGTCGCCCCCTCTGTAA

      TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCys
8941  TATCACAGCGTGTCTCATGCCCGGCCCCGCTGGATCTGGTTTTGCCC
      ATAGTGTCGCACAGAGTACGGGCCGGGGCGACCTAGACCAAAACGGG
```

5'   3'
CCGGGCGAGGGGGCAGTGCAGTGCATGAACCGCCTGATAGCCTTCGCCTCCCGGCGGAAC
    CGCTCCCCGTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTG
3'   5'

5'   3'
CATGTTTCCCCCTAATGAG
GTACAAAGGGGGATTACTCAGC
3'   5'

FIGURE 73

Argument Map in DNA Strand ssc31
from the 'standard restriction' file.
Translation shown at frame 1.

Sequence too long or too many sites for condensed map.

```
        GluPheGlySerValIleProThrSerGlyAspValValValValAlaThrAspAlaLeu
     1  GAATTCGGGTCCGTCATCCCGACCAGCGGCGATGTTGTCGTCGTGGCAACCGATGCCCTC
        CTTAAGCCCAGGCAGTAGGGCTGGTCGCCGCTACAACAGCAGCACCGTTGGCTACGGGAG

        1 ECOR1, 7 NLA1V, 8 AVA2 SAU96, 15 FOK1, 24 NSPB11, 26 FNU4H
        1, 52 SFAN1, 57 MNL1, 60 NLA111,


        MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
    61  ATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTGCAATACGTGTGTCACCCAG
        TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTC

        65 HPA11, 74 HPA11, 83 TAQ1, 85 HINF1, 90 HPH, 106 AFL111 MA
        E2, 112 MAE3, 113 HPH,


        ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
   121  ACAGTCGATTTCAGCCTTGACCCTACCTTCACCATTGAGACAATCACGCTCCCCCAAGAT
        TGTCAGCTAAAGTCGGAACTGGGATGGAAGTGGTAACTCTGTTAGTGCGAGGGGGTTCTA

        125 TAQ1, 149 HPH, 178 SFAN1,


        AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
   181  GCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGGGAAGCCAGGCATCTACAGA
        CGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGACCGTCCCCCTTCGGTCCGTAGATGTCT

        198 MAE2, 226 ECOR11 SCRF1, 230 SFAN1,


        PheValAlaProGlyGluArgProProAlaCysSerThrArgProSerSerValSerAla
   241  TTTGTGGCACCGGGGGGAGCGCCCTCCGGCATGTTCGACTCGTCCGTCCTCTGTGAGTGCC
        AAACACCGTGGCCCCCTCGCGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCACGG

        246 BAN1 NLA1V, 250 HPA11 NCI1 SCRF1, 257 HAE11, 258 HHA1, 2
        62 MNL1, 265 HPA11, 268 NSPC1, 269 NLA111, 274 TAQ1, 276 HIN
        F1, 287 MNL1, 296 BSP1286,


        ArgIle
   301  CGAATTC
        GCTTAAG

        302 ECOR1,


   361
```

FIGURE 74

Translation of DNA p131jh

```
--------------------Overlap with 6k--------------------
     TyrHisSerValSerHisAlaArgProArgTrpIleTrpPheCysLeuLeuLeuLeuAla
  1  TTATCACAGCGGTGTCTCATGCCCGGCCCCGGCTGGATCTGGTTTTGCCTACTCCTGCTTGC
     AATAGTGTCGCACAGAGTACGGGCCGGGGCCGACCTAGACCAAAACGGATGAGGACGAACG

     AlaGlyValGlyIleTyrLeuLeuProAsnArgOP
 61  TGCAGGGGGTAGGCATCTACCTCCTCCCAACCGATGAAGGTTGGGGTAAACACTCCGGCC
     ACGTCCCCATCCGTAGATGGAGGAGGGGTTGGCTACTTCCAACCCCATTTGTGAGGCCGG

121  T
     A
```

FIGURE 75

FIGURE 76

FIGURE 77

Flow Chart for Construction of Expression Vector p Scd #9
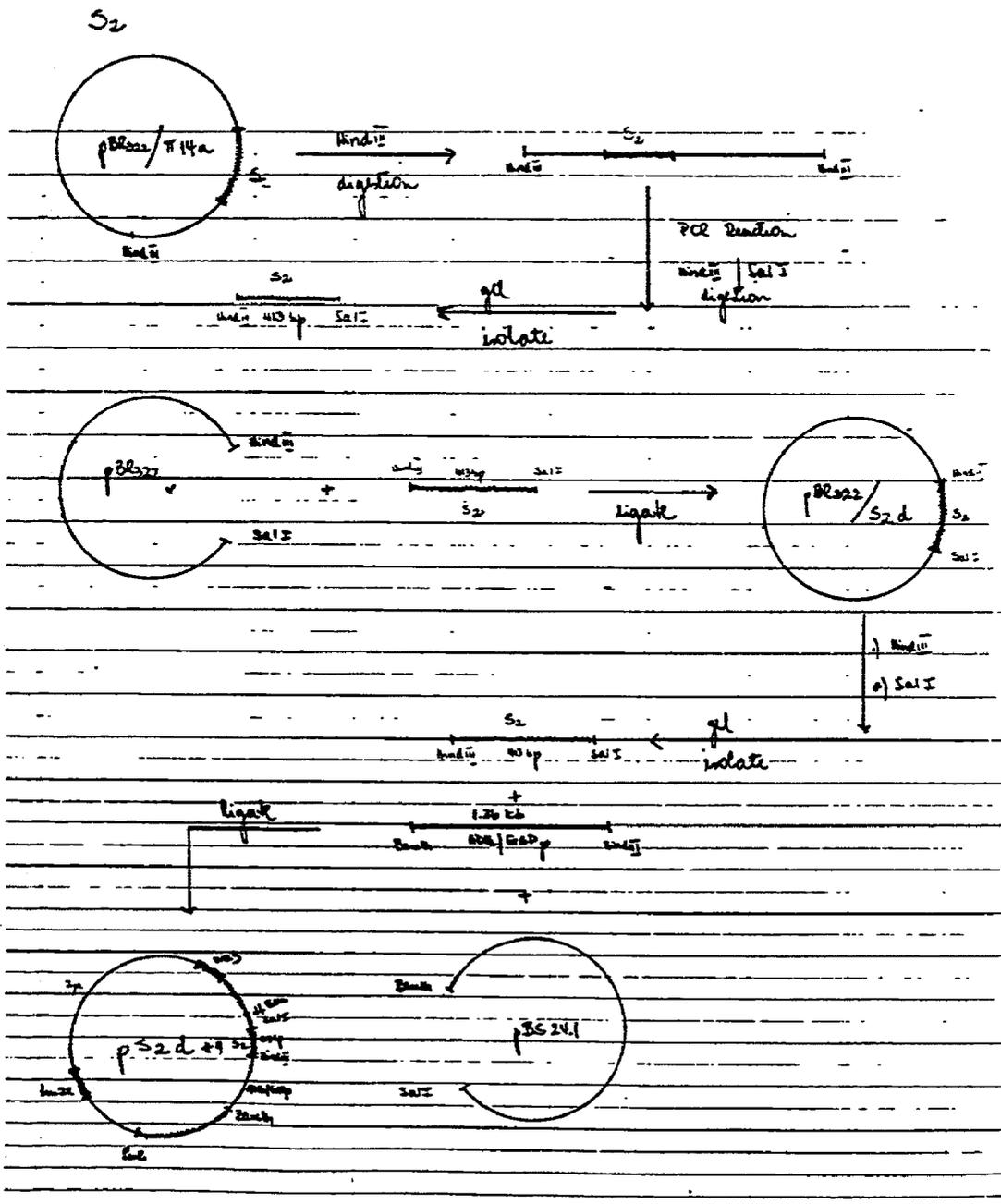
FIGURE 78

Flow Chart For Construction of the
Expression Vector pNS11d;13
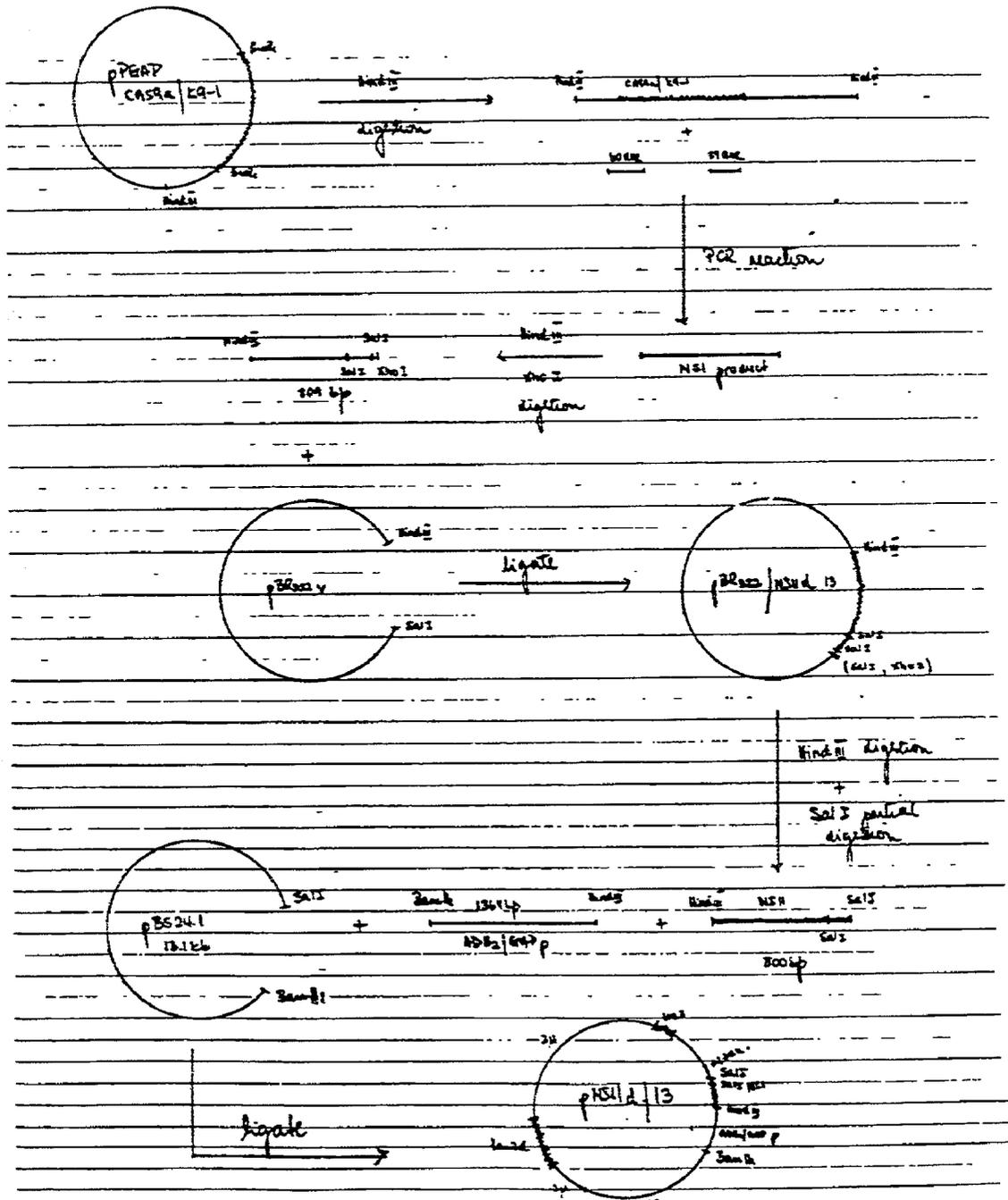
FIGURE 79-1

```
          AlaValAspPheIleProValGluAsnLeuGluThrThrMetArgSerProValPheThr
     2    GCGGTGGACTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCACG
          CGCCACCTGAAATAGGGACACCTCTTGGATCTCTGTTGGTACTCCAGGGGCCACAAGTGC
```

29 MAE1, 40 NLA111, 43 MNL1, 45 AVA2 NLA1V SAU96, 49 NCI1 SC
RF1, 50 HPA11,

```
          AspAsnSerSerProProValValProGlnSerPheGlnValAlaHisLeuHisAlaPro
    62    GATAACTCCTCTCCACCAGTAGTGCCCCAGAGCTTCCAGGTGGCTCACCTCCATGCTCCC
          CTATTGAGGAGAGGTGGTCATCACGGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGGG
```

69 MNL1, 83 BSP1286, 92 ALU1, 97 ECOR11 SCRF1, 106 HPH, 109
MNL1, 113 NLA111,

```
          ThrGlySerGlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysVal
   122    ACAGGCAGCGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGTG
          TGTCCGTCGCCGTTTTCGTGGTTCCAGGGCCGACGTATACGTCGAGTCCCGATATTCCAC
```

126 BBV FNU4H1, 127 NSPB11, 129 FNU4H1, 145 AVA2 NLA1V SAU96
, 148 NCI1 SCRF1, 149 HPA11, 152 BBV FNU4H1, 156 NDE1, 161 B
BV FNU4H1, 163 ALU1, 165 DDE1,

```
          LeuValLeuAsnProSerValAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAla
   182    CTAGTACTCAACCCCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCAAGGCT
          GATCATGAGTTGGGGAGACAACGACGTTGTGACCCGAAACCACGAATGTACAGGTTCCGA
```

182 MAE1, 184 SCA1, 185 RSA1, 195 MNL1, 203 BBV FNU4H1, 228
AFL111 NSPC1, 229 NLA111,

```
          HisGlyIleAspProAsnIleArgThrGlyValArgThrIleThrThrGlySerProIle
   242    CATGGGATCGATCCTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCCCCATC
          GTACCCTAGCTAGGATTGTAGTCCTGGCCCCACTCTTGTTAATGGTGACCGTCGGGGTAG
```

242 NLA111, 246 BIN1, 247 MBO1 SAU3A, 248 CLA1, 249 TAQ1, 25
1 BIN1 MBO1 SAU3A, 264 AVA2 SAU96, 267 HPA11 NCI1 SCRF1, 271
HPH, 291 BBV FNU4H1,

```
          ThrTyrSerThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAsp
   302    ACGTACTCCACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGCGCTTATGAC
          TGCATGAGGTGGATGCCGTTCAAGGAACGGCTGCCGCCCACGAGCCCCCCGCGAATACTG
```

302 MAE2, 304 RSA1, 3) BSP1286 HGIA, 343 AVA1, 350 HAE11, 3
51 HHA1,

```
          IleIleIleCysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThr
   362    ATAATAATTTGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATTGGCACT
          TATTATTAAACACTGCTCACGGTGAGGTGCCTACGGTGTAGGTAGAACCCGTAACCGTGA
```

372 MAE3, 391 FOK1, 392 SFAN1, 399 FOK1,

```
          ValLeuAspGlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrPro
   422    GTCCTTGACCAAGCAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCACCCCT
          CAGGAACTGGTTCGTCTCTGACGCCCCCGCTCTGACCAACACGAGCGGTGGCGGTGGGGA
```

431 TTHIII2, 435 ALWN1, 461 BSP1286 HGIA, 479 MNL1,

FIGURE 79-2

```
       ProGlySerValThrValProHisProAsnIleGluGluValAlaLeuSerThrThrGly
  482  CCGGGCTCCGTCACTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCACCGGA
       GGCCCGAGGCAGTGACACGGGGTAGGGTTGTAGCTCCTCCAACGAGACAGGTGGTGGCCT
```

482 HPA11 NCI1 SCRF1, 484 BAN11 BSP1286, 485 NLA1V, 491 MAE3
, 497 BSP1286, 503 FOK1, 513 TAQ1, 515 MNL1, 518 MNL1, 537 H
PA11,

```
       GluIleProPheTyrGlyLysAlaIleProLeuGluValIleLysGlyGlyArgHisLeu
  542  GAGATCCCTTTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGGAGACATCTC
       CTCTAGGGAAAAATGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCCCTCTGTAGAG
```

543 XHO2, 544 BIN1 MBO1 SAU3A, 571 MNL1, 573 TAQ1,

```
       IlePheCysHisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGly
  602  ATCTTCTGTCATTCAAAGAAGAAGTGCGACGAACTCGCCGCAAAGCTGGTCGCATTGGGC
       TAGAAGACAGTAAGTTTCTTCTTCACGCTGCTTGAGCGGCGTTTCGACCAGCGTAACCCG.
```

603 MBO11, 619 MBO11, 638 FNU4H1, 645 ALU1, 660 SFAN1,

```
       IleAsnAlaValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAsp
  662  ATCAATGCCGTGGCCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCGGCGAT
       TAGTTACGGCACCGGATGATGGCGCCAGAACTGCACAGGCAGTAGGGCTGGTCGCCGCTA
```

672 HAE1, 673 HAE111, 682 NSPB11 SAC2, 683 THA1, 693 AFL111
MAE2, 703 FOK1, 712 NSPB11, 714 FNU4H1,

```
       ValValValValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerVal
  722  GTTGTCGTCGTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACTCGGTG
       CAACAGCAGCACCGTTGGCTACGGGAGTACTGGCCGATATGGCCGCTGAAGCTGAGCCAC
```

740 SFAN1, 745 MNL1, 748 NLA111, 753 HPA11, 762 HPA11, 771 T
AQ1, 773 HINF1, 778 HPH,

```
       IleAspCysAsnThrCysValThrGlnThrValAspPheSerLeuAspProThrPheThr
  782  ATAGACTGCAATACGTGTGTCACCCAGACAGTCGATTTCAGCCTTGACCCTACCTTCACC
       TATCTGACGTTATGCACACAGTGGGTCTGTCAGCTAAAGTCGGAACTGGGATGGAAGTGG
```

794 AFL111 MAE2, 800 MAE3, 801 HPH, 813 TAQ1, 837 HPH,

```
       IleGluThrIleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArgThr
  842  ATTGAGACAATCACGCTCCCCCAAGATGCTGTCTCCCGCACTCAACGTCGGGGCAGGACT
       TAACTCTGTTAGTGCGAGGGGGTTCTACGACAGAGGGCGTGAGTTGCAGCCCCGTCCTGA
```

866 SFAN1, 886 MAE2,

```
       GlyArgGlyLysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGlyMet
  902  GGCAGGGGGAAGCCAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCGGCATG
       CCGTCCCCCTTCGGTCCGTAGATGTCTAAACACCGTGGCCCCCTCGCGGGGAGGCCGTAC
```

914 ECOR11 SCRF1, 918 SFAN1, 934 BAN1 NLA1V, 938 HPA11 NCI1
SCRF1, 945 HAE11, 946 HHA1, 948 BGL1, 951 MNL1, 954 HPA11, 9
57 NSPC1, 958 NLA111,

```
       PheAspSerSerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeuThr
  962  TTCGACTCGTCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTCACG
       AAGCTGAGCAGGCAGGAGACACTCACGATACTGCGTCCGACACGAACCATACTCGAGTGC
```

963 TAQ1, 965 HINF1, 976 MNL1, 992 HGA1, 1003 TTHIII2, 1013
BAN11 BSP1286 HGIA SAC1, 1014 ALU1,

```
       ProAlaGluThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProValCys
 1022  CCCGCCGAGACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGCTTCCCGTGTGC
       GGGCGGCTCTGATGTCAATCCGATGCTCGCATGTACTTGTGGGGCCCCGAAGGGCACACG
```

FIGURE 79-3

```
     1051 RSAl, 1054 NLAlll, 1063 AVAl NCIl SCRFl SMAl, 1064 HPAl
     1 NCIl SCRFl, 1081 ECORll SCRFl,

        GlnAspHisLeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAlaHis
1082    CAGGACCATCTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCCCAC
        GTCCTGGTAGAACTTAAAACCCTCCCGCAGAAATGTCCGGAGTGAGTATATCTACGGGTG

        1084 AVA2 SAU96, 1103 MNLl, 1106 AHAll, 1107 HGAl, 1117 HAEl
         STUl, 1118 HAElll, 1120 MNLl, 1133 SFANl,

        PheLeuSerGlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGlnAla
1142    TTTCTATCCCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAAGCC
        AAAGATAGGGTCTGTTTCGTCTCACCCCTCTTGGAAGGAATGGACCATCGCATGGTTCGG

        1183 ECORll SCRFl, 1192 RSAl, 1201 DRA3,

        ThrValCysAlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeu
1202    ACCGTGTGCGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGTTTG-
        TGGCACACGCGATCCCGAGTTCGGGGAGGGGGTAGCACCCTGGTCTACACCTTCACAAAC

        1209 HHAl, 1212 MAEl, 1215 BANll BSP1286, 1226 MNLl, 1239 NL
        AlV, 1240 AVA2 SAU96, 1256 TTHIII2, 1261 HINFl,

        IleArgLeuLysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaVal
1262    ATTCGCCTCAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTT
        TAAGCGGAGTTCGGGTGGGAGGTACCCGGTTGTGGGGACGATATGTCTGACCCGCGACAA

        1267 MNLl, 1279 MNLl, 1282 NCOl, 1283 NLAlll, 1286 SAU96, 12
        87 HAElll, 1313 HAEll, 1314 HHAl,

        GlnAsnGluIleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSerAla
1322    CAGAATGAAATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCGGCC
        GTCTTACTTTAGTGGGACTGCGTGGGTCAGTGGTTTATGTAGTACTGTACGTACAGCCGG

        1332 HPH, 1339 HGAl, 1349 MAE3, 1350 HPH, 1363 NLAlll, 1367
        NSPCl, 1368 NLAlll, 1369 AVA3 NSIl, 1371 NSPCl, 1372 NLAlll,
         1377 CFRl XMA3, 1378 HAElll,

        AspLeuGluValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeuAla
1382    GACCTGGAGGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCC
        CTGGACCTCCAGCAGTGCTCGTGGACCCACGAGCAACCGCCGCAGGACCGACGAAACCGG

        1384 ECORll SCRFl, 1385 GSUl, 1388 MNLl, 1394 MAE3, 1399 BSP
        1286 HGIA, 1404 ECORll SCRFl, 1409 BSP1286 HGIA, 1419 FNU4Hl
        , 1421 AHAll, 1422 HGAl, 1426 ECORll SCRFl, 1430 BBV FNU4Hl,
         1437 CFRl, 1438 HAElll, 1439 FNU4Hl, 1441 THAl,

        AlaTyrCysLeuSerThrGlyCysValVal IleValGlyArgValValLeuSerGlyLys
1442    GCGTATTGCCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGCTCGTCTTGTCCGGGAAG
        CGCATAACGGACAGTTGTCCGACGCACCAGTATCACCCGTCCCAGCAGAACAGGCCCTTC

        1453 HINCll, 1461 BBV FNU4Hl, 1494 HPAll NCIl SCRFl, 1501 NA
        El,

        ProAlaIleIleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGluCys
1502    CCGGCAATCATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGC
        GGCCGTTAGTATGGACTGTCCCTTCAGGAGATGGCTCTCAAGCTACTCTACCTTCTCACG

        1502 HPAll, 1528 MNLl, 1542 TAQl, 1553 MBOll, 1558 BSP1286 H
        GIA,

        SerGlnHisLeuProTyrIleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLys
1562    TCTCAGCACTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAG
        AGAGTCGTGAATGGCATGTAGCTCGTTCCCTACTACGAGCGGCTCGTCAAGTTCGTCTTC

     - -
```

FIGURE 79-4

TTHIII2, 1621 HAE111 SAU96,

```
        AlaLeuGlyLeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaValGln
 1622   GCCCTCGGCCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAG
        CGGGAGCCGGAGGACGTCTGGCGCAGGGCAGTCCGTCTCCAATAGCGGGGACGACAGGTC
```

1624 MNL1, 1628 HAE111, 1630 MNL1, 1634 PST1, 1639 TTHIII1,
1642 THA1, 1643 HGA1, 1658 MNL1,

```
        ThrAsnTrpGlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGly
 1682   ACCAACTGGCAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGG
        TGGTTGACCGTTTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTTGAAGTAGTCACCC
```

1697 AVA1 XHO1, 1698 TAQ1, 1718 NDE1,

```
        IleGlnTyrLeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMet
 1742   ATACAATACTTGGCGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATG
        TATGTTATGAACCGCCCGAACAGTTGCGACGGACCATTGGGGCGGTAACGAAGTAACTAC-
```

1762 HINC11, 1768 BBV FNU4H1, 1772 ECOR11 SCRF1, 1775 BSTE2,
 1776 MAE3,

```
        AlaPheThrAlaAlaValThrSerProLeuThrThrSerGlnThrLeuLeuPheAsnIle
 1802   GCTTTTACAGCTGCTGTCACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCAACATA
        CGAAAATGTCGACGACAGTGGTCGGGTGATTGGTGATCGGTTTGGGAGGAGAAGTTGTAT
```

1809 ALWN1 NSPB11 PVU11, 1810 ALU1, 1811 BBV FNU4H1, 1817 MA
E3, 1818 HPH, 1836 MAE1, 1846 MNL1, 1849 MNL1, 1851 MBO11,

```
        LeuGlyGlyTrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGly-
 1862   TTGGGGGGGTGGGTGGCTGCCCAGCTCGCCGCCCCGGTGCCGCTACTGCCTTTGTGGGC
        AACCCCCCCACCCACCGACGGGTCGAGCGGCGGGGGCCACGGCGATGACGGAAACACCCG
```

1877 BBV FNU4H1, 1884 ALU1, 1889 FNU4H1, 1895 NCI1 SCRF1, 18
96 HPA11, 1898 BAN1 NLA1V, 1901 FNU4H1, 1919 HAE11, 1920 HHA
1,

```
        AlaGlyLeuAlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIle
 1922   GCTGGCTTAGCTGGCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAGACATC
        CGACCGAATCGACCGCGGCGGTAGCCGTCACAACCTGACCCCTTCCAGGAGTATCTGTAG
```

1927 DDE1, 1930 ALU1, 1934 AHA11 BAN1 HAE11 NAR1 NLA1V, 1935
 HHA1, 1937 FNU4H1, 1966 AVA2 SAU96, 1969 MNL1, 1978 FOK1,

```
        LeuAlaGlyTyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGly
 1982   CTTGCAGGGTATGGCGCGGGCGTGGCGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGT
        GAACGTCCCATACCGCGCCCGCACCGCCCTCGAGAACACCGTAAGTTCTAGTACTCGCCA
```

1995 HHA1, 1996 TFA1, 2010 BAN11 BSP1286 HGIA SAC1, 2011 ALU
1, 2021 BSM1, 2029 MBO1 SAU3A, 2032 NLA111, 2039 HPH,

```
        GluValProSerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAla
 2042   GAGGTCCCCTCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCC
        CTCCAGGGGAGGTGCCTCCTGGACCAGTTAGATGACGGGCGGTAGGAGAGCGGGCCTCGG
```

2042 MNL1, 2044 AVA2 NLA1V SAU96, 2049 MNL1, 2057 MNL1, 2059
 AVA2 SAU96, 2060 TTHIII1, 2062 ECOR11 SCRF1, 2083 FOK1, 208
6 MNL1, 2093 NCI1 SCRF1, 2094 HPA11, 2096 NLA1V, 2097 BAN11
BSP1286, 2101 MNL1,

```
        LeuValValGlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGly
 2102   CTCGTAGTCGGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCGAGGGG
        GAGCATCAGCCGCACCAGACACGTCGTTATGACGCGGCCGTGCAACCGGGCCCGCTCCCC
```

2123 BBV FNU4H1, 2134 HHA1, 2136 NAE1, 2137 HPA11, 2142 MAE2

FIGURE 79-5

CI1 SCRF1, 2156 MNL1,

AlaValGlnTrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSer
2162 GCAGTGCAGTGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCC
CGTCACGTCACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTTGGTACAAAGGGG

2172 FOK1, 2179 HPA11, 2196 MNL1, 2199 AVA1 NCI1 SCRF1 SMA1,
2200 HPA11 NCI1 SCRF1, 2205 NLA1V, 2210 NLA111,

2222

FIGURE 80

Human 23

```
       GlyPheAlaAspLeuMetGlyTyrIleProLeuValGlyAlaProLeuGlyGlyArgAla
  1    GGCTTCGCCGACCTCATGGGGTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCCGTGCC

       ArgAlaLeuAlaHisGlyValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsn
 61    AGGGCCCTGGCGCACGGCGTCCGGGTTTTGGAAGACGGCGTGAACTATGCAACAGGGAAC
                CG    A

       LeuProGlyCysSerPheSerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValPro
121    CTTCCTGGTTGCTCCTTTTCTATCTTCCTTCTGGCCCTACTCTCTTGCCTGACCGTGCCC
                           GA                      T

       AlaSerAlaTyrGlnValArgAsnSerThrGlyLeuTyrHisValThrAsnAspCysPro
181    GCTTCAGCCTACCAAGTGCGCAACTCTACGGGGCTTTACCATGTCACCAATGATTGCCCT

       AsnSerSerIleValTyrGluAlaAlaAspAlaIleLeuHisAlaProGlyCysValPro
241    AACTCGAGTATTGTGTACGAGGCGGCCGATGCCATCCTGCACGCTCCGGGGTGTGTCCCT
                         T                                   C

       CysValArgGluAspAsnValSerArgCysTrpValAlaValThrProThrValAlaThr
301    TGCGTTCGCGAGGATAACGTCTCGAGATGTTGGGTGGCGGTGACCCCCACGGTGGCCACC
               G                                                  T

       LysAspGlyLysLeuProThrThrGlnLeuArgArgHisIleAspLeuLeuValGlySer
361    AAGGACGGCAAACTCCCCACAACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGC
                                                    C   · A

       AlaThrLeuCysSerAlaLeuTyrValGlyAspLeuCysGlySerIlePheLeuValGly
421    GCCACCCTCTGCTCGGCCCTCTACGTGGGGGACCTTTGCGGGTCCATCTTTCTTGTCGGT
               T

       GlnLeuPheThrPheSerProArgArgHisTrpThrThrGlnAspCysAsnCysSerIle
481    CAACTGTTTACCTTCTCTCCCAGGCGCCACTGGACGACGCAGGACTGCAACTGTTCTATC
                                       C

       TyrProGlyHisIleThrGlyHisArgMetAlaTrpAspMetMetMetAsnTrpSerPro
541    TATCCCGGCCATATAACGGGTCACCGCATGGCATGGGATATGATGATGAACTGGTCCCCT
                                                        G   ·

       ThrAlaAlaLeuValValAlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIle
601    ACGGCGGCATTGGTAGTAGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATC
                 G    AG

       AlaGlyAlaHisTrpGlyValLeuAlaGlyMetAlaTyrPheSerMetValGlyAsnTrp
661    GCTGGTGCTCACTGGGGAGTCCTGGCGGGCATGGCGTATTTCTCCATGGTGGGGAACTGG
                                                  G

       AlaLysValLeuValValLeuLeuLeuPheAlaGlyValAspAlaGluThrHisArgThr
721    GCGAAGGTCCTGGTAGTGCTGCTTCTATTTGCCGGCGTCGACGCGGAAACCCACCGTACC
                                                  G          ·

       GlyGlySerAlaAlaArgSerThrAlaGlyValAlaSerLeuPheThrProGlyAlaArg
781    GGGGGAAGTGCCGCCCGCAGCACGGCTGGAGTTGCTAGTCTCTTCACACCAGGCGCTAGG
       C           T   A

       GlnAsnIleGlnLeuIleAsnThrAsnGlySerTrpHisIleAsnSerThrAlaLeuAsn
841    CAGAACATCCAGCTGATCAACACCAACGGCAGTTGGCACATCAATAGTACGGCCTTGAAC
                                                            AT

       CysAsnAspSerLeuThrThrGlyTrpLeuAlaGlyLeuPheTyrHisHisLysPheAsn
901    TGCAATGACAGCCTTACCACCGGCTGGTTAGCGGGGCTTTTCTATCACCATAAATTCAAC
       A                                        A
```

FIGURE 81

Human 27

```
      GlyPheAlaAspLeuMetGlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAla
  1   GGCTTCGCCGACCTCATGGGGTACATTCCGCTCGTCGGCGCTCCTCTTGGGGGCGCTGCC

      ArgAlaLeuAlaHisGlyValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsn
 61   AGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAAC

      LeuProGlyCysSerPheSerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValPro
121   CTTCCTGGTTGCTCTTTCTCTATCTTCCTTCTGGCTCTGCTCTCTTGCCTGACCGTGCCC

      AlaSerAlaTyrGlnValArgAsnSerSerGlyIleTyrHisValThrAsnAspCysPro
181   GCATCGGCCTACCAAGTACGCAACTCCTCGGGCATTTACCATGTCACCAATGATTGCCCT

      AsnSerSerIleValTyrGluThrAlaAspThrIleLeuHisSerProGlyCysValPro
241   AATTCGAGTATTGTGTACGAGACGGCCGACACCATCCTACACTCTCCGGGGTGTGTCCCT  -
         C           C

      CysValArgGluGlyAsnAlaSerLysCysTrpValProValAlaProThrValAlaThr
301   TGCGTTCGCGAGGGTAACGCCTCGAAATGTTGGGTGCCGGTAGCCCCCACAGTGGCCACT
                  G

      ArgAspGlyAsnLeuProAlaThrGlnLeuArgArgHisIleAspLeuLeuValGlySer
361   AGGGACGGCAACCTCCCCGCAACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGT
                                    G           G

      AlaThrLeuCysSerAlaLeuTyrValGlyAspLeuCysGlySerValPheLeuValGly
421   GCCACCCTTTGCTCGGCCCTCTATGTGGGGGACTTGTGCGGGTCTGTCTTTCTTGTCGGT
         C                                                C

      GlnLeuPheThrPheSerProArgArgHisTrpThrThrGlnAspCysAsnCysSerIle
481   CAACTGTTCACTTTCTCCCCCAGGCGCCACTGGACAACGCAAGATTGCAACTGCTCTATC
                -      A

      TyrProGlyHisIleThrGlyHisArgMetAlaTrpAspMetMetMetAsnTrpSerPro
541   TACCCCGGCCATATAACGGGACACCGCATGGCATGGGATATGATGATGAACTGGTCCCCT

      ThrAlaAlaLeuValMetAlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIle
601   ACAGCAGCGCTGGTAATGGCTCAGCTGCTCAGGATCCCGCAAGCCATCTTGGACATGATC
         G

      AlaGlyAlaHisTrpGlyValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrp
661   GCTGGTGCTCACTGGGGAGTCCTAGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGG

      AlaLysValLeuValValLeuLeuLeuPheAlaGlyValAspAlaThrThrTyrThrThr
721   GCGAAGGTCCTGGTGGTGCTGTTGCTGTTTGCCGGCGTCGATGCGACAACCTATACCACC

      GlyGlyAsnAlaAlaArgThrThrGlnAlaLeuThrSerPhePheSerProGlyAlaLys
781   GGGGGGAATGCTGCCAGGACCACGCAGGCGCTCACCAGTTTTTTCAGCCCAGGCGCCAAG

      GlnAspIleGlnLeuIleAsnThrAsnGlySerTrpHisIleAsnArgThrAlaLeuAsn
841   CAGGATATCCAGCTGATCAACACCAACGGCAGTTGGCACATCAATCGCACGGCCTTGAAC
         G                             T

      CysAsnAlaSerLeuAspThrGlyTrpValAlaGlyLeuPheTyrTyrHisLysPheAsn
901   TGTAATGCGAGCCTCGACACTGGCTGGGTAGCGGGGCTCTTCTATTACCACAAATTCAAC
         T              G

      SerSerGlyCysProGluArgMetAlaSerCysArgProLeuAlaAspPheAspGln
961   TCTTCAGGCTGCCCCGAGAGGATGGCCAGCTGTAGGCCCCTTGCCGATTTCGACCAGG
                                                              C
```

FIGURE 82-1

```
1.  human 27
2.  HCV 1
3.  human 23
```

```
  1 CGGCTTCGCCGACCTCATGGGGTACATtCCGCTCGTCGGCGCtCCTCTTGGgGGCGCTGCCAGGGCCCTGGC
    *********************************** ******* ***********************
  1 CGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGC
    *************************************************** ***************
  1 CGGCTTCGCCGACCTCATGGGGTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCcgTGCCAGGGCCCTGGC


 73 GCATGGCGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTCTAT
    ****************************************************t***********************
 73 GCATGGCGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTCTAT
    *** ************* *************************************** ** *****
 73 GCAcGGCGTCCGGGTTtTGGAAGACGGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCcTTtTCTAT


145 CTTCCTTCTGGCtCTGCTCTCTTGCcTGACcGTGCCCGCaTCGGCCTACCAAGTaCGCAACTCCtCGGGcaT
    *********** *********** **** ******* ************** ********* ****   *
145 CTTCCTTCTGGCCCTGCTCTCTTGCtTGACtGTGCCCGCTTCGGCCTACCAAGTGCGCAACTCCACGGGGCT
    **************** ********* **** ********** *********************** ********
145 CTTCCTTCTGGCCCTaCTCTCTTGCcTGACcGTGCCCGCTTCaGCCTACCAAGTGCGCAACTCtACGGGGCT


217 TTACCAtGTCACCAATGATTGCCCTAAtTCGAGTATTGTGTACGAGaCGGCCGAcaCCATCCTaCACtCTCC
    ***** ********************** ****************** ******   ******* *** ****
217 TTACCAcGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGCGGCCGATGCCATCCTGCACaCTCC
    ****** ************************************************************ ****
217 TTACCAtGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGCGGCCGATGCCATCCTGCACgCTCC


289 GGGGTGtGTCCCTTGCGTTCGcGAGGGtAACGCCTCGAaaTGTTGGGTGcCGgTagCCCCcACaGTGGCCAC
    ****** ************** ***** ********* ******** ** *  **** ** *******
289 GGGGTGcGTCCCTTGCGTTCGtGAGGGcAACGCCTCGAGgTGTTGGGTGGCGaTGACCCCtACGGTGGCCAC
    ****** *************** **** **** ****** ************** ******* ***********
289 GGGGTGtGTCCCTTGCGTTCGcGAGGatAACGtCTCGAGaTGTTGGGTGGCGgTGACCCCcACGGTGGCCAC


361 CAGGGAcGGCAAcCTCCCCGCaACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGtGCCACCCTtTG
    ****** ***** ******* ************************************* ******* **
361 CAGGGAtGGCAAACTCCCCGCgACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGCGCCACCCTCTG
    ** *** *********** * ***********************************************
361 CAaGGAcGGCAAACTCCCCaCaACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGCGCCACCCTCTG


433 cTCGGCCCTCTAtGTGGGGGACttTGTGCGGGTCTGTCTTTCTtGTCGGtCAACTGTTCACtTTCTCcCCAG
    ********** ******** ************************* ********* ***** *****
433 tTCGGCCCTCTACGTGGGGGACCTGTGCGGGTCTGTCTTTCTTGTCGGcCAACTGTTCACCTTCTCTCCCAG
    ************************ ******** ************** ******* ***************
433 cTCGGCCCTCTACGTGGGGGACCTtTGCGGGTCcaTCTTTCTTGTCGGtCAACTGTTtACCTTCTCTCCCAG


505 GCGCCACTGGACaACGCAAGaTTGCAAcTGCTCTATCTAcCCCGGCCATATAACGGGaCACCGCATGGCATG
    ********** ******* ***** ********** ****************** **************
505 GCGCCACTGGACGACGCAAGgTTGCAAtTGCTCTATCTATCCCGGCCATATAACGGGTCACCGCATGGCATG
    **************** * ***** ** ****************************************
505 GCGCCACTGGACGACGCAgGacTGCAAcTGtTCTATCTATCCCGGCCATATAACGGGTCACCGCATGGCATG


577 GGATATGATGATGAACTGGTCCCCTACAgCaGCGcTGGTAATGGCTCAGCTGCTCaGGATCCCgCAAGCCAT
    ******************* * *** ********************** ****** *******
577 GGATATGATGATGAACTGGTCCCCTACGaCGGCGTTGGTAATGGCTCAGCTGCTCcGGATCCACAAGCCAT
```

FIGURE 82-2

```
649 CTTGGACATGATCGCTGGTGCTCACTGGGGAGTCCTaGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTG
    ************************************* ********************************
649 CTTGGACATGATCGCTGGTGCTCACTGGGGAGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTG
    ************************************* ********************************
649 CTTGGACATGATCGCTGGTGCTCACTGGGGAGTCCTGGCGGGCATgGCGTATTTCTCCATGGTGGGGAACTG


721 GGCGAAGGTCCTGGTgGTGCTGtTGCTgTTTGCCGGCGTCGAtGCGacAACCtAtacCACCGGGGGgAaTGC
    ************** ****** **** ************** *** **** * ******** * ***
721 GGCGAAGGTCCTGGTAGTGCTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACgtCACCGGGGGAAGTGC
    ************************** *************************** **************
721 GGCGAAGGTCCTGGTAGTGCTGCTtCTATTTGCCGGCGTCGACGCGGAAACCCACcgtACCGGGGGAAGTGC


793 tGcCaggACcacGcagGcgcTcaccAGttTtttTCagcCCAGGCGCCAAGCAGgAtaTCCAGCTGATCAACAC
    * *  **   *  *  *   **  *  **  ************** * ****************
793 CGgCCaCACtgtGtCTGGAtTTGtTAGcCTCcTCgCACCAGGCGCCAAGCAGAACgTCCAGCTGATCAACAC
    ** ** **    * ***** *** *** *** ** ********** * ****** ******************
793 CGcCCgCAgcacGgCTGGAgTTGcTAGtCTCtTCaCACCAGGCGCtAgGCAGAACaTCCAGCTGATCAACAC


865 CAACGGCAGTTGGCACaTCAATcGCACGGCCttTGAACTGtAATGCgAGCCTCgACACtGGCTGGgTaGCgGG
    ***************** ***** ******* ****** **** ***** **** ****** * ** **
865 CAACGGCAGTTGGCACcTCAATAGCACGGCCcTGAACTGCAATGAtAGCCTCAACACCGGCTGGTTgGCaGG
    ***************** ****** ****** ************* ***** * ************* ** **
865 CAACGGCAGTTGGCACaTCAATAGtACGGCCtTGAACTGCAATGAcAGCCTtAcCACCGGCTGGTTaGCgGG


937 GCTcTTCTATtACCACAAaTTCAACTCTTCAGGCTGcCCcGAGAGGaTgGCCAGCTGtaGgCCCCTTgCCGA
    *** ****** ****** ****************** ** ****** * ******* * ****** ****
937 GCTTTTCTATCACCACAAgTTCAACTCTTCAGGCTGTCCtGAGAGGcTaGCCAGCTGCCGACCCCTTACCGA
    ************** ** ***************** ***** * ****************** *****
937 GCTTTTCTATCACCAtAAaTTCAACTCTTCAGGCTGTCCgGAGAGGtTgGCCAGCTGCCGACCCCTcACCGA


1009 TTTcGACCAGG
     *** *******
1009 TTTTGACCAGG
     ***** *****
1009 TTTTGcCCAGG
```

FIGURE 83

```
1.  human 27
2.  HCV 1
3.  human 23
```

```
  1 GFADLMGYIPLVGAPLGGAARALAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSsGi
    ****************************************************************** *
  1 GFADLMGYIPLVGAPLGGAARALAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSTGL
    ***************** ***************************************************
  1 GFADLMGYIPLVGAPLGGrARALAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSTGL


 73 YHVTNDCPNSSIVYEtADtILHsPGCVPCVREGnASkCWVpvaPTVATRDGnLPATQLRRHIDLLVGSATLC
    **************** ** *** **************** ***     ******* ********************
 73 YHVTNDCPNSSIVYEAADAILHtPGCVPCVREGNASRCWVAmTPTVATRDGKLPATQLRRHIDLLVGSATLC
    ************************ ********* * ****** ****** ***** ********************
 73 YHVTNDCPNSSIVYEAADAILHaPGCVPCVREdNvSRCWVAvTPTVATkDGKLPtTQLRRHIDLLVGSATLC


145 SALYVGDLCGSVFLVGQLFTFSPRRHWTTQdCNCSIYPGHITGHRMAWDMMMNWSPTaALVMAQLLRIPQAI
    ****************************** ************************* **************
145 SALYVGDLCGSVFLVGQLFTFSPRRHWTTQgCNCSIYPGHITGHRMAWDMMMNWSPTtALVMAQLLRIPQAI
    ********** ****************** ************************ *** *********
145 SALYVGDLCGSiFLVGQLFTFSPRRHWTTQdCNCSIYPGHITGHRMAWDMMMNWSPTaALVvAQLLRIPQAI


217 LDMIAGAHWGVLAGIAYFSMVGNWAKVLVVLLLFAGVDAtTytTGGnAarTtqaltSffsPGAKQdiQLINT
    ****************************************** * *** * *     *   ***** *****
217 LDMIAGAHWGVLAGIAYFSMVGNWAKVLVVLLLFAGVDAETHvTGGSAghTvsGfvSLlaPGAKQNvQLINT
    ************** ************************** *****     *  **  *** ** *****
217 LDMIAGAHWGVLAGmAYFSMVGNWAKVLVVLLLFAGVDAETHrTGGSAarstaGvaSLftPGArQNiQLINT


289 NGSWHiNrTALNCNaSLdTGWvAGLFYyHKFNSSGCPERmASCRPLaDFDQ
    ***** * _****** ** *** ***** ********** ****** ****
289 NGSWHlNSTALNCNDSLnTGWLAGLFYHHKFNSSGCPERLASCRPLTDFDQ
    ***** ********** ****************************** *
289 NGSWHiNSTALNCNDSLtTGWLAGLFYHHKFNSSGCPERLASCRPLTDFaQ
```

FIGURE 84

FIGURE C5-1

CLUSTERED PAIR-WISE 'REGION' ALIGNMENT
in 'identity (no translation)' alphabet of:

        1. ssThorn#8.r (1-587)
        2. ssEC1#2.r   (1-587)
        3. ssHCT18#7.r (1-587)
        4. env1.hcv   (1-1657)


```
  1                                                                            GA
                                                                               ||
  1                                                                            GA
                                                                               ||
  1                                                                            GA
                                                                               |
289 gggtgggogggatggctoctgtotoccogtggctctcggcctagctggggcccacagaccccggcgtaGg


  3 ATTCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATGGGGTACATACCGCTC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  3 ATTCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATGGGGTACATACCGCTC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
  3 ATTCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATGGGGTATATACCGCTC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
361 tcgCGCAATTTCGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCATGGGGTAcATACCGCTC


 75 GTCGGCGCCCCTCTTGGgGGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGACGGCGTGAAC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 75 GTCGGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGACGGCGTGAAC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 75 GTCGGCGCCCCTCTTGGACGCGCCTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGACGGCGTGAAC
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
433 GTCGGCGCCCCTCTTGGACGCGCCTGCCAGGGCCCTGGCGCATGGCGTCCGGCGTTCTGGAAGACGGCGTGAAC


147 TATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTCTCTCTTCCTTCTGGCCCTGCTCTCTTGtcTGACCGTG
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||| |||
147 TATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTtTATCTTCCTTCTGGCCtTGCTCTCTTGCtTGACTGTG
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
147 TATGC CAGGGAACCTTCCTGGTTGCTCTTTCTCTATCTTCCTTCTGGCCCTGCTCTCTTGCcTGACTGTG
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
505 TATGCAACAGGGAACCTTCCTGGTTGCTCTTTCTCTATCTTCCTTCTGGCCCTGCTCTCTTGCtTGACTGTG


219 CCCGCTTCAGCCTACCAAGTGCGCAACTCCaCGGGGCTTTACCATGTCACCAAcGATTGCCCcAACTCGAGt
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||
219 CCCGCTTCAGCCTACCAACTGCGCCAACTCCtCGGGGCTTTACCATGTCACCAATGATTGCCCtAACTCGAGc
    |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
219 CCCGCTTCAGCCaCCAAGTGCGCCAACTCCACGGGGCTTTACCATGTCACCAATGATTGCCCcAACTCGAGT
    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
577 CCCGCTTCgGCCtACCAAGTGCGCAACTCCACGGGGCTTTACCAcGTCACCAATGATTGCCCtAACTCGAGT
```

FIGURE 95-2

```
291 ATTGTGTACGAGGCGGCCGATGCtATCCTGCACgCTCCGGGGTGTGTCCCTTGCGTTCgCGAGCCtAACGcC
      |||||||| ||||| ||| ||||| |||||  ||||  |||  |||||||| |    ||| |||  ||| |
291 ATTGTGTACGAGGCGGCCGATGCCATCCTGCACACTCCGGGGTGTGTCCCTTGCGTTCACGAGGGCCAACGTC
      ||| ||| ||||  |||| ||||| ||||||||  || ||||||||||| ||||| ||||  |||||| ||| |
291 ATTGTaTACGAaGCGGCCGACGCCATCCTGCACACTCCGGGGTGTGTCCCTTGCGTTCACGAGGGCAACGTC
      |||| ||| |||  |||| ||| |||||||||| || |||||| |||  |||  ||| ||| |||||  || |
649 ATTGTgTACGAgGCGGCCGAtGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTCgtGAGGGCAACGcC
```

```
363 TCGAGGTGTTGGGTGGCGATGACCCCCACGGTGGCCgCCAGGGaCGGCAgAcTCCCCACAACGCAGCTgCCA
      ||||| || |||||| |||||| |||||||||||| ||  ||||| ||||  |  ||||||||||||||||  |||
363 TCGAGGTGTTGGGTGGCGATGACCCCCACGGTGGCCACCAGGGgCGGCAAACTCCCCACAACGCAGCTtCGA
      ||||||||| ||||||| || |||||||||||||||||| ||||| |||| ||||||||||||||||||||| |||
363 TCGAGGTGTTGGGTGGCGgtGACCCCCACGGTGGCCACCAGGGATGGCAAACTCCCCACAACGCAGCTtCGA
      |||| ||| ||||| ||||| |||||||||||||||||||||||| || || |||||||||||||||| |||
721 TCGAGGTGTtGGGTGGCGatGACCCCtACGGTGGCCACCAGGGATGGCAAACTCCCCgCgACGCAGCTtCGA
```

```
435 CGTCACATCGATCTGCTTGTCGGGAGCGCcACCCTCTGCTCGGCCCTCtACGTGGGGGACcTGtGcGGGTCc
      ||||||||||||| || ||||||||||||| ||||| |||||||||||| || |||||||| |||| | |||||||
435 CGTCACATCGATCtGCTTGTCGGGAGCGCtACCCTCTGCTCGGCCCTCTACGTGGGGGACCTGTGCGGGTCt
      ||||||| || ||| ||| || ||||||||| ||||| |||||| ||||| || ||||||| ||||| | |||||
435 CGTCACATCGAtCtGCTTGTCGGGAGCGCCACCCTCTGCTCGGCCCTCtAtGTGGGGGACtTGtGcGGGTCt
      |||||||||| || |||||| ||||| ||||| |||| ||| ||||| || ||||||| |||| || |||||||
793 CGTCACATCGATCtGCTTGTCGGGAGCGCCACCCTCTGtTCGGCCCTCTACGTGGGGGACcTaTGCGGGTCt
```

```
507 aTCtTtCTTGTCGGTCAACTGTTCACCTTCTCTCCCAGGCGCCACTGGACGACGCAAGGtTGCAATTGcTCT
      ||||  ||| | |||||||||||||||  ||||| ||||||||||||||||||||||||||||||||| | |||
507 GTCTTcCTTGTCGGTCAACTGTTcACCTTCTCTCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCT
      |||| || ||| || | |||||||||| ||||||||||||||||||||||||||||||||||||||||||||
507 GTCTTCTTGTCGGCCAACTGTTTACCTTCTCTCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCT
      ||||||| || || |||||| ||| |||||||||||||||||||||||||||||||||||||||||||||||
865 GTCTTCTtGTCGGCCAACTGTTcACCTTCTCTCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCT
```

```
579 ATCGAATTC
      ||| ||||| 
579 ATCGAATTC
      ||  |||||
579 ATCGAATTC
      ||| |
937 ATCtAtccC
```

```
                     10        20        30        40
                GAATTCGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATAT
                      X:::::::::::::::::::::::::::::::::::::::::::::::
1;../ssp  CTCTCCCAGGCGCCACTGGACGACCCAAGGTTGCAATTGCTCTATCTATCCCGGCCATAT
              550       560       570       580       590       600


      50        60        70        80    ▲   90        100
      AACAGGTCACCGCATGGCATGGGATATGATGATGAACTGGTCCCCTACGACGGCGTTAGT
      :::  :::::::::::::::::::::::::::::::::::::::::::::::::::::  ::
      AACGGGTCACCGCATGGCATGGGATATGATGATGAACTGGTCCCCTACGACGGCGTTGGT
          610       620       630       640       650       660


   110       120       130       140       150       160
  _ GGTAGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTG
     :  :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      AATGGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTG
          670       680       690       700       710       720


   170       180       190       200       210       220
    GGGAGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGGGCGAAGGTCTTGGC
    :::::::::::::::::::::::::::::::::::::::::::::::::::::::::  :::
    GGGAGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGT
        730       740       750       760    _  770       780


 230       240       250       260       270       280
   AGTGCTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACGTCACTGGGGGGATCGCCGC
   :::::::::::::::::::::::::::::::::::::::::::::  :::::  :  ::::
   AGTGCTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGGAAGTGCCGG
       790       800       810       820       830       840


 290       300       310       320       330       340
_ CAAAACTACGGCTAGCCTTACTGGTCTCTTCAATTTAGGTGCCAAGCAGAACATCCAGCT
   :  :  :::   :  ::  :   ::   :  :  ::  ::     :::  :::::::::::::  ::::::
   CCACACTGTGTCTGGATTTGTTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCT
       850       860       870       880       890       900


 350       360       370       380       390       400
   GATCAACACCAACGGCAGTTGGCACATCAACAGGACGGCCTTGAACTGCAATGATAGCCT
   ::::::::::::::::::::::::::::::  ::::  ::  :::::  ::::::::::::::::::::::::
   GATCAACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCT
       910       920       930       940       950       960


 410       420
   CAACACCGGCTGGAATTC
   ::::::::::::::X
   CAACACCGGCTGGTTGGCAGGGCTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCC
       970       980       990       1000      1010      1020
```

FIGURE 86

FIGURE F-7

AA #117-308 (putative envelope region)

1) HCT #18 (USA)   3 clones sequenced
2) JH23 (USA)   ?
3) JH 27 (USA)   ?
4) PBL-Th (USA)   2 clones sequenced
5) EC1 (Italy)   3 clones sequenced
6) HCV-1 (chimpanzee)   multiple

       C/M ←─┬─→ S
1)   (P)
2)
3)
4)
5)
6)RNLGKVIDTLTCGFADLMGYIPLVGAPLGGAARALAHGVRVLEDGVNYATGNL

1)   H
2)
3)   S   T   T
4)   L
5)   (F)   S
6)PGCSFSIFLLALLSCLTVPASAYQVRNSTGLYHVTNDCPNSSIVYEAADAILH

            Y
1)   (H)   V   V   T
2)A   D V   V   K   T
3)S   PVA   N
4)A   A   R   T
5)   H   V   T
6)TPGCVPCVREGNASRCWVAMTPTVATRDGKLPATQLRRHIDLLVGSATLCS

1)
2)   I   D
3)   D
4)
5)   I
6)ALYVGDLCGSVFLVGQLFTFSPRRHWTTQGCNCSI

SUMMARY: "S" AA117-308 (93%)

HCT#18, PBL-Th, EC1(Italy) have 97% homology with HCV-1

JH23 and JH 27 have 96% and 95% homology with HCV-1, respectively

FIGURE 68

AA#300-438 (C-terminal region of the putative envelope region and amino ~1/3 of NSI)

1) JH23	?
2) JH27	?
3) Japanese isolate (T. Miyamura)	?
4) EC10 (Italy)	2 clones sequenced (one nt difference, which did not result in an amino acid change)
5) HCV-1 (chimpanzee)	multiple

S ←——→ NS I

1)   D   A   V
2)   D   A
3)   V S   VM   V
4)
5)TTQGCNCSIYPGHITGHRMAWDMMMNWSPTTALVMAQLLRIPQAILDMIAGA

1)   M   R   A RST A VA
2)   T YT   N A R TQALT F
3)   L   Y   I M   GH R   VQ   VT TLT
4)   A   1 AK TASLTA
5)HWGVLAGIAYFSMVGNWAKVLVVLLLFAGVDAETHVTGGSAGHTVSGFVSL

1)FS   P   I   I   T   V
2)FT   D I   I R   A D
3)FR   S KI V   I R   Q   F
4)FNL   I   I R   N
5)LAPGAKQNVQLINTNGSWHLNSTALNCNDSLNTGWL

SUMMARY: NS I AA 330-660

"Isolate"	%Homology (AA330-438)	%Homology (AA353-460)
JH23	83	57
JH27	80	39
Japanese	73	48
EC10 (Italy)	84	48

FIGURE 89-1

SEQUENCE OF THE HCV GENOME
————————————————————————————

(AS DEDUCED FROM OVERLAPPING CLONES b114a/18g/ag30a/CA205a/
CA290a/CA216a/pi14a/CA167b/CA156e/CA84a/CA59a/K9-1/26j/13i/
12f/14i/11b/7f/7e/8h/33c/40b/37b/35/36/81/32/33b/25c/14c/8f/
33f/33g/39c/35f/19g/26g/15e/b5a/16jh/6k/131jh)

```
                          5'terminus——————————————————————————————
CACTCCACCATGAATCACTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAG
CCATGGCGTTAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATA
GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGGTCCTTTCTTGGA
TCAACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGT
GTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATAGGGTGCTTGCGAGTGCCCCGGGAG-300

       (Putative initiator methionine codon)
                         |                     G        C
GTCTCGTAGACCGTGCACCATGAGCACGAATCCTAAACCTCAAAAAAAAAACAAACGTAA
CACCAACCGTCGCCCACAGGACGTCAAGTTCCCGGGTGGCGGTCAGATCGTTGGTGGAGT
TTACTTGTTGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCCGA
GCGGTCGCAACCTCGAGGTAGACGTCAGCCTATCCCCAAGGCTCGTCGGCCCGAGGGCAG
GACCTGGGCTCAGCCCGGGTACCCTTGGCCCCTCTATGGCAATGAGGGCTGCGGGTGGGC-600
GGGATGGCTCCTGTCTCCCCGTGGCTCTCGGCCTAGCTGGGGCCCCACAGACCCCCGGCG
TAGGTCGCGCAATTTGGGTAAGGTCATCGATACCCTTACGTGCGGCTTCGCCGACCTCAT
GGGGTACATACCGCTCGTCGGCGCCCCTCTTGGAGGCGCTGCCAGGGCCCTGGCGCATGG
CGTCCGGGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAACCTTCCTGGTTGCTCTTT

       C
CTCTATCTTCCTTCTGGCCCTGCTCTCTTGCTTGACTGTGCCCGCTTCGGCCTACCAAGT-900
GCGCAACTCCACGGGGCTTTACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTA
CGAGGCGGCCGATGCCATCCTGCACACTCCGGGGTGCGTCCCTTGCGTTCGTGAGGGCAA
CGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCC
CGCGACGCAGCTTCGACGTCACATCGATCTGCTTGTCGGGAGCGCCACCCTCTGTTCGGC
CCTCTACGTGGGGGGACCTATGCGGGTCTGTCTTTCTTGTCGGCCAACTGTTCACCTTCTC-1200
TCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAAC

                                                             G
GGGTCACCGCATGGCATGGGATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAAT
GGCTCAGCTGCTCCGGATCCCACAAGCCATCTTGGACATGATCGCTGGTGCTCACTGGGG
AGTCCTGGCGGGCATAGCGTATTTCTCCATGGTGGGGAACTGGGCGAAGGTCCTGGTAGT
GCTGCTGCTATTTGCCGGCGTCGACGCGGAAACCCACGTCACCGGGGGGAAGTGCCGGCCA-1500
CACTGTGTCTGGATTTGTTAGCCTCCTCGCACCAGGCGCCAAGCAGAACGTCCAGCTGAT
CAACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAACTGCAATGATAGCCTCAA
CACCGGCTGGTTGGCAGGGCTTTTCTATCACCACAAGTTCAACTCTTCAGGCTGTCCTGA
GAGGCTAGCCAGCTGCCGACCCCTTACCGATTTTGACCAGGGCTGGGGCCCTATCAGTTA
TGCCAACGGAAGCGGCCCCGACCAGCGCCCCTACTGCTGGCACTACCCCAAAACCTTG-1800
CGGTATTGTGCCCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGT
GGTGGTGGGAACGACCGACAGGTCGGGCGCGCCCACCTACAGCTGGGGTGAAAATGATAC
GGACGTCTTCGTCCTTAACAATACCAGGCCACCGCTGGGCAATTGGTTCGGTTGTACCTG
GATGAACTCAACTGGATTCACCAAAGTGTGCGGAGCGCCTCCTTGTGTCATCGGAGGGGC
GGGCAACAACACCCTGCACTGCCCCACTGATTGCTTCCGCAAGCATCCGGACGCCACATA-2100

                           C
CTCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCCTGGTCGACTACCCGTATAG
GCTTTGGCATTATCCTTGTACCATCAACTACACCATATTTAAAAATCAGGATGTACGTGGG
AGGGGTCGAACACAGGCTGGAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCT
GGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCAGGT
CCTCCCGTGTTCCTTCACAACCCTACCAGCCTTGTCCACCGGCCTCATCCACCTCCACCA-2400
GAACATTGTGGACGTGCAGTACTTGTACGGGGTGGGGTCAAGCATCGCGTCCTGGGCCAT
TAAGTGGGAGTACGTCGTTCTCCTGTTCCTTCTGCTTGCAGACGCGCGCGTCTGCTCCTG
CTTGTGGATGATGCTACTCATATCCCAACGGGAGGCGGCTTTGGAGAACCTCGTAATACT
TAATGCAGCATCCCTGGCCGGGACGCACGGTCTTGTATCCTTCCTCGTGTTCTTCTGCTT
TGCATGGTATTTGAAGGGTAAGTGGGTGCCCGGAGCGGTCTACACCTTCTACGGGATGTG-2700
GCCTCTCCTCCTGCTCCTGTTGGCGTTGCCCCAGCGGGCGTACGCGCTGGACACGGAGGT
GGCCGCGTCGTGTGGCGGTGTTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATA
TTACAAGCGCTATATCAGCTGGTGCTTGTGGTGGCTTCAGTATTTTCTGACCAGAGTGGA
```

FIGURE 89-2

```
AGCGCAACTGCACGTGTGGATTCCCCCCCCTCAACGTCCGAGGGGGGCGCGACGCCGTCAT
CTTACTCATGTGTGCTGTACACCCGACTCTGGTATTTGACATCACCAAATTGCTGCTGGC-3000
CGTCTTCGGACCCCTTTGGATTCTTCAAGCCAGTTTGCTTAAAGTACCCTACTTTGTGCG
CGTCCAAGGCCTTCTCCGGTTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGT
GCAAATGGTCATCATTAAGTTAGGGGCGCTTACTGGCACCTATGTTTATAACCATCTCAC
TCCTCTTCGGGACTGGGCGCACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGT
CGTCTTCTCCCAAATGGAGACCAAGCTCATCACGTGGGGGGCAGATACCGCCGCGTGCGG-3300
TGACATCATCAACGGCTTGCCTGTTTCCGCCCGCAGGGGCCGGGAGATACTGCTCGGGGCC
AGCCGATGGAATGGTCTCCAAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCCA
GCAGACAAGGGGCCTCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCA
AGTGGAGGGTGAGGTCCAGATTGTGTCAACTGCTGCCCAAACCTTCCTGGCAACGTGCAT
CAATGGGGTGTGCTGGACTGTCTACCACGGGGCCGGAACGAGGACCATCGCGTCACCCAA-3600

                                                             T
GGGTCCTGTCATCCAGATGTATACCAATGTAGACCAAGACCTTGTGGGCTGGCCCGCTCC

             C
GCAAGGTAGCCGCTCATTGACACCCTGCACTTGCGGCTCCTCGGACCTTTACCTGGTCAC
GAGGCACGCCGATGTCATTCCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTC
GCCCCGGCCCATTTCCTACTTGAAAGGCTCCTCGGGGGGTCCGCTGTTGTGCCCCGCGGG
GCACGCCGTGGGCATATTTAGGGCCGCGGTGTGCACCCGTGGAGTGGCTAAGGCGGTGGA-3900
CTTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTC
CTCTCCACCAGTAGTGCCCCAGAGCCTTCCAGGTGGCTCACCTCCATGCTCCCACAGGCAG
CGGCAAAAGCACCAAGGTCCCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACT
CAACCCCTCTGTTGCTGCAACACTGGGCTTTGGTGCTTACATGTCCAAGGCTCATGGGAT

      T
CGATCCTAACATCAGGACCGGGGTGAGAACAATTACCACTGGCAGCCCCATCACGTACTC-4200
CACCTACGGCAAGTTCCTTGCCGACGGCGGGTGCTCGGGGGGGCGCTTATGACATAATAAT
TTGTGACGAGTGCCACTCCACGGATGCCACATCCATCTTGGGCATCGGCACTGTCCTTGA
CCAAGCAGAGACTGCGGGGGCGAGACTGGTTGTGCTCGCCACCGCCACCCCTCCGGGCTC
CGTCACTGTGCCCCATCCCAACATCGAGGAGGTTGCTCTGTCCACCACCGGAGAGATCCC
TTTTTACGGCAAGGCTATCCCCCTCGAAGTAATCAAGGGGGGGAGACATCTCATCTTCTG-4500
TCATTCAAAGAAGAAGTGCGACGAACTCGCCGCAAAGCTGGTCGCATTGGGCATCAATGC
CGTGGCCTACTACCGCGGTCTTGACGTGTCCGTCATCCCGACCAGCGGCGATGTTGTCGT

                                                             A
CGTGGCAACCGATGCCCTCATGACCGGCTATACCGGCGACTTCGACTCGGTGATAGACTG
CAATACGTGTGTCACCCAGACAGTCGATTTCAGCCTTGACCCTACCTTCACCATTGAGAC
AATCACGCTCCCCCAGGATGCTGTCTCCCGCACTCAACGTCGGGGCAGGACTGGCAGGGG-4800
GAAGCCAGGCATCTACAGATTTGTGGCACCGGGGGAGCGCCCCTCCGGCATGTTCGACTC
GTCCGTCCTCTGTGAGTGCTATGACGCAGGCTGTGCTTGGTATGAGCTCACGCCCGCCGA
GACTACAGTTAGGCTACGAGCGTACATGAACACCCCGGGGGCTTCCCGTGTGCCAGGACCA
TCTTGAATTTTGGGAGGGCGTCTTTACAGGCCTCACTCATATAGATGCCCACTTTCTATC
CCAGACAAAGCAGAGTGGGGAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTG-5100
CGCTAGGGCTCAAGCCCCTCCCCCATCGTGGGACCAGATGTGGAAGTGTTTGATTCGCCT
CAAGCCCACCCTCCATGGGCCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGA
AATCACCCTGACGCACCCAGTCACCAAATACATCATGACATGCATGTCGGCCGACTTGGA
GGTCGTCACGAGCACCTGGGTGCTCGTTGGCGGCGTCCTGGCTGCTTTGGCCGCGTATTG
CCTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTCGTCTTGTCCGGGAAGCCGGCAAT-5400
CATACCTGACAGGGAAGTCCTCTACCGAGAGTTCGATGAGATGGAAGAGTGCTCTCAGCA
CTTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGG
CCTCCTGCAGACCGCGTCCCGTCAGGCAGAGGTTATCGCCCCTGCTGTCCAGACCAACTG
GCAAAAACTCGAGACCTTCTGGGCGAAGCATATGTGGAACTTCATCAGTGGGATACAATA
CTTGGCGGGGCTTGTCAACGCTGCCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTAC-5700
AGCTGCTGTCTACCAGCCCACTAACCACTAGCCAAACCCTCCTCTTCAACATATTGGGGGG
GTGGGTGGCTGCCCAGCTCGCCGCCCCCGGTGCCGCTACTGCCTTTGTGGGCGCTGGCTT
AGCTGGCGCCGCCATCGGCAGTGTTGGACTGGGGAAGGTCCTCATAGACATCCTTGCAGG
GTATGGCGCGGGCGTGGCGGGGAGCTCTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCC
CTCCACGGAGGACCTGGTCAATCTACTGCCCGCCATCCTCTCGCCCGGAGCCCTCGTAGT-6000
CGGCGTGGTCTGTGCAGCAATACTGCGCCGGCACGTTGGCCCGGGCGAGGGGGCAGTGCA
GTGGATGAACCGGCTGATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCCCACGCACTA
CGTGCCCGGAGAGCGCATGCAGCTGCCCGTCACTGCCATACTCAGCAGCCTCACTGTAAC
CCAGCTCCTGAGGCGACTGCACCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGG
TTCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGTTGAGCGACTTTAAGACCTG-6300
GCTAAAAGCTAAGCTCATGCCACAGCTGCCTGGGATCCCCTTTGTGTCCTGCCAGCGCGG
```

FIGURE 89-3

```
GTATAAGGGGGGTCTGGCGAGTGGACGGCATCATGCACACTCGCTGCCACTGTGGAGCTGA
GATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAA
CATGTGGAGTGGGACCTTCCCCATTAATGCCTACACCACGGGCCCCTGTACCCCCCTTCC
TGCGCCGAACTACACGTTCGCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAG-6600
GCAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATCTCAAATGCCCGTG
CCAGGTCCCATCGCCCGAATTTTTCACAGAATTGGACGGGGTGCGCCTACATAGGTTTGC
GCCCCCCTGCAAGCCCTTGCTGCGGGAGGAGGTATCATTCAGAGTAGGACTCCACGAATA
CCCGGTAGGGTCGCAATTACCTTGCGAGCCCGAACCGGACGTGGCCGTGTTGACGTCCAT
GCTCACTGATCCCTCCCATATAACAGCAGAGGCGGCCGGGCGAAGGTTGGCGAGGGGATC-6900
ACCCCCCTCTGTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCTCAAGGCAAC
TTGCACCGCTAACCATGACTCCCCTGATGCTGAGCTCATAGAGGCCAACCTCCTATGGAG
GCAGGAGATGGGCGGCAACATCACCAGGGTTGAGTCAGAAAACAAAGTGGTGATTCTGGA
CTCCTTCGATCCGCTTGTGGCGGAGGAGGACGAGCGGGAGATCTCCGTACCCGCAGAAAT
CCTGCGGAAGTCTCGGAGATTCGCCCAGGCCCTGCCCGTTTGGGCGCGGCCGGACTATAA-7200
CCCCCCGCTAGTGGAGACGTGGAAAAAGCCCGACTACGAACCACCTGTGGTCCATGGCTG
TCCGCTTCCACCTCCAAAGTCCCCTCCTGTGCCTCCGCCTCGGAAGAAGCGGACGGTGGT
CCTCACTGAATCAACCCTATCTACTGCCTTGGCCGAGCTCGCCACCAGAAGCTTTGGCAG
CTCCTCAACTTCCGGCATTACGGGCGACAATACGACAACATCCTCTGAGCCCGCCCCTTC
TGGCTGCCCCCCCGACTCCGACGCTGAGTCCTATTCCTCCATGCCCCCCCTGGAGGGGGA-7500
GCCTGGGGATCCGGATCTTAGCGACGGGTCATGGTCAACGGTCAGTAGTGAGGCCAACGC
GGAGGATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTG
CGCCGCGGAAGAACAGAAACTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCA
CAATTTGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATT
TGACAGACTGCAAGTTCTGGACAGCCATTACCAGGACGTACTCAAGGAGGTTAAAGCAGC-7800
GGCGTCAAAAGTGAAGGCTAACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCCCC
ACACTCAGCCAAATCCAAGTTTGGTTATGGGGCAAAAGACGTCCGTTGCCATGCCAGAAA
GGCCGTAACCCACATCAACTCCGTGTGGAAAGACCTTCTGGAAGACAATGTAACACCAAT
AGACACTACCATCATGGCTAAGAACGAGGTTTTCTGCGTTCAGCCTGAGAAGGGGGGTCG
TAAGCCAGCTCGTCTCATCGTGTTCCCCGATCTGGGCGTGCGCGTGTGCGAAAAGATGGC-8100
TTTGTACGACGTGGTTACAAAGCTCCCCTTGGCCGTGATGGGAAGCTCCTACGGATTCCA
ATACTCACCAGGACAGCGGGTTGAATTCCTCGTGCAAGCGTGGAAGTCCAAGAAAACCCC
AATGGGGTTCTCGTATGATACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCCG
TACGGAGGAGGCAATCTACCAATGTTGTGACCTCGACCCCCAAGCCCGCGTGGCCATCAA
GTCCCTCACCGAGAGGCTTTATGTTGGGGGCCCTCTTACCAATTCAAGGGGGGGAGAACTG-8400
CGGCTATCGCAGGTGCCGCGCGAGCGGCGTACTGACAACTAGCTGTGGTAACACCCTCAC
TTGCTACATCAAGGCCCGGGCAGCCTGTCGAGCCGCAGGGCTCCAGGACTGCACCATGCT
CGTGTGTGGCGACGACTTAGTCGTTATCTGTGAAAGCGCGGGGGTCCAGGAGGACGCGGC
GAGCCTGAGAGCCTTCACGGAGGCTATGACCAGGTACTCCGCCCCCCCTGGGGACCCCCC
ACAACCAGAATACGACTTGGAGCTCATAACATCATGCTCCTCCAACGTGTCAGTCGCCCA-8700
CGACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAG
AGCTGCGTGGGAGACAGCAAGACACACTCCAGTCAATTCCTGGCTAGGCAACATAATCAT
GTTTGCCCCCACACTGTGGGCGAGGATGATACTGATGACCCATTTCTTTAGCGTCCTTAT
AGCCAGGGACCAGCTTGAACAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCAT
AGAACCACTTGATCTACCTCCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTTCACT-9000
CCACAGTTACTCTCCAGGTGAAATTAATAGGGTGGCCGCATGCCTCAGAAAACTTGGGGT
```

```
                 G
ACCGCCCTTGCGAGCTTGGAGACACCGGGCCCGGAGCGTCCGCGCTAGGCTTCTGGCCAG
AGGAGGCAGGGCTGCCATATGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCT
CAAACTCACTCCAATAGCGGCCGCTGGCCAGCTGGACTTGTCCGGCTGGTTCACGGCTGG
CTACAGCGGGGGAGACATTTATCACAGCGTGTCTCATGCCCGGCCCCGCTGGATCTGGTT-9300
TTGCCTACTCCTGCTTGCTGCAGGGGTAGGCATCTACCTCCTCCCCAACCGATGAAGGTT
GGGGTAAACACTCCGGCCT------------------------------3'terminus
```

FIGURE 90-1

Structure of the HCV polyprotein

(From the putative initiator methionine)

       R T
MSTNPKPQKKNKRNTNRRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATR
KTSERSQPRGRRQPIPKARRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSP-100
RGSRPSWGPTDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPLGGAARA

             T
LAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSTGL-200
YHVTNDCPNSSIVYEAADAILHTPGCVPCVREGNASRCWVAMTPTVATRD
GKLPATQLRRHIDLLVGSATLCSALYVGDLCGSVFLVGQLFTFSPRRHWT-300

                  V
TQGCNCSIYPGHITGHRMAWDMMMNWSPTTALVMAQLLRIPQAILDMIAG
AHWGVLAGIAYFSMVGNWAKVLVVLLLFAGVDAETHVTGGSAGHTVSGFV-400
SLLAPGAKQNVQLINTNGSWHLNSTALNCNDSLNTGWLAGLFYHHKFNSS
GCPERLASCRPLTDFDQGWGPISYANGSGPDQRPYCWHYPPKPCGIVPAK-500
SVCGPVYCFTPSPVVVGTTDRSGAPTYSWGENDTDVFVLNNTRPPLGNWF
GCTWMNSTGFTKVCGAPPCVIGGAGNNTLHCPTDCFRKHPDATYSRCGSG-600

   I
PWLTPRCLVDYPYRLWHYPCTINYTIFKIRMYVGGVEHRLEAACNWTRGE
RCDLEDRDRSELSPLLLTTTQWQVLPCSFTTLPALSTGLIHLHQNIVDVQ-700
YLYGVGSSIASWAIKWEYVVLLFLLLADARVCSCLWMMLLISQAEAALEN
LVILNAASLAGTHGLVSFLVFFCFAWYLKGKWVPGAVYTFYGMWPLLLLL-800

                        (N)
LALPQRAYALDTEVAASCGGVVLVGIMALTLSPYYKRYISWCLWWLQYFL
TRVEAQLHVWIPPLNVRGGRDAVILLMCAVHPTLVFDITKLLLAVFGPLW-900
ILQASLLKVPYFVRVQGLLRFCALARKMIGGHYVQMVIIKLGALTGTYVY
NHLTPLRDWAHNGLRDLAVAVEPVVFSQMETKLITWGADTAACGDIINGL-1000
PVSARRGREILLGPADGMVSKGWRLLAPITAYAQQTRGLLGCIITSLTGR
DKNQVEGEVQIVSTAAQTFLATCINGVCWTVYHGAGTRTIASPKGPVIQM-1100

           S T
YTNVDQDLVGWPAPQGSRSLTPCTCGSSDLYLVTRHADVIPVRRRGDSRG
SLLSPRPISYLKGSSGGPLLCPAGHAVGIFRAAVCTRGVAKAVDFIPVEN-1200
LETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYK

                L
VLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFL-1300

ADGGCSGGAYDIIICDECHSTDATSILGIGTVLDQAETAGARLVVLATAT
PPGSVTVPHPNIEEVALSTTGEIPFYGKAIPLEVIKGGRHLIFCHSKKKC-1400
DELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVATDALMTGYTGDFDS

    Y   (S)
VIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRTQRRGRTGRGKPGIYR-1500
FVAPGERPSGMFDSSVLCECYDAGCAWYELTPAETTVRLRAYMNTPGLPV
CQDHLEFWEGVFTGLTHIDAHFLSQTKQSGENLPYLVAYQATVCARAQAP-1600
PPSWDQMWKCLIRLKPTLHGPTPLLYRLGAVQNEITLTHPVTKYIMTCMS
ADLEVVTSTWVLVGGVLAALAAYCLSTGCVVIVGRVVLSGKPAIIPDREV-1700
LYREFDEMEECSQHLPYIEQGMMLAEQFKQKALGLLQTASRQAEVIAPAV
QTNWQKLETFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTAAVTSP-1800
LTTSQTLLFNILGGWVAAQLAAPGAATAFVGAGLAGAAIGSVGLGKVLID

                 (G)
ILAGYGAGVAGALVAFKIMSGEVPSTEDLVNLLPAILSPGALVVGVVCAA-1900

                  (HC)
ILRRHVGPGEGAVQWMNRLIAFASRGNHVSPTHYVPESDAAARVTAILSS

FIGURE 90-2

```
LTVTQLLRRLHQWISSECTTPCSGSWLRDIWDWICEVLSDFKTWLKAKLM-2000

                              (V)
PQLPGIPFVSCQRGYKGVWRGDGIMHTRCHCGAEITGHVKNGTMRIVGPR
TCRNMWSGTFPINAYTTGPCTPLPAPNYTFALWRVSAEEYVEIRQVGDFH-2100
YVTGMTTDNLKCPCQVPSPEFFTELDGVRLHRFAPPCKPLLREEVSFRVG
LHEYPVGSQLPCEPEPDVAVLTSMLTDPSHITAEAAGRRLARGSPPSVAS-2200
SSASQLSAPSLKATCTANHDSPDAELIEANLLWRQEMGGNITRVESENKV
VILDSFDPLVAEEDEREISVPAEILRKSRRFAQALPVWARPDYNPPLVET-2300

                                      (S)
WKKPDYEPPVVHGCPLPPPKSPPVPPPRKKRTVVLTESTLSTALAELATR

                       (FA)
SFGSSSTSGITGDNTTTSSEPAPSGCPPDSDAESYSSMPPLEGEPGDPDL-2400
SDGSWSTVSSEANAEDVVCCSMSYSWTGALVTPCAAEEQKLPINALSNSL
LRHHNLVYSTTSRSACQRQKKVTFDRLQVLDSHYQDVLKEVKAAASKVKA-2500

(F)
NLLSVEEACSLTPPHSAKSKFGYGAKDVRCHARKAVTHINSVWKDLLEDN
VTPIDTTIMAKNEVFCVQPEKGGRKPARLIVFPDLGVRVCEKMALYDVVT-2600
KLPLAVMGSSYGFQYSPGQRVEFLVQAWKSKKTPMGFSYDTRCFDSTVTE

                              (G)
SDIRTEEAIYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENCGYRRCR-2700
ASGVLTTSCGNTLTCYIKARAACRAAGLQDCTMLVCGDDLVVICESAGVQ
EDAASLRAFTEAMTRYSAPPGDPPQPEYDLELITSCSSNVSVAHDGAGKR-2800
VYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMFAPTLWARMILMTHFF
SVLIARDQLEQALDCEIYGACYSIEPLDLPPIIQRLHGLSAFSLHSYSPG-2900

                    G
EINRVAACLRKLGVPPLRAWRHRARSVRARLLARGGRAAICGKYLFNWAV

                                  (P)
RTKLKLTPIAAAGQLDLSGWFTAGYSGGDIYHSVSHARPRWIWFCLLLLA-3000
AGVGIYLLPNRQ-3011
            |
        Stop codon
```

( ) = Heterogeneity due possibly
      to 5' or 3' terminal cloning
      artefact.

FIGURE 91

FLOW CHART FOR THE PREPARATION OF EXPRESSION VECTOR pC22
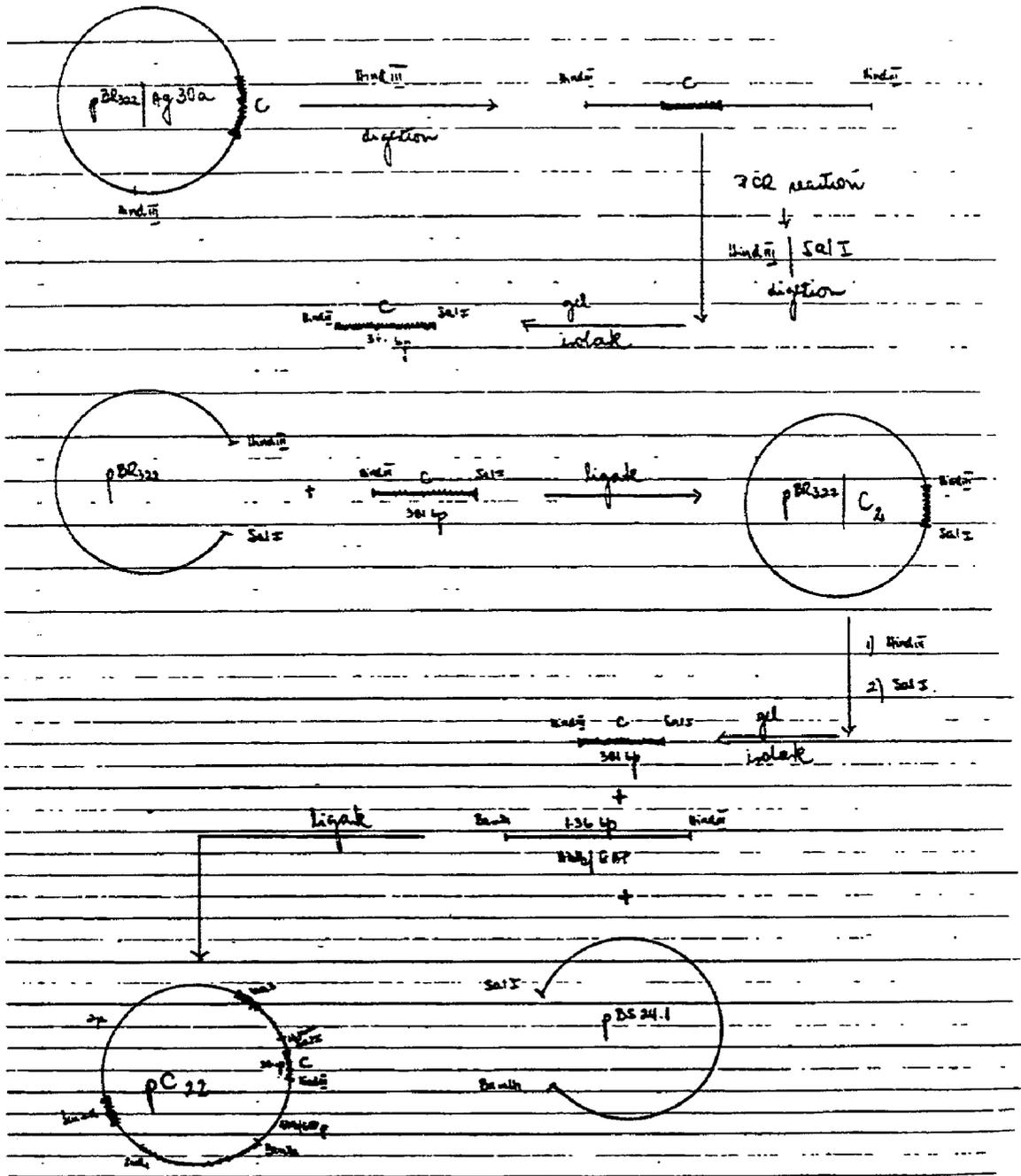
METHOD OF DETECTING ANTIBODIES TO HCV

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of attorney docket 2300-0063.28 (U.S. Ser. No. 07/355,002) filed May 18, 1989; which is a continuation-in-part of attorney docket number 2300-0063.29 (U.S. Ser. No. 07/341,334)filed Apr. 20, 1989; which is a continuation-in-part of attorney docket number 2300-0063.59 (PCT/US88/04125) filed Nov. 18, 1988, converted to U.S. National phase on Apr. 21, 1989 and assigned attorney docket number 2300-0063.26 (U.S. Ser. No. 353,896) and a continuation-in-part of attorney docket number 2300-0063.25 (U.S. Ser. No. 07/325,338) filed Mar. 17, 1989 (now abandoned); which are continuations-in-part of attorney docket number 2300-0063.24 (U.S. Ser. No. 271,450) filed Nov. 14, 1988, now abandoned; which is a continuation-in-part of attorney docket number 2300-0063.23 (U.S. Ser. No. 263,584) filed Oct. 26, 1988, now abandoned; which is a continuation-in-part of attorney docket number 2300-0063.22 (formerly 2300-0237, U.S. Ser. No. 191,263) filed May 6, 1988, now abandoned; which is a continuation-in-part of attorney docket number 2300-0063.21 (formerly 2300-0228, U.S. Ser. No. 151,072) filed Feb. 26, 1988, now abandoned; which is a continuation-in-part of attorney docket number 2300-0063.20 (formerly 2300-0219, U.S. Ser. No. 139,886)filed Dec. 30, 1987, now abandoned; which is a continuation-in-part of attorney docket number 2300-0063 (formerly 2300-0203, U.S. Ser. No. 122,714) ailed Nov. 18, 1987, now abandoned; the aforementioned applications are, in their entirety, incorporated herein by reference.

TECHNICAL FIELD

[0002] The invention relates to materials and methodologies for managing the spread of non-A, non-B hepatitis virus (NANBV) infection. More specifically, it relates to diagnostic DNA fragments, diagnostic proteins, diagnostic antibodies and protective antigens and antibodies for an etiologic agent of NANB hepatitis, i.e., hepatitis C virus.

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BACKGROUND ART

[0120] Non-A, Non-B hepatitis (NANBH) is a transmissible disease or family of diseases that are believed to be viral-induced, and that are distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH is due to a transmissible infectious agent or agents. However, the transmissible agent responsible for NANBH is still unidentified and the number of agents which are causative of the disease are unknown.

[0121] Epidemiologic evidence is suggestive that there may be three types of NANBH: the water-borne epidemic type; the blood or needle associated type; and the sporadically occurring (community acquired) type. However, the number of agents which may be the causative of NANBSH are unknown.

[0122] Clinical diagnosis and identification of NANBH has been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBV antigens and antibodies are agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However,

none of these assays has proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

[0123] Until now there has been neither clarity nor agreement as to the identity or specificity of the antigen antibody systems associated with agents of NANBH. This is due, at least in part, to the prior or co-infection of HBV with NANBV in individuals, and to the known complexity of the soluble and particulate antigens associated with HBV, as well as to the integration of HBV DNA into the genome of liver cells. In addition, there is the possibility that NANBH is caused by more than one infectious agent, as well as the possibility that NANBH has been misdiagnosed. Moreover, it is unclear what the serological assays detect in the serum of patients with NANBH. It has been postulated that the agar-gel diffusion and counterimmunoelectrophoresis assays detect autoimmune responses or nonspecific protein interactions that sometimes occur between serum specimens, and that they do not represent specific NANBV antigen-antibody reactions. The immunofluorescence, and enzyme-linked immunosorbent, and radioimmunoassays appear to detect low levels of a rheumatoid-factor-like material that is frequently present in the serum of patients with NANBH as well as in patients with other hepatic and nonhepatic diseases. Some of the reactivity detected may represent antibody to host-determined cytoplasmic antigens.

[0124] There are a number of alleged candidate NANBV. See, for example the reviews by Prince (1983), Feinstone and Hoofnagle (1984), and Overby (1985, 1986, 1987) and the article by Iwarson (1987). However, the field has not accepted that any of these candidates represent the etiological agent of NANBH.

[0125] The demand for sensitive, specific methods for screening and identifying carriers of NANBV and NANBV contaminated blood or blood products is significant. Posttransfusion hepatitis (PTH) occurs in approximately 10% of transfused patients, and NANBH accounts for up to 90% of these cases. The major problem in this disease is the frequent progression to chronic liver damage (25-55%).

[0126] Patient care as well as the prevention of transmission of NANBH by blood and blood products or by close personal contact require reliable diagnostic and prognostic tools to detect nucleic acids, antigens and antibodies related to NANBV. In addition, there is also a need for effective vaccines and immunotherapeutic therapeutic agents for the prevention and/or treatment of the disease.

DISCLOSURE OF THE INVENTION

[0127] The invention pertains to the isolation and characterization of a newly discovered etiologic agent of NANBH, hepatitis C virus (HCV), its nucleotide sequences, its protein sequences and resulting polynucleotides, polypeptides and antibodies derived therefrom. The inventions described herein were made possible by the discovery of cDNA replicas isolated by a technique which included a novel step of screening expression products from cDNA libraries created from a particulate agent in infected tissue with sera from patients with NANBH to detect newly synthesized antigens derived from the genome of the heretofore unisolated and uncharacterized viral agent, and of selecting clones which produced products which reacted immunologically only with sera from infected individuals as compared to non-infected individuals.

[0128] Studies of the nature of the genome of the HCV, utilizing probes derived from the HCV cDNA, as well as sequence information contained within the HCV cDNA, are suggestive that HCV is a positive-stranded RNA virus which appears to be distantly related to the flaviviridae family, and to the pestiviruses.

[0129] Portions of the cDNA sequences derived from HCV are useful as probes to diagnose the presence of virus in samples, and to isolate naturally occurring variants of the virus. These cDNAs also make available polypeptide sequences of HCV antigens encoded within the HCV genome(s) and permits the production of polypeptides which are useful as standards or reagents in diagnostic tests and/or as components of vaccines. Antibodies, including for example both polyclonal and monoclonal, directed against HCV epitopes contained within these polypeptide sequences are also useful for diagnostic tests, as therapeutic agents, for screening of antiviral agents, and for the isolation of the NANBV agent from which these cDNAs derive. In addition, by utilizing probes derived from these cDNAs it is possible to isolate and sequence other portions of the HCV genome, thus giving rise to additional probes and polypeptides which are useful in the diagnosis and/or treatment, both prophylactic and therapeutic, of NANBH.

[0130] Accordingly with respect to polynucleotides, some aspects of the invention are: a purified HCV polynucleotide; a recombinant HCV polynucleotide; a recombinant polynucleotide comprising a sequence derived from an HCV genome or from HCV cDNA; a recombinant polynucleotide encoding an epitope of HCV; a recombinant vector containing the any of the above recombinant polynucleotides, and a host cell transformed with any of these vectors.

[0131] Other aspects of the invention are: a recombinant expression system comprising an open reading frame (ORF) of DNA derived from an HCV genome or from HCV cDNA, wherein the ORF is operably linked to a control sequence compatible with a desired host, a cell transformed with the recombinant expression system, and a polypeptide produced by the transformed cell.

[0132] Still other aspects of the invention are: purified HCV, a preparation of polypeptides from the purified HCV; a purified HCV polypeptide; a purified polypeptide comprising an epitope which is immunologically identifiable with an epitope contained in HCV.

[0133] Included aspects of the invention are a recombinant HCV polypeptide; a recombinant polypeptide comprised of a sequence derived from an HCV genome or from HCV cDNA; a recombinant polypeptide comprised of an HCV epitope; and a fusion polypeptide comprised of an HCV polypeptide.

[0134] Also included in the invention are a monoclonal antibody directed against an HCV epitope; and a purified preparation of polyclonal antibodies directed against an HCV epitope; and an anti-idiotype antibody comprising a region which mimics an HCV epitope.

[0135] Another aspect of the invention is a particle which is immunogenic against HCV infection comprising a non-HCV polypeptide having an amino acid sequence capable of forming a particle when said sequence is produced in a eukaryotic host, and an HCV epitope.

[0136] Still another aspect of the invention is a polynucleotide probe for HCV.

[0137] Aspects of the invention which pertain to kits are those for: analyzing samples for the presence of polynucleotides derived from HCV comprising a polynucleotide probe containing a nucleotide sequence from HCV of about 8 or more nucleotides, in a suitable container; analyzing samples for the presence of an HCV antigen comprising an antibody directed against the HCV antigen to be detected, in a suitable container; analyzing samples for the presence of an antibodies directed against an HCV antigen comprising a polypeptide containing an HCV epitope present in the HCV antigen, in a suitable container.

[0138] Other aspects of the invention are: a polypeptide comprised of an HCV epitope, attached to a solid substrate; and an antibody to an HCV epitope, attached to a solid substrate.

[0139] Still other aspects of the invention are: a method for producing a polypeptide containing an HCV epitope comprising incubating host cells transformed with an expression vector containing a sequence encoding a polypeptide containing an HCV epitope under conditions which allow expression of said polypeptide; and a polypeptide containing an HCV epitope produced by this method.

[0140] The invention also includes a method for detecting HCV nucleic acids in a sample comprising reacting nucleic acids of the sample with a probe for an HCV polynucleotide under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample; and detecting a polynucleotide duplex which contains the probe.

[0141] Immunoassays are also included in the invention. These include an immunoassay for detecting an HCV antigen comprising incubating a sample suspected of containing an HCV antigen with a probe antibody directed against the HCV antigen to be detected under conditions which allow the formation of an antigen-antibody complex; and detecting an antigen-antibody complex containing the probe antibody. An immunoassay for detecting antibodies directed against an HCV antigen comprising incubating a sample suspected of containing anti-HCV antibodies with a probe polypeptide which contains an epitope of the HCV, under conditions which allow the formation of an antibody-antigen complex; and detecting the antibody-antigen complex containing the probe antigen.

[0142] Also included in the invention are vaccines for treatment of HCV infection comprising an immunogenic peptide containing an HCV epitope, or an inactivated preparation of HCV, or an attenuated preparation of HCV.

[0143] Another aspect of the invention is a tissue culture grown cell infected with HCV.

[0144] Yet another aspect of the invention is a method for producing antibodies to HCV comprising administering to an individual an isolated immunogenic polypeptide containing an HCV epitope in an amount sufficient to produce an immune response.

BRIEF DESCRIPTION OF THE DRAWINGS

[0145] FIG. 1 shows the double-stranded nucleotide sequence of the HCV cDNA insert in clone 5-1-1, and the putative amino acid sequence of the polypeptide encoded therein.

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[0146] **FIG. 2** shows the homologies of the overlapping HCV cDNA sequences in clones 5-1-1, 81, 1-2, and 91.

[0147] **FIG. 3** shows a composite sequence of HCV cDNA derived from overlapping clones 81, 1-2, and 91, and the amino acid sequence encoded therein.

[0148] **FIG. 4** shows the double-stranded nucleotide sequence of the HCV cDNA insert in clone 81, and the putative amino acid sequence of the polypeptide encoded therein.

[0149] **FIG. 5** shows the HCV cDNA sequence in clone 36, the segment which overlaps the NANBV cDNA of clone 81, and the polypeptide sequence encoded within clone 36.

[0150] **FIG. 6** shows the combined ORF of HCV cDNAs in clones 36 and 81, and the polypeptide encoded therein.

[0151] **FIG. 7** shows the HCV cDNA sequence in clone 32, the segment which overlaps clone 81, and the polypeptide encoded therein.

[0152] **FIG. 8** shows the HCV cDNA sequence in clone 35, the segment which overlaps clone 36, and the polypeptide encoded therein.

[0153] **FIG. 9** shows the combined ORF of HCV cDNAs in clones 35, 36, 81, and 32, and the polypeptide encoded therein.

[0154] **FIG. 10** shows the HCV cDNA sequence in clone 37b, the segment which overlaps clone 35, and the polypeptide encoded therein.

[0155] **FIG. 11** shows the HCV cDNA sequence in clone 33b, the segment which overlaps clone 32, and the polypeptide encoded therein.

[0156] **FIG. 12** shows the HCV cDNA sequence in clone 40b, the segment which overlaps clone 37b, and the polypeptide encoded therein.

[0157] **FIG. 13** shows the HCV cDNA sequence in clone 25c, the segment which overlaps clone 33b, and the polypeptide encoded therein.

[0158] **FIG. 14** shows the nucleotide sequence and polypeptide encoded therein of the ORF which extends through the HCV cDNAs in clones 40b, 37b, 35, 36, 81, 32, 33b, and 25c.

[0159] **FIG. 15** shows the HCV cDNA sequence in clone 33c, the segment which overlaps clones 40b and 33c, and the amino acids encoded therein.

[0160] **FIG. 16** shows the HCV cDNA sequence in clone 8h, the segment which overlaps clone 33c, and the amino acids encoded therein.

[0161] **FIG. 17** shows the HCV cDNA sequence in clone 7e, the segment which overlaps clone 8h, and the amino acids encoded therein.

[0162] **FIG. 18** shows the HCV cDNA sequence in clone 14c, the segment which overlaps clone 25c, and the amino acids encoded therein.

[0163] **FIG. 19** shows the HCV cDNA sequence in clone 8f, the segment which overlaps clone 14c, and the amino acids encoded therein.

[0164] **FIG. 20** shows the HCV cDNA sequence in clone 33f, the segment which overlaps clone 8f, and the amino acids encoded therein.

[0165] **FIG. 21** shows the HCV cDNA sequence in clone 33g, the segment which overlaps clone 33f, and the amino acids encoded therein.

[0166] **FIG. 22** shows the HCV cDNA sequence in clone 7f, the segment which overlaps the sequence in clone 7e, and the amino acids encoded therein.

[0167] **FIG. 23** shows the HCV cDNA sequence in clone 11b, the segment which overlaps the sequence in clone 7f, and the amino acids encoded therein.

[0168] **FIG. 24** shows the HCV cDNA sequence in clone 14i, the segment which overlaps the sequence in clone 11b, and the amino acids encoded therein.

[0169] **FIG. 25** shows the HCV cDNA sequence in clone 39c, the segment which overlaps the sequence in clone 33g, and the amino acids encoded therein.

[0170] **FIG. 26** shows a composite HCV cDNA sequence derived from the aligned cDNAs in clones 14i, 11b, 7f, 7e, 8h, 33c 40b 37b 35 36, 81, 32, 33b, 25c, 14c, 8f, 33f, and 33g; also shown is the amino acid sequence of the polypeptide encoded in the extended ORF in the derived sequence.

[0171] **FIG. 27** shows the sequence of the HCV cDNA in clone 12f, the segment which overlaps clone 14i, and the amino acids encoded therein.

[0172] **FIG. 28** shows the sequence of the HCV cDNA in clone 35f, the segment which overlaps clone 39c, and the amino acids encoded therein.

[0173] **FIG. 29** shows the sequence of the HCV cDNA in clone 19g, the segment which overlaps clone 35f, and the amino acids encoded therein.

[0174] **FIG. 30** shows the sequence of clone 26g, the segment which overlaps clone 19g, and the amino acids encoded therein.

[0175] **FIG. 31** shows the sequence of clone 15e, the segment which overlaps clone 26g, and the amino acids encoded therein.

[0176] **FIG. 32** shows the sequence in a composite cDNA, which was derived by aligning clones 12f through 15e in the 5' to 3' direction; it also shows the amino acids encoded in the continuous ORF.

[0177] **FIG. 33** shows a photograph of Western blots of a fusion protein, SOD-NANB$_{5-1-1}$, with chimpanzee serum from chimpanzees infected with BB-NANB, HAV, and HBV.

[0178] **FIG. 34** shows a photograph of Western blots of a fusion protein, SOD-NANB$_{5-1-1}$, with serum from humans infected with NANBV, HAV, HBV, and from control humans.

[0179] **FIG. 35** is a map showing the significant features of the vector pAB24.

[0180] **FIG. 36** shows the putative amino acid sequence of the carboxy-terminus of the fusion polypeptide C100-3 and the nucleotide sequence encoding it.

[0181] FIG. 37A is a photograph of a coomassie blue stained polyacrylamide gel which identifies C100-3 expressed in yeast.

[0182] FIG. 37B shows a Western blot of C100-3 with serum from a NANBV infected human.

[0183] FIG. 38 shows an autoradiograph of a Northern blot of RNA isolated from the liver of a BB-NANBV infected chimpanzee, probed with BB-NANBV cDNA of clone 81.

[0184] FIG. 39 shows an autoradiograph of NANBV nucleic acid treated with RNase A or DNase I, and probed with BB-NANBV cDNA of clone 81.

[0185] FIG. 40 shows an autoradiograph of nucleic acids extracted from NANBV particles captured from infected plasma with anti-NANB$_{5-1-1}$, and probed with ^{32}P-labeled NANBV cDNA from clone 81.

[0186] FIG. 41 shows autoradiographs of filters containing isolated NANBV nucleic acids, probed with ^{32}P-labeled plus and minus strand DNA probes derived from NANBV cDNA in clone 81.

[0187] FIG. 42 shows the homologies between a polypeptide encoded in HCV cDNA and an NS protein from Dengue flavivirus.

[0188] FIG. 43 shows a histogram of the distribution of HCV infection in random samples, as determined by an ELISA screening.

[0189] FIG. 44 shows a histogram of the distribution of HCV infection in random samples using two configurations of immunoglobulin-enzyme conjugate in an ELISA assay.

[0190] FIG. 45 shows the sequences in a primer mix, derived from a conserved sequence in NS1 of flaviviruses.

[0191] FIG. 46 shows the HCV cDNA sequence in clone k9-1, the segment which overlaps the cDNA in FIG. 26, and the amino acids encoded therein.

[0192] FIG. 47 shows the sequence in a composite cDNA which was derived by aligning clones k9-1 through 15e in the 5' to 3' direction; it also shows the amino acids encoded in the continuous ORF.

[0193] FIG. 48 shows the nucleotide sequence of HCV cDNA in clone 13i, the amino acids encoded therein, and the sequences which overlap with clone 12f.

[0194] FIG. 49 shows the nucleotide sequence of HCV cDNA in clone 26j, the amino acids encoded therein, and the sequences which overlap clone 13i.

[0195] FIG. 50 shows the nucleotide sequence of HCV cDNA in clone CA59a, the amino acids encoded therein, and the sequences which overlap with clones 26j and K9-1.

[0196] FIG. 51 shows the nucleotide sequence of HCV cDNA in clone CA84a, the amino acids encoded therein, and the sequences which overlap with clone CA59a.

[0197] FIG. 52 shows the nucleotide sequence of HCV cDNA in clone CA156e, the amino acids encoded therein, and the sequences which overlap with CA84a.

[0198] FIG. 53 shows the nucleotide sequence of HCV cDNA in clone CA167b, the amino acids encoded therein, and the sequences which overlap CA156e.

[0199] FIG. 54 shows the ORF of HCV cDNA derived from clones pi14a, CA167b, CA156e, CA84a, CA59a, K9-1, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, and 15e.

[0200] FIG. 55 shows the hydrophobicity profiles of polyproteins encoded in HCV and in West Nile virus.

[0201] FIG. 56 shows the nucleotide sequence of HCV cDNA in clone CA216a, the amino acids encoded therein, and the overlap with clone CA167b.

[0202] FIG. 57 shows the nucleotide sequence of HCV cDNA in clone CA290a, the amino acids encoded therein, and the overlap with clone CA216a.

[0203] FIG. 58 shows the nucleotide sequence of HCV cDNA in clone ag30a and the overlap with clone CA290a.

[0204] FIG. 59 shows the nucleotide sequence of HCV cDNA in clone CA205a, and the overlap with the HCV cDNA sequence in clone CA290a.

[0205] FIG. 60 shows the nucleotide sequence of HCV cDNA in clone 18g, and the overlap with the HCV cDNA sequence in clone ag30a.

[0206] FIG. 61 shows the nucleotide sequence of HCV cDNA in clone 16jh, the amino acids encoded therein, and the overlap of nucleotides with the HCV cDNA sequence in clone 15e.

[0207] FIG. 62 shows the composite sequence of the HCV cDNA sense strand deduced from overlapping clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, and 16jh.

[0208] FIG. 62A shows the sequence of FIG. 62, but includes the complementary cDNA strand.

[0209] FIG. 63 shows the relative positions of the clones from which HCV cDNA was isolated for expression and antigenic mapping of the putative HCV polyprotein.

[0210] FIG. 64 is a diagram of the immunological colony screening method used in antigenic mapping studies.

[0211] FIG. 65 presents the antigenicity of polypeptides expressed from HCV cDNA clones used in an antigenic mapping study of the putative HCV polyprotein.

[0212] FIG. 66 presents the amino acid sequence of the putative polyprotein encoded in a composite HCV cDNA sequence shown in FIG. 62.

[0213] FIG. 67 is a tracing of the hydrophilicity/hydrophobicity profile and of the antigenic index of the putative HCV polyprotein.

[0214] FIG. 68 shows the conserved co-linear peptides in HCV and Flaviviruses.

[0215] FIG. 69 shows schematic alignment of a flaviviral polyprotein and a putative HCV polyprotein encoded in the major ORF of the HCV genome. Also indicated in the figure are the possible functions of the flaviviral polypeptides cleaved from the flaviral polyprotein. In addition, the relative placements of the HCV polypeptides, NANB$_{5-1-1}$ and C100, with respect to the putative HCV polyprotein are indicated.

[0216] **FIG. 70** shows relevant characteristics of AcNPV transfer vectors used for high level expression of nonfused foreign proteins. It also shows a restriction endonuclease map of the transfer vector pAc373.

[0217] **FIG. 71** shows the nucleotide sequence of clone 6k, the part of the sequence which overlaps clone 16jh, and the amino acids encoded therein.

[0218] **FIG. 72** shows a composite cDNA sequence derived from overlapping clones clones bi14a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, 16jh and 6k; also shown are the amino acids encoded in the positive strand of the cDNA (which is the equivalent of the HCV RNA).

[0219] **FIG. 73** shows the linkers used in the construction of pS3-56$_{C100m}$.

[0220] **FIG. 74** shows the nucleotide sequence of the HCV cDNA in clone 31, the amino acids encoded therein, and putative restriction enzyme sites encoded therein.

[0221] **FIG. 75** shows the nucleotide sequence of the HCV cDNA in clone p131jh, and its overlap with the nucleotide sequence in clone 6k.

[0222] **FIG. 76** shows a flow chart for construction of the expression vector pC100⁻d#3.

[0223] **FIG. 77** shows a flow chart for construction of the expression vector pS2d#9.

[0224] **FIG. 78** shows a flow chart for construction of the expression vector pNS11d/13.

[0225] **FIG. 79** shows the nucleotide sequence of HCV cDNA in the C200-C100 construct, the amino acids encoded therein, and putative restriction enzyme sites encoded therein.

[0226] **FIG. 80** shows the nucleotide consensus sequence of human isolate 23, variant sequences are shown below the sequence line. The amino acids encoded in the consensus sequence are also shown.

[0227] **FIG. 81** shows the nucleotide consensus sequence of human isolate 27, variant sequences are shown below the sequence line. The amino acids encoded in the consensus sequence are also shown.

[0228] **FIG. 82** shows the aligned nucleotide sequences of human isolates 23 and 27 and of HCV1. Homologous sequences are indicated by the symbol (*). Non homologous sequences are in small letters.

[0229] **FIG. 83** shows the aligned amino acid sequences of human isolates 23 and 27 and of HCV1. Homologous sequences are indicated by the symbol (*). Non homologous sequences are in small letters.

[0230] **FIG. 84** is a graph showing the relationship of the EnvL and EnvR primers to the model flavivirus polyprotein and putative HCV polyprotein.

[0231] **FIG. 85** shows a comparison of the composite aligned nucleotide sequences of isolates Thorn, EC1, HCT #18, and HCV1.

[0232] **FIG. 86** shows a comparison of the nucleotide sequences of EC10 and a composite of the HCV1 sequence; the EC10 sequence is on the line above the dots, and the HCV1 sequence is on the line below the dots.

[0233] **FIG. 87** shows a comparison of the amino acid sequences 117-308 (relative to HCV1) encoded in the "EnvL" regions of the consensus sequences of human isolates HCT #18, JH23, JH27, Thorne, EC1, and of HCV1.

[0234] **FIG. 88** shows a comparison of the amino acid sequences 330-360 (relative to HCV1) encoded in the "EnvR" regions of the consensus sequences of human isolates HCT #18, JH23, JH 27, Thorne, EC1. and of HCV1.

[0235] **FIG. 89** shows a composite cDNA sequence for HCV1, deduced from overlapping clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, 16jh, 6k, and 131jh.

[0236] **FIG. 90** shows a putative polyprotein encoded in the HCV cDNA shown in **FIG. 89**.

[0237] **FIG. 91** is a flow chart for the preparation of expression vector pC22.

MODES FOR CARRYING OUT THE INVENTION

[0238] I. Definitions

[0239] The term "hepatitis C virus" has been reserved by workers in the field for an heretofore unknown etiologic agent of NANBH. Accordingly, as used herein, "hepatitis C virus" (HCV) refers to an agent causitive of NANBH, which was formerly referred to as NANBV and/or BB-NANBV. The terms HCV, NANBV, and BB-NANBV are used interchangeably herein. As an extension of this terminology, the disease caused by HCV, formerly called NANB hepatitis (NANBH), is called hepatitis C. The terms NANBH and hepatitis C may be used interchangeably herein.

[0240] HCV is a viral species of which pathogenic strains cause NANBH, and attenuated strains or defective interfering particles derived therefrom. As shown infra, the HCV genome is comprised of RNA. It is known that RNA containing viruses have relatively high rates of spontaneous mutation, i.e., reportedly on the order of 10^{-3} to 10^{-4} per incorporated nucleotide (Fields & Knipe (1986)). Therefore, since heterogeneity and fluidity of genotype are inherent in RNA viruses, there are multiple strains/isolates, which may be virulent or avirulent, within the HCV species. The compositions and methods described herein, enable the propagation, identification, detection, and isolation of the various HCV strains or isolates. Moreover, the disclosure herein allows the preparation of diagnostics and vaccines for the various strains/isolates, as well as compositions and methods that have utility in screening procedures for antiviral agents for pharmacologic use, such as agents that inhibit replication of HCV.

[0241] Information on several different strains/isolates of HCV is disclosed herein, particularly strain or isolate CDC/HCVI (also called HCV1). Information from one strain or isolate, such as a partial genomic sequence, is sufficient to allow those skilled in the art using standard techniques to

isolate new strains/isolates and to identify whether such new strains/isolates are HCV. For example, several different strains/isolates are described in Section IV.H.7., infra. These strains, which were obtained from a number of human sera (and from different geographical areas), were isolated utilizing the information from the genomic sequence of HCV1.

[0242] The information provided herein is indicative that HCV may be distantly related to the flaviviridae. The Flavivirus family contains a large number of viruses which are small, enveloped pathogens of man. The morphology and composition of Flavivirus particles are known, and are discussed in Brinton (1986). Generally, with respect to morphology, Flaviviruses contain a central nucleocapsid surrounded by a lipid bilayer. Virions are spherical and have a diameter of about 40-50 nm. Their cores are about 25-30 nm in diameter. Along the outer surface of the virion envelope are projections that are about 5-10 nm long with terminal knobs about 2 nm in diameter. Typical examples of the family include Yellow Fever virus, West Nile virus, and Dengue Fever virus. They possess positive-stranded RNA genomes (~11,000 nucleotides) that are slightly larger than that of HCV and encode a polyprotein precursor of about 3500 amino acids. Individual viral proteins are cleaved from this precursor polypeptide.

[0243] Using the techniques derived infra, the genomic structure and the nucleotide sequence of HCV1 genomic RNA has been deduced. The genome appears to be single-stranded RNA containing ~10,000 nucleotides. The genome is positive-stranded, and possesses a continuous, translational open reading frame (ORF) that encodes a polyprotein of about 3,000 amino acids. In the ORF, the structural protein(s) appear to be encoded in approximately the first quarter of the N-terminus region, with the majority of the polyprotein responsible for non-structural proteins. When compared with all known viral sequences, small but significant co-linear homologies are observed with the non-structural proteins of the flavivirus family, and with the pestiviruses (which are now also considered to be part of the Flavirus family).

[0244] A schematic alignment of possible regions of a flaviviral polyprotein (using Yellow Fever Virus as an example), and of a putative polyprotein encoded in the major ORF of the HCV genome, is shown in Fig. **FIG. 69**. In the figure the possible domains of the HCV polyprotein are indicated. The flavivirus polyprotein contains, from the amino terminus to the carboxy terminus, the nucleocapsid protein (C), the matrix protein (M), the envelope protein (E), and the non-structural proteins 1, 2 (a+b), 3, 4 (a+b), and 5 (NS1, NS2, NS3, NS4, and NS5). Based upon the putative amino acids encoded in the nucleotide sequence of HCV1, a small domain at the extreme N-terminus of the HCV polyprotein appears similar both in size and high content of basic residues to the nucleocapsid protein (C) found at the N-terminus of flaviviral polyproteins. The non-structural proteins 2,3,4, and 5 (NS2-5) of HCV and of yellow fever virus (YFV) appear to have counter parts of similar size and hydropathicity, although there is divergence of the amino acid sequences. However, the region of HCV which would correspond to the regions of YFV polyprotein which contains the M, E, and NS1 protein not only differs in sequence, but also appears to be quite different both in size and hydropathicity. Thus, while certain domains of the HCV genome may be referred to herein as, for example, NS1, or

NS2, it should be borne in mind that these designations are speculative; there may be considerable differences between the HCV family and flaviviruses that have yet to be appreciated.

[0245] Based upon the nucleotide sequences encoding the polypeptides NANB$_{5-1-1}$ and HCV C100, and the sequence of the ORF, the relative placements of the 5-1-1 polypeptide and the C100 polypeptide with respect to the putative HCV polyprotein have been calculated. These are also shown in **FIG. 69**.

[0246] Different strains, isolates or subtypes of HCV are expected to contain variations at the amino acid and nucleic acids compared with HCV1. Many isolates are expected to show much (i.e., more than about 40%) homology in the total amino acid sequence compared with HCV1. However, it may also be found that there are other less homologous HCV isolates. These would be defined as HCV according to various criteria such as, for example, an ORF of approximately 9,000 nucleotides to approximately 12,000 nucleotides, encoding a polyprotein similar in size to that of HCV1, an encoded polyprotein of similar hydrophobic and/or antigenic character to that of HCV1, and the presence of co-linear peptide sequences that are conserved with HCV1. In addition, the genome would be a positive-stranded RNA.

[0247] HCV encodes at least one epitope which is immunologically identifiable with an epitope in the HCV genome from which the cDNAs described herein are derived; preferably the epitope is contained in an amino acid sequence described herein. The epitope is unique to HCV when compared to previously known Flaviviruses. The uniqueness of the epitope may be determined by its immunological reactivity with anti-HCV antibodies and lack of immunological reactivity with antibodies to known Flavivirus species. Methods for determining immunological reactivity are known in the art, for example, by radioimmunoassay, by Elisa assay, by hemagglutination, and several examples of suitable techniques for assays are provided herein.

[0248] In addition to the above, the following parameters of nucleic acid homology and amino acid homology are applicable, either alone or in combination, in identifying a strain/isolate as HCV. Since HCV strains and isolates are evolutionarily related, it is expected that the overall homology of the genomes at the nucleotide level may be about 10% or greater, probably will be about 40% or greater, probably about 60% or greater, and even more probably about 80% or greater; and in addition that there will be corresponding contiguous sequences of at least about 13 nucleotides. It should be noted, as shown infra, that there are variable and hypervariable regions within the HCV genome; therefore, the homology in these regions is expected to be significantly less than that in the overall genome. The correspondence between the putative HCV strain genomic sequence and, for example, the CDC/HCV1 cDNA sequence can be determined by techniques known in the art. For example, they can be determined by a direct comparison of the sequence information of the polynucleotide from the putative HCV, and the HCV cDNA sequencers) described herein. For example, also, they can be determined by hybridization of the polynucleotides under conditions which form stable duplexes between homologous regions (for example, those which would be used prior to S$_1$ digestion), followed

by digestion with single stranded specific nuclease(s), followed by size determination of the digested fragments.

[0249] Because of the evolutionary relationship of the strains or isolates of HCV, putative HCV strains or isolates are identifiable by their homology at the polypeptide level. Generally, HCV strains or isolates are expected to be at least 10% homologous, more than about 40% homologous, probably more than about 70% homologous, and even more probably more than about 80% homologous, and some may even be more than about 90% homologous at the polypeptide level. The techniques for determining amino acid sequence homology are known in the art. For example, the amino acid sequence may be determined directly and compared to the sequences provided herein. Alternatively the nucleotide sequence of the genomic material of the putative HCV may be determined (usually via a cDNA intermediate), the amino acid sequence encoded therein can be determined, and the corresponding regions compared.

[0250] As used herein, a polynucleotide "derived from" a designated sequence refers to a polynucleotide sequence which is comprised of a sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding to a region of the designated nucleotide sequence. "Corresponding" means homologous to or complementary to the designated sequence. Preferably, the sequence of the region from which the polynucleotide is derived is homologous to or complementary to a sequence which is unique to an HCV genome. Whether or not a sequence is unique to the HCV genome can be determined by techniques known to those of skill in the art. For example, the sequence can be compared to sequences in databanks, e.g., Genebank, to determine whether it is present in the uninfected host or other organisms. The sequence can also be compared to the known sequences of other viral agents, including those which are known to induce hepatitis, e.g., HAV, HBV, and HDV, and to members of the Flaviviridae. The correspondence or non-correspondence of the derived sequence to other sequences can also be determined by hybridization under the appropriate stringency conditions. Hybridization techniques for determining the complementarity of nucleic acid sequences are known in the art, and are discussed infra. See also, for example, Maniatis et al. (1982). In addition, mismatches of duplex polynucleotides formed by hybridization can be determined by known techniques, including for example, digestion with a nuclease such as S1 that specifically digests single-stranded areas in duplex polynucleotides. Regions from which typical DNA sequences may be "derived" include but are not limited to, for example, regions encoding specific epitopes, as well as non-transcribed and/or non-translated regions.

[0251] The derived polynucleotide is not necessarily physically derived from the nucleotide sequence shown, but may be generated in any manner, including for example, chemical synthesis or DNA replication or reverse transcription or transcription. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use.

[0252] Similarly, a polypeptide or amino acid sequence "derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least 3-5 amino acids, and more preferably at least 8-10 amino acids, and even more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence. This terminology also includes a polypeptide expressed from a designated nucleic acid sequence.

[0253] A recombinant or derived polypeptide is not necessarily translated from a designated nucleic acid sequence, for example, the sequences in Section IV.A, or from an HCV genome; it may be generated in any manner, including for example, chemical synthesis, or expression of a recombinant expression system, or isolation from HCV, including mutated HCV. A recombinant or derived polypeptide may include one or more analogs of amino acids or unnatural amino acids in its sequence. Methods of inserting analogs of amino acids into a sequence are known in the art. It also may include one or more labels, which are known to those of skill in the art.

[0254] The term "recombinant polynucleotide" as used herein intends a polynucleotide of genomic, cDNA, semi-synthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide other than that to which it is linked in nature, or (3) does not occur in nature.

[0255] The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA and RNA. It also includes known types of modifications, for example, labels which are known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

[0256] The term "purified viral polynucleotide" refers to an HCV genome or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and even more preferably less than about 90% of polypeptides with which the viral polynucleotide is naturally associated. Techniques for purifying viral polynucleotides from viral particles are known in the art, and include for example, disruption of the particle with a chaotropic agent, differential extraction and separation of the polynucleotide(s) and polypeptides by ion-exchange chromatography, affinity chromatography, and sedimentation according to density.

[0257] The term "purified viral polypeptide" refers to an HCV polypeptide or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than

about 70%, and even more preferably less than about 90%, of cellular components with which the viral polypeptide is naturally associated. Techniques for purifying viral polypeptides are known in the art, and examples of these techniques are discussed infra.

[0258] "Recombinant host cells", "host cells", "cells", "cell lines", "cell cultures", and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

[0259] A "replicon" is any genetic element, e.g., a plasmid, a chromosome, a virus, a cosmid, etc. that behaves as an autonomous unit of polynucleotide replication within a cell; i.e., capable of replication under its own control.

[0260] A "vector" is a replicon in which another polynucleotide segment is attached, so as to bring about the replication and/or expression of the attached segment.

[0261] "Control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and terminators; in eukaryotes, generally, such control sequences include promoters, terminators and, in some instances, enhancers. The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression, and may also include additional components whose presence is advantageous, for example, leader sequences.

[0262] "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[0263] An "open reading frame" (ORF) is a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

[0264] A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, and recombinant polynucleotide sequences.

[0265] "Immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptides(s) which are also present in the designated polypeptide(s), usually HCV proteins. Immunological identity may be determined by

antibody binding and/or competition in binding; these techniques are known to those of average skill in the art, and are also illustrated infra.

[0266] As used herein, "epitope" refers to an antigenic determinant of a polypeptide. An epitope could comprise 3 amino acids in a spatial conformation which is unique to the epitope. Generally an epitope consists of at least 5 such amino acids, and more usually, consists of at least 8-10 such amino acids. Methods of determining the spatial conformation of amino acids are known in the art, and include, for example, x-ray crystallography and 2dimensional nuclear magnetic resonance.

[0267] A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by competition in binding using as competitors a known polypeptide(s) containing an epitope against which the antibody is directed. The techniques for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

[0268] As used herein, the term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one antibody combining site. An "antibody combining site" or "binding domain" is formed from the folding of variable domains of an antibody molecule(s) to form three-dimensional binding spaces with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows an immunological reaction with the antigen. An antibody combining site may be formed from a heavy and/or a light chain domain (VH and VL, respectively), which form hypervariable loops which contribute to antigen binding. The term "antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, altered antibodies, univalent antibodies, the Fab proteins, and single domain antibodies.

[0269] As used herein, a "single domain antibody" (dAb) is an antibody which is comprised of an VH domain, which reacts immunologically with a designated antigen. A dAB does not contain a VL domain, but may contain other antigen binding domains known to exist in antibodies, for example, the kappa and lambda domains. Methods for preparing dABs are known in the art. See, for example, Ward et al. (1989).

[0270] Antibodies may also be comprised of VH and VL domains, as well as other known antigen binding domains. Examples of these types of antibodies and methods for their preparation are known in the art (see, e.g., U.S. Pat. No. 4,816,467, which is incorporated herein by reference), and include the following. For example, "vertebrate antibodies" refers to antibodies which are tetramers or aggregates thereof, comprising light and heavy chains which are usually aggregated in a "Y" configuration and which may or may not have covalent linkages between the chains. In vertebrate antibodies, the amino acid sequences of all the chains of a particular antibody are homologous with the chains found in one antibody produced by the lymphocyte which produces that antibody in situ, or in vitro (for example, in hybridomas). Vertebrate antibodies typicallly include native antibodies, for example, purified polyclonal antibodies and

monoclonal antibodies. Examples of the methods for the preparation of these antibodies are described infra.

[0271] "Hybrid antibodies" are antibodies wherein one pair of heavy and light chains is homologous to those in a first antibody, while the other pair of heavy and light chains is homologous to those in a different second antibody. Typically, each of these two pairs will bind different epitopes, particularly on different antigens. This results in the property of "divalence", i.e., the ability to bind two antigens simultaneously. Such hybrids may also be formed using chimeric chains, as set forth below.

[0272] "Chimeric antibodies", are antibodies in which the heavy and/or light chains are fusion proteins. Typically the constant domain of the chains is from one particular species and/or class, and the variable domains are from a different species and/or class. Also included is any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be differing classes, or different species of origin, and whether or not the fusion point is at the variable/constant boundary. Thus, it is possible to produce antibodies in which neither the constant nor the variable region mimic known antibody sequences. It then becomes possible, for example, to construct antibodies whose variable region has a higher specific affinity for a particular antigen, or whose constant region can elicit enhanced complement fixation, or to make other improvements in properties possessed by a particular constant region.

[0273] Another example is "altered antibodies", which refers to antibodies in which the naturally occurring amino acid sequence in a vertebrate antibody has been varied. Utilizing recombinant DNA techniques, antibodies can be redesigned to obtain desired characteristics. The possible variations are many, and range from the changing of one or more amino acids to the complete redesign of a region, for example, the constant region. Changes in the constant region, in general, to attain desired cellular process characteristics, e.g., changes in complement fixation, interaction with membranes, and other effector functions. Changes in the variable region may be made to alter antigen binding characeristics. The antibody may also be engineered to aid the specific delivery of a molecule or substance to a specific cell or tissue site. The desired alterations may be made by known techniques in molecular biology, e.g., recombinant techniques, site directed mutagenesis, etc.

[0274] Yet another example are "univalent antibodies", which are aggregates comprised of a heavy chain/light chain dimer bound to the Fc (i.e., constant) region of a second heavy chain. This type of antibody escapes antigenic modulation. See, e.g., Glennie et al. (1982).

[0275] Included also within the definition of antibodies are "Fab" fragments of antibodies. The "Fab" region refers to those portions of the heavy and light chains which are roughly equivalent, or analogous, to the sequences which comprise the branch portion of the heavy and light chains, and which have been shown to exhibit immunological binding to a specified antigen, but which lack the effector Fc portion . "Fab" includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers containing the 2H and 2L chains (referred to as F(ab)$_2$), which are capable of selectively reacting with a designated antigen or antigen family. "Fab" antibodies may be divided into subsets analogous to those described above, i.e, "vertebrate Fab", "hybrid Fa", "chimeric Fab", and "altered Fab". Methods of producing "Fab" fragments of antibodies are known within the art and include, for example, proteolysis, and synthesis by recombinant techniques.

[0276] As used herein, the term "immunogenic polypeptide" is a polypeptide that elicits a cellular and/or humoral immune response, whether alone or linked to a carrier in the presence or absence of an adjuvant.

[0277] The term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

[0278] "Transformation", as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, direct uptake, transduction, f-mating or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

[0279] "Treatment" as used herein refers to prophylaxis and/or therapy.

[0280] An "individual", as used herein, refers to vertebrates, particularly members of the mammalian species, and includes but is not limited to domestic animals, sports animals, and primates, including humans.

[0281] As used herein, the "sense strand"0 of a nucleic acid contains the sequence that has sequence homology to that of mRNA. The "anti-sense strand" contains a sequence which is complementary to that of the "sense strand".

[0282] As used herein, a "positive stranded genome" of a virus is one in which the genome, whether RNA or DNA, is single-stranded and which encodes a viral polypeptide(s). Examples of positive stranded RNA viruses include Togaviridae, Coronaviridae, Retroviridae, Picornaviridae, and Caliciviridae. Included also, are the Flaviviridae, which were formerly classified as Togaviradae. See Fields & Knipe (1986).

[0283] As used herein, "antibody containing body component" refers to a component of an individuals body which is a source of the antibodies of interest. Antibody containing body components are known in the art, and include but are not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

[0284] As used herein, "purified HCV" refers to a preparation of HCV which has been isolated from the cellular constituents with which the virus is normally associated, and from other types of viruses which may be present in the infected tissue. The techniques for isolating viruses are

known to those of skill in the art, and include, for example, centrifugation and affinity chromatography; a method of preparing purified HCV is discussed infra.

[0285] The term "HCV particles" as used herein include entire virion as well as particles which are intermediates in virion formation. HCV particles generally have one or more HCV proteins associated with the HCV nucleic acid.

[0286] As used herein, the term "probe" refers to a polynucleotide which forms a hybrid structure with a sequence in a target region, due to complementarity of at least one sequence in-the probe with a sequence in the target region.

[0287] As used herein, the term "target region" refers to a region of the nucleic acid which is to be amplified and/or detected.

[0288] As used herein, the term "viral RNA", which includes HCV RNA, refers to RNA from the viral genome, fragments thereof, transcripts thereof, and mutant sequences derived therefrom.

[0289] As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from an individual, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs, and also samples of in vitro cell culture constituents (including but not limited to conditioned medium resulting from the growth of cells in cell culture medium, putatively virally infected cells, recombinant cells, and cell components).

[0290] II. Description of the Invention

[0291] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See e.g., Maniatis, Fitsch & Sambrook, MOLECULAR CLONING; A LABORATORY MANUAL (1982); DNA CLONING, VOLUMES I AND II (D. N Glover ed. 1985); OLIGO-NUCLEOTIDE SYNTHESIS (M. J. Gait ed, 1984); NUCLEIC ACID HYBRIDIZATION (B. D. Hames & S. J. Higgins eds. 1984); TRANSCRIPTION AND TRANSLA-TION (B. D. Hames & S. J. Higgins eds. 1984); ANIMAL CELL CULTURE (R. I. Freshney ed. 1986); IMMOBI-LIZED CELLS AND ENZYMES (IRL Press, 1986); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLON-ING (1984); the series, METHODS IN ENZYMOLOGY (Academic Press, Inc.); GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (J. H. Miller and M. P. Calos eds. 1987, Cold Spring Harbor Laboratory), Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively), Mayer and Walker, eds. (1987, IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London), Scopes, (1987), PROTEIN PURIFICATION: PRINCIPLES AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D. M. Weir and C. C. Blackwell eds 1986). All patents, patent applications, and publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

[0292] The useful materials and processes of the present invention are made possible by the provision of a family of closely homologous nucleotide sequences isolated from a cDNA library derived from nucleic acid sequences present in the plasma of an HCV infected chimpanzee. This family of nucleotide sequences is not of human or chimpanzee origin, since it hybridizes to neither human nor chimpanzee genomic DNA from uninfected individuals, since nucleotides of this family of sequences are present only in liver and plasma of chimpanzees with HCV infection, and since the sequence is not present in Genebank. In addition, the family of sequences shows no significant homology to sequences contained within the HBV genome.

[0293] The sequence of one member of the family, contained within clone 5-1-1, has one continuous open reading frame (ORF) which encodes a polypeptide of approximately 50 amino acids. Sera from HCV infected humans contain antibodies which bind to this polypeptide, whereas sera from non-infected humans do not contain antibodies to this polypeptide. Moreover, whereas the sera from uninfected chimpanzees do not contain antibodies to this polypeptide, the antibodies are induced in chimpanzees following acute NANBH infection. In addition, antibodies to this polypeptide are not detected in chimps and humans infected with HAV and HBV. By these criteria the sequence is a cDNA to a viral sequence, wherein the virus causes or is associated with NANBH; this cDNA sequence is shown in **FIG. 1**. As discussed infra, the cDNA sequence in clone 5-1-1 differs from that of the other isolated cDNAs in that it contains 28 extra base pairs.

[0294] A composite of other identified members of the cDNA family, which were isolated using as a probe a synthetic sequence equivalent to a fragment of the cDNA in clone 5-1-1, is shown in **FIG. 3**. A member of the cDNA family which was isolated using a synthetic sequence derived from the cDNA in clone 81 is shown in **FIG. 5**, and the composite of this sequence with that of clone 81 is shown in **FIG. 6**. Other members of the cDNA family are described in Section IV.A. A composite of the cDNAs in these clones is shown in **FIG. 62**. The composite cDNA shows that it contains one continuous ORF, and thus encodes a polyprotein. This data is consistent with the suggestion, discussed infra., that HCV is a flavi-like virus.

[0295] The availability of the family of cDNAs shown herein in Section IV.A permits the construction of DNA probes and polypeptides useful in diagnosing NANBH due to HCV infection and in screening blood donors as well as donated blood and blood products for infection. For example, from the sequences it is possible to synthesize DNA oligomers of about 8-10 nucleotides, or larger, which are useful as hybridization probes to detect the presence of HCV RNA in, for example, sera of subjects suspected of harboring the virus, or for screening donated blood for the presence of the virus. The family of cDNA sequences also allows the design and production of HCV specific polypeptides which are useful as diagnostic reagents for the presence of antibodies raised during NANBH. Antibodies to purified polypeptides derived from the cDNAs may also be used to detect viral antigens in infected individuals and in blood.

[0296] Knowledge of these cDNA sequences also enables the design and production of polypeptides which may be used as vaccines against HCV and also for the production of antibodies, which in turn may be used for protection against the disease, and/or for therapy of HCV infected individuals.

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[0297] Moreover, the family of cDNA sequences enables further characterization of the HCV genome. Polynucleotide probes derived from these sequences may be used to screen cDNA libraries for additional overlapping cDNA sequences, which, in turn, may be used to obtain more overlapping sequences. Unless the genome is segmented and the segments lack common sequences, this technique may be used to gain the sequence of the entire genome. However, if the genome is segmented, other segments of the genome can be obtained by repeating the lambda-gt11 serological screening procedure used to isolate the cDNA clones described herein, or alternatively by isolating the genome from purified HCV particles.

[0298] The family of cDNA sequences and the polypeptides derived from these sequences, as well as antibodies directed against these polypeptides are also useful in the isolation and identification of the BB-NANBV agent(s). For example, antibodies directed against HCV epitopes contained in polypeptides derived from the cDNAs may be used in processes based upon affinity chromatography to isolate the virus. Alternatively, the antibodies may be used to identify viral particles isolated by other techniques. The viral antigens and the genomic material within the isolated viral particles may then be further characterized.

[0299] The information obtained from further sequencing of the HCV genome(s), as well as from further characterization of the HCV antigens and characterization of the genome enables the design and synthesis of additional probes and polypeptides and antibodies which may be used for diagnosis, for prevention, and for therapy of HCV induced NANBH, and for screening for infected blood and blood-related products.

[0300] The availability of probes for HCV, including antigens and antibodies, and polynucleotides derived from the genome from which the family of cDNAs is derived also allows for the development of tissue culture systems which will be of major use in elucidating the biology of HCV. This in turn, may lead to the development of new treatment regimens based upon antiviral compounds which preferentially inhibit the replication of, or infection by HCV.

[0301] In addition to the above, the information provided infra allows the identification of additional HCV strains or isolates. The isolation and characterization of the additional HCV strains or isolates may be accomplished by isolating the nucleic acids from body components which contain viral particles and/or viral RNA, creating cDNA libraries using polynucleotide probes based on the HCV cDNA probes described infra., screening the libraries for clones containing HCV cDNA sequences described infra., and comparing the HCV cDNAs from the new isolates with the cDNAs described infra. The polypeptides encoded therein, or in the viral genome, may be monitored for immunological cross-reactivity utilizing the polypeptides and antibodies described supra. Strains or isolates which fit within the parameters of HCV, as described in the Definitions section, supra., are readily identifiable. Other methods for identifying HCV strains will be obvious to those of skill in the art, based upon the information provided herein.

[0302] The method used to identify and isolate the etiologic agent for NANBH may be applicable to the identification and/or isolation of heretofore uncharacterized agents which contain a genome, and which are associated with a variety of diseases, including those induced by viruses, viroids, bacteria, fungi and parasites. In this method, a cDNA library was created from the nucleic acids present in infected tissue from an infected individual. The library was created in a vector which allowed the expression of polypeptides encoded in the cDNA. Clones of host cells containing the vector, which expressed an immunologically reactive fragment of a polypeptide of the etiologic agent, were selected by immunological screening of the expression products of the library with an antibody-containing body component from another individual previously infected with the putative agent. The steps in the immunological screening technique included interacting the expression products of the cDNA containing vectors with the antibody-containing body component of a second infected individual, and detecting the formation of antibody-antigen complexes between the expression product(s) and antibodies of the second infected individual. The isolated clones are screened further immunologically by interacting their expression products with the antibody-containing body components of other individuals infected with the putative agent and with control individuals uninfected with the putative agent, and detecting the formation of antigen-antibody complexes with antibodies from the infected individuals; and the cDNA containing vectors which encode polypeptides which react immunologically with antibodies from infected individuals and individuals suspected of being infected with the agent, but not with control individuals are isolated. The infected individuals used for the construction of the cDNA library, and for the immunological screening need not be of the same species.

[0303] The cDNAs isolated as a result of this method, and their expression products, and antibodies directed against the expression products, are useful in characterizing and/or capturing the etiologic agent. As described in more detail infra, this method has been used successfully to isolate a family of cDNAs derived from the HCV genome.

[0304] II.A. Preparation of the cDNA Sequences

[0305] Pooled serum from a chimpanzee with chronic HCV infection and containing a high titer of the virus, i.e., at least 10^6 chimp infectious doses/ml (CID/ml) was used to isolate viral particles; nucleic acids isolated from these particles was used as the template in the construction of a cDNA library to the viral genome. The procedures for isolation of putative HCV particles and for constructing the cDNA library in lambda-gt11 is discussed in Section IV.A.1. Lambda-gt11 is a vector that has been developed specifically to express inserted cDNAs as fusion polypeptides with beta-galactosidase and to screen large numbers of recombinant phage with specific antisera raised against a defined antigen. Huynh, T. V. et al. (1985). The lambda-gt11 cDNA library generated from a cDNA pool containing cDNA of approximate mean size of 200 base pairs was screened for encoded epitopes that could bind specifically with sera derived from patients who had previously experienced NANB hepatitis. Approximately 10^6 phages were screened, and five positive phages were identified, purified, and then further tested for specificity of binding to sera from different humans and chimpanzees previously infected with the HCV agent. One of the phages, 5-1-1, bound 5 of the 8 human sera tested. This binding appeared selective for sera derived from patients with prior NANB hepatitis infections since 7 normal blood donor sera did not exhibit such binding.

[0306] The sequence of the cDNA in recombinant phage 5-1-1 was determined, and is shown in **FIG. 1**. The polypeptide encoded by this cloned cDNA, which is in the same translational frame as the N-terminal beta-Galactosidase moiety of the fusion polypeptide is shown above the nucleotide sequence. This translational ORF, therefore, encodes an epitope(s) specifically recognized by sera from patients with NANB hepatitis infections.

[0307] The availability of the cDNA in recombinant phage 5-1-1 has allowed for the isolation of other clones containing additional segments and/or alternative segments of cDNA to the viral genome. The lambda-gt11 cDNA library described supra, was screened using a synthetic polynucleotide derived from the sequence of the cloned 5-1-1 cDNA. This screening yielded three other clones, which were identified as 81, 1-2 and 91; the cDNAs contained within these clones were sequenced. See Sections IV.A.3. and IV.A.4. The homologies between the four independent clones are shown in **FIG. 2**, where the homologies are indicated by the vertical lines. Sequences of nucleotides present uniquely in clones 5-1-1, 81, and 91 are indicated by small letters.

[0308] The cloned cDNAs present in recombinant phages in clones 5-1-1, 81, 1-2, and 91 are highly homologous, and differ in only two regions. First, nucleotide number 67 in clone 1-2 is a thymidine, whereas the other three clones contain a cytidine residue in this position. This substitution, however, does not alter the nature of the encoded amino acid.

[0309] The second difference between the clones is that clone 5-1-1 contains 28 base pairs at its 5'-terminus which are not present in the other clones. The extra sequence may be a 5'-terminal cloning artifact; 5'-terminal cloning artifacts are commonly observed in the products of cDNA methods.

[0310] Synthetic sequences derived from the 5'-region and the 3'-region of the HCV cDNA in clone 81 were used to screen and isolate cDNAs from the lambda-gt11 NANBV cDNA library, which overlapped clone 81 cDNA (Section IV.A.5.). The sequences of the resulting cDNAs, which are in clone 36 and clone 32, respectively, are shown in **FIG. 5** and **FIG. 7**.

[0311] Similarly, a synthetic polynucleotide based on the 5'-region of clone 36 was used to screen and isolate cDNAs from the lambda gt-11 NANBV cDNA library which overlapped clone 36 cDNA (Section IV.A.8.). A purified clone of recombinant phage-containing cDNA which hybridized to the synthetic polynucleotide probe was named clone 35 and the NANBV cDNA sequence contained within this clone is shown in **FIG. 8**.

[0312] By utilizing the technique of isolating overlapping cDNA sequences, clones containing additional upstream and downstream HCV cDNA sequences have been obtained. The isolation of these clones, is described infra in Section IV.A.

[0313] Analysis of the nucleotide sequences of the HCV cDNAs encoded within the isolated clones show that the composite cDNA contains one long continuous ORF. **FIG. 62** shows the sequence of the composite cDNA from these clones, along with the putative HCV polypeptide encoded therein.

[0314] The description of the method to retrieve the cDNA sequences is mostly of historical interest. The resultant

sequences (and their complements) are provided herein, and the sequences, or any portion thereof, could be prepared using synthetic methods, or by a combination of synthetic methods with retrieval of partial sequences using methods similar to those described herein.

[0315] The description above, of "walking" the genome by isolating overlapping cDNA sequences from the HCV lambda gt-11 library provides one method by which cDNAs corresponding to the entire HCV genome may be isolated. However, given the information provided herein, other methods for isolating these cDNAs are obvious to one of skill in the art. Some of these methods are described in Section IV.A., infra.

[0316] II.B. Preparation of Viral Polypeptides and Fragments

[0317] The availability of cDNA sequences, either those isolated by utilizing the cDNA sequences described in Section IV.A, as discussed infra, or nucleotide sequences derived therefrom (including segments and modifications of the sequence), permits the construction of expression vectors encoding antigenically active regions of the polypeptide encoded in either strand. These antigenically active regions may be derived from coat or envelope antigens or from core antigens, or from antigens which are non-structural including, for example, polynucleotide binding proteins, polynucleotide polymerase(s), and other viral proteins required for the replication and/or assembly of the virus particle. Fragments encoding the desired polypeptides are derived from the cDNA clones using conventional restriction digestion or by synthetic methods, and are ligated into vectors which may, for example, contain portions of fusion sequences such as beta-Galactosidase or superoxide dismutase (SOD), preferably SOD. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European Patent Office Publication number 0196056, published Oct. 1, 1986. Vectors encoding fusion polypeptides of SOD and HCV polypeptides, i.e., $NANB_{5-1-1}$, $NANB_{81}$, and C100-3, which is encoded in a composite of HCV cDNAs, are described in Sections IV.B.1, IV.B.2, and IV.B.4, respectively. Any desired portion of the HCV cDNA containing an open reading frame, in either sense strand, can be obtained as a recombinant polypeptide, such as a mature or fusion protein; alternatively, a polypeptide encoded in the cDNA can be provided by chemical synthesis.

[0318] The DNA encoding the desired polypeptide, whether in fused or mature form, and whether or not containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems are presently used in forming recombinant polypeptides, and a summary of some of the more common control systems and host cell lines is given in Section III.A., infra. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification may be by techniques known in the art, for example, differential extraction, salt fractionation, chromatography on ion exchange resins, affinity chromatography, centrifugation, and the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins. Such polypeptides can be used as diagnostics, or those which give rise to neutralizing antibodies may be formulated

into vaccines. Antibodies raised against these polypeptides can also be used as diagnostics, or for passive immunotherapy. In addition, as discussed in Section-II.J. herein below, antibodies to these polypeptides are useful for isolating and identifying HCV particles.

[0319] The HCV antigens may also be isolated from HCV virions. The virions may be grown in HCV infected cells in tissue culture, or in an infected host.

[0320] II.C. Preparation of Antigenic Polypeptides and Conjugation with Carrier

[0321] An antigenic region of a polypeptide is generally relatively small—typically 8 to 10 amino acids or less in length. Fragments of as few as 5 amino acids may characterize an antigenic region. These segments may correspond to regions of HCV antigen. Accordingly, using the cDNAs of HCV as a basis, DNAs encoding short segments of HCV polypeptides can be expressed recombinantly either as fusion proteins, or as isolated polypeptides. In addition, short amino acid sequences can be conveniently obtained by chemical synthesis. In instances wherein the synthesized polypeptide is correctly configured so as to provide the correct epitope, but is too small to be immunogenic, the polypeptide may be linked to a suitable carrier.

[0322] A number of techniques for obtaining such linkage are known in the art, including the formation of disulfide linkages using N-succinimidyl-3-(2-pyridyl-thio)propionate (SPDP) and succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC) obtained from Pierce Company, Rockford, Ill., (if the peptide lacks a sulfhydryl group, this can be provided by addition of a cysteine residue.) These reagents create a disulfide linkage between themselves and peptide cysteine residues on one protein and an amide linkage through the epsilon-amino on a lysine, or other free amino group in the other. A variety of such disulfide/amide-forming agents are known. See, for example, Immun. Rev. (1982) 62:185. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thio-ether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimido-methyl)cyclohexane-1-carboxylic acid, and the like. The carboxyl groups can be activated by combining them with succinimide or l-hydroxy-2-nitro-4-sulfonic acid, sodium salt. Additional methods of coupling antigens employs the rotavirus/"binding peptide" system described in EPO Pub. No. 259, 149, the disclosure of which is incorporated herein by reference. The foregoing list is not meant to be exhaustive, and modifications of the named compounds can clearly be used.

[0323] Any carrier may be used which does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles, see, for example, section II.D. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those skilled in the art.

[0324] In addition to full-length viral proteins, polypeptides comprising truncated HCV amino acid sequences encoding at least one viral epitope are useful immunological reagents. For example, polypeptides comprising such truncated sequences can be used as reagents in an immunoassay. These polypeptides also are candidate subunit antigens in compositions for antiserum production or vaccines. While these truncated sequences can be produced by various known treatments of native viral protein, it is generally preferred to make synthetic or recombinant polypeptides comprising an HCV sequence. Polypeptides comprising these truncated HCV sequences can be made up entirely of HCV sequences (one or more epitopes, either contiguous or noncontiguous), or HCV sequences and heterologous sequences in a fusion protein. Useful heterologous sequences include sequences that provide for secretion from a recombinant host, enhance the immunological reactivity of the HCV epitope(s), or facilitate the coupling of the polypeptide to an immunoassay support or a vaccine carrier. See, e.g., EPO Pub. No. 116,201; U.S. Pat. No. 4,722,840; EPO Pub. No. 259,149; U.S. Pat. No. 4,629,783, the disclosures of which are incorporated herein by reference.

[0325] The size of polypeptides comprising the truncated HCV sequences can vary widely, the minimum size being a sequence of sufficient size to provide an HCV epitope, while the maximum size is not critical. For convenience, the maximum size usually is not substantially greater than that required to provide the desired HCV epitopes and functions of the heterologous sequence, if any. Typically, the truncated HCV amino acid sequence will range from about 5 to about 100 amino acids in length. More typically, however, the HCV sequence will be a maximum of about 50 amino acids in length, preferably a maximum of about 30 amino acids. It is usually desirable to select HCV sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids.

[0326] Truncated HCV amino acid sequences comprising epitopes can be identified in a number of ways. For example, the entire viral protein sequence can be screened by preparing a series of short peptides that together span the entire protein sequence. By starting with, for example, 100mer polypeptides, it would be routine to test each polypeptide for the presence of epitope(s) showing a desired reactivity, and then testing progressively smaller and overlapping fragments from an identified 100mer to map the epitope of interest. Screening such peptides in an immunoassay is within the skill of the art. It is also known to carry out a computer analysis of a protein sequence to identify potential epitopes, and then prepare oligopeptides comprising the identified regions for screening. Such a computer analysis of the HCV amino acid sequence is shown in **FIG. 67**, where the hydrophilic/hydrophobic character is displayed above the antigen index. The amino acids are numbered from the starting MET (position 1) as shown in **FIG. 66**. It is appreciated by those of skill in the art that such computer analysis of antigenicity does not always identify an epitope that actually exists, and can also incorrectly identify a region of the protein as containing an epitope.

[0327] Examples of HCV amino acid sequences that may be useful as described herein are set forth below. It is to be understood that these peptides do not necessarily precisely map one epitope, but may also contain HCV sequence that is not immunogenic. These non-immunogenic portions of the sequence can be defined as described above using conventional techniques and deleted from the described

sequences. Further, additional truncated HCV amino acid sequences that comprise an epitope or are immunogenic can be identified as described above. The following sequences are given by amino acid number (i.e., "AAn") where n is the amino acid number as shown in **FIG. 66**:

AA1–AA25; AA1–AA50; AA1–AA84; AA9–AA177; AA1–AA10;

AA5–AA20; AA20–AA25; AA35–AA45; AA50–AA100;

AA40–AA90; AA45–AA65; AA65–AA75; AA80–90;

AA99–AA120; AA95–AA110; AA105–AA120; AA100–AA150;

AA150–AA200; AA155–AA170; AA190–AA210;

AA200–AA250; AA220–AA240; AA245–AA265;

AA250–AA300; AA290–AA330; AA290–305;

AA300–AA350; AA310–AA330; AA350–AA400;

AA380–AA395; AA405–AA495; AA400–AA450;

AA405–AA415; AA415–AA425; AA425–AA435;

AA437–AA582; AA450–AA500; AA440–AA460;

AA460–AA470; AA475–AA495; AA500–AA550;

AA511–AA690; AA515–AA550; AA550–AA600;

AA550–AA625; AA575–AA605; AA585–AA600;

AA600–AA650; AA600–AA625; AA635–AA665;

AA650–AA700; AA645–AA680; AA700–AA750;

AA700–AA725; AA700–AA750; AA725–AA775;

AA770–AA790; AA750–AA800; AA800–AA815;

AA825–AA850; AA850–AA875; AA800–AA850;

AA920–AA990; AA850–AA900; AA920–AA945;

AA940–AA965; AA970–AA990; AA950–AA1000;

AA1000–AA1060; AA1000–AA1025; AA1000–AA1050;

AA1025–AA1040; AA1040–AA1055; AA1075–AA1175;

AA1050–AA1200; AA1070–AA1100; AA1100–AA1130;

AA1140–AA1165; AA1192–AA1457; AA1195–AA1250;

AA1200–AA1225; AA1225–AA1250; AA1250–AA1300;

AA1260–AA1310; AA1260–AA1280; AA1266–AA1428;

AA1300–AA1350; AA1290–AA1310; AA1310–AA1340;

AA1345–AA1405; AA1345–AA1365; AA1350–AA1400;

AA1365–AA1350; AA1380–AA1405; AA1400–AA1450;

AA1450–AA1500; AA1460–AA1475; AA1475–AA1515;

AA1475–AA1500; AA1500–AA1550; AA1500–AA1515;

AA1515–AA1550; AA1550–AA1600; AA1545–AA1560;

AA1569–AA1931; AA1570–AA1590; AA1595–AA1610;

AA1590–AA1650; AA1610–AA1645; AA1G50–AA1690;

–continued

AA1685–AA1770; AA1689–AA1805; AA1690–AA1720;

AA1694–AA1735; AA1720–AA1745; AA1745–AA1770;

AA1750–AA1800; AA1775–AA1810; AA1795–AA1850;

AA1850–AA1900; AA1900–AA1950; AA1900–AA1920;

AA1916–AA2021; AA1920–AA1940; AA1949–AA2124;

AA1950–AA2000; AA1950–AA1985; AA1980–AA2000;

AA2000–AA2050; AA2005–AA2025; AA2020–AA2045;

AA2045–AA2100; AA2045–AA2070; AA2054–AA2223;

AA2070–AA2100; AA2100–AA2150; AA2150–AA2200;

AA2200–AA2250; AA2200–AA2325; AA2250–AA2330;

AA2255–AA2270; AA2265–AA2280; AA2280–AA2290;

AA2287–AA2385; AA2300–AA2350; AA2290–AA2310;

AA2310–AA2330; AA2330–AA2350; AA2350–AA2400;

AA2348–AA2464; AA2345–AA2415; AA2345–AA2375;

AA2370–AA2410; AA2371–AA2502; AA2400–AA2450;

AA2400–AA2425; AA2415–AA2450; AA2445–AA2500;

AA2445–AA2475; AA2470–AA2490; AA2500–AA2550;

AA2505–AA2540; AA2535–AA2560; AA2550–AA2600;

AA2560–AA2580; AA2600–AA2650; AA2605–AA2620;

AA2620–AA2650; AA2640–AA2660; AA2650–AA2700;

AA2655–AA2670; AA2670–AA2700; AA2700–AA2750;

AA2740–AA2760; AA2750–AA2800; AA2755–AA2780;

AA2780–AA2830; AA2785–AA2810; AA2796–AA2886;

AA2810–AA2825; AA2800–AA2850; AA2850–AA2900;

AA2850–AA2865; AA2885–AA2905; AA2900–AA2950;

AA2910–AA2930; AA2925–AA2950; AA2945–end(C'

terminal).

[0328] The above HCV amino acid sequences can be prepared as discrete peptides or incorporated into a larger polypeptide, and may find use as described herein. Additional polypeptides comprising truncated HCV sequences are described in the examples.

[0329] The observed relationship of the putative polyproteins of HCV and the Flaviviruses allows a prediction of the putative domains of the HCV "non-structural" (NS) proteins. The locations of the individual NS proteins in the putative Flavirus precursor polyprotein are fairly well-known. Moreover, these also coincide with observed gross fluctuations in the hydrophobicity profile of the polyprotein. It is established that NS5 of Flaviviruses encodes the virion polymerase, and that NS1 corresponds with a complement fixation antigen which has been shown to be an effective vaccine in animals. Recently, it has been shown that a flaviviral protease function resides in NS3. Due to the observed similarities between HCV and the Flaviviruses, deductions concerning the approximate locations of the

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corresponding protein domains and functions in the HCV polyprotein are possible (see Section IV.R.6). The expression of polypeptides containing these domains in a variety of recombinant host cells, including, for example, bacteria, yeast, insect, and vertebrate cells, should give rise to important immunological reagents which can be used for diagnosis, detection, and vaccines.

[0330] Although the non-structural protein region of the putative polyproteins of the HCV isolate described herein and of Flaviviruses appears to be generally similar, there is less similarity between the putative structural regions which are towards the N-terminus. In this region, there is a greater divergence in sequence, and in addition, the hydrophobic profile of the two regions show less similarity. This "divergence" begins in the N-terminal region of the putative NS1 domain in HCV, and extends to the presumed N-terminus. Nevertheless, it is still possible to predict the approximate locations of the putative nucleocapsid (N-terminal basic domain) and E (generally hydrophobic) domains within the HCV polyprotein. In Section IV.H.6., the predictions are based on the changes observed in the hydrophobic profile of the HCV polyprotein, and on a knowledge of the location and character of the flaviviral proteins. From these predictions it may be possible to identify approximate regions of the HCV polyprotein that could correspond with useful immunological reagents. For example, the E and NS1 proteins of Flaviviruses are known to have efficacy as protective vaccines. These regions, as well as some which are shown to be antigenic in the HCV isolate described herein, for example those within putative NS3, C, and NS5, etc., should also provide diagnostic reagents. Moreover, the location and expression of viral-encoded enzymes may also allow the evaluation of anti-viral enzyme inhibitors, i.e., for example, inhibitors which prevent enzyme activity by virtue of an interaction with the enzyme itself, or substances which may prevent expression of the enzyme, (for example, anti-sense RNA, or other drugs which interfere with expression).

[0331] II.D. Preparation of Hybrid Particle Immunogens Containing HCV Epitopes

[0332] The immunogenicity of the epitopes of HCV may also be enhanced by preparing them in mammalian or yeast systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. See, e.g., U.S. Pat. No. 4,722,840. Constructs wherein the NANBV epitope is linked directly to the particle-forming protein coding sequences produce hybrids which are immunogenic with respect to the HCV epitope. In addition, all of the vectors prepared include epitopes specific to HBV, having various degrees of immunogenicity, such as, for example, the pre-S peptide. Thus, particles constructed from particle forming protein which include HCV sequences are immunogenic with respect to HCV and HBV.

[0333] Hepatitis surface antigen (HBSAg) has been shown to be formed and assembled into particles in *S. cerevisiae* (Valenzuela et al. (1982)), as well as in, for example, mammalian cells (Valenzuela, P., et al. (1984)). The formation of such particles has been shown to enhance the immunogenicity of the monomer subunit. The constructs may also include the immunodominant epitope of HBSAg, comprising the 55 amino acids of the presurface (pre-S) region. Neurath et al. (1984). Constructs of the pre-S-HBSAg particle expressible in yeast are disclosed in EPO

174,444, published Mar. 19, 1986; hybrids including heterologous viral sequences for yeast expression are disclosed in EPO 175,261, published Mar. 26, 1966. These constructs may also be expressed in mammalian cells such as Chinese hamster ovary (CHO) cells using an SV40dihydrofolate reductase vector (Michelle et al. (1984)).

[0334] In addition, portions of the particle-forming protein coding sequence may be replaced with codons encoding an HCV epitope. In this replacement, regions which are not required to mediate the aggregation of the units to form immunogenic particles in yeast or mammals can be deleted, thus eliminating additional HBV antigenic sites from competition with the HCV epitope.

[0335] II.E. Preparation of Vaccines

[0336] Vaccines may be prepared from one or more immunogenic polypeptides derived from HCV. The observed homology between HCV and Flaviviruses provides information concerning the polypeptides which are likely to be most effective as vaccines, as well as the regions of the genome in which they are encoded. The general structure of the Flavivirus genome is discussed in Rice et al (1986). The flavivirus genomic RNA is believed to be the only virus-specific mRNA species, and it is translated into the three viral structural proteins, i.e., C, M, and E, as well as two large nonstructural proteins, NS4 and NS5, and a complex set of smaller nonstructural proteins. It is known that major neutralizing epitopes for Flaviviruses reside in the E (envelope) protein (Roehrig (1986)). The corresponding HCV E gene and polypeptide encoding region may be predicted, based upon the homology to Flaviviruses. Thus, vaccines may be comprised of recombinant polypeptides containing epitopes of HCV E. These polypeptides may be expressed in various host cells (e.g., bacteria, yeast, insect, or mammalian cells), or alternatively may be isolated from viral preparations. It is also anticipated that the other structural proteins may also contain epitopes which give rise to protective anti-HCV antibodies. Thus, polypeptides containing the epitopes of E, C, and M may also be used, whether singly or in combination, in HCV vaccines.

[0337] In addition to the above, it has been shown that immunization with NS1 (nonstructural protein 1), results in protection against yellow fever (Schlesinger et al (1986)). This is true even though the immunization does not give rise to neutralizing antibodies. Thus, particularly since this protein appears to be highly conserved among Flaviviruses, it is likely that HCV NS1 will also be protective against HCV infection. Moreover, it also shows that nonstructural proteins may provide protection against viral pathogenicity, even if they do not cause the production of neutralizing antibodies.

[0338] In view of the above, multivalent vaccines against HCV may be comprised of one or more epitopes from one or more structural proteins, and/or one or more epitopes from one or more nonstructural proteins. These vaccines may be comprised of, for example, recombinant HCV polypeptides and/or polypeptides isolated from the virions. In particular, vaccines are contemplated comprising one or more of the following HCV proteins, or subunit antigens derived therefrom: E, NS1, C, NS2, NS3, NS4 and NS5. Particularly preferred are vaccines comprising E and/or NS1, or subunits thereof. In addition, it may be possible to use inactivated HCV in vaccines; inactivation may be by the

preparation of viral lysates, or by other means known in the art to cause inactivation of Flaviviruses, for example, treatment with organic solvents or detergents, or treatment with formalin. Moreover, vaccines may also be prepared from attenuated HCV strains. The preparation of attenuated HCV strains is described infra.

[0339] It is known that some of the proteins in Flaviviruses contain highly conserved regions. Thus, some immunological cross-reactivity is possible between HCV and other Flaviviruses. It is possible that shared epitopes between the Flaviviruses and HCV will give rise to protective antibodies against one or more of the disorders caused by these pathogenic agents. Thus, it may be possible to design multipurpose vaccines based upon this knowledge.

[0340] In addition to the above, it is also possible to prepare live vaccines of attenuated microorganisms which express one or more recombinant HCV polypeptides. Suitable attenuated microorganisms are known in the art and include, for example, viruses (e.g., vaccinia virus (see Brown et al. (1986)), as well as bacteria. The preparation of vaccines which contain an immunogenic polypeptide(s) as active ingredients, is known to one skilled in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the protein encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against an immunogenic polypeptide containing an HCV antigenic sequence resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

[0341] The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccha-

rine, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%.

[0342] The proteins may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[0343] II.F. Dosage and Administration of Vaccines

[0344] The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 5 micrograms to 250 micrograms of antigen per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be peculiar to each subject.

[0345] The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reenforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the individual and be dependent upon the judgment of the practitioner.

[0346] In addition, the vaccine containing the immunogenic HCV antigen(s) may be administered in conjunction with other immunoregulatory agents, for example, immune globulins.

[0347] II.G. Preparation of Antibodies Against HCV Epitopes

[0348] The immunogenic polypeptides prepared as described above are used to produce antibodies, including polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an immunogenic polypeptide bearing an HCV epitope(s). Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an HCV epitope contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art, see for example, Mayer and Walker (1987).

[0349] Alternatively, polyclonal antibodies may be isolated from a mammal which has been previously infected with HCV. A. example of a method for purifying antibodies

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to HCV epitopes from serum from an infected individual, based upon affinity chromatography and utilizing a fusion polypeptide of SOD and a polypeptide encoded within cDNA clone 5-1-1, is presented in Section V.E.

[0350] Monoclonal antibodies directed against HCV epitopes can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al. (1980); Hammerling et al. (1981); Kennett et al. (1980); see also, U.S. Pat. Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against HCV epitopes can be screened for various properties; i.e., for isotype, epitope affinity, etc.

[0351] Antibodies, both monoclonal and polyclonal, which are directed against HCV epitopes are particularly useful in diagnosis, and those which are neutralizing are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies.

[0352] Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the infectious agent against which protection is desired. See, for example, Nisonoff, A., et al. (1981) and Dreesman et al. (1985). Techniques for raising anti-idiotype antibodies are known in the art. See, for example, Grzych (1985), Mac-Namara et al. (1984), and Uytdehaag et al. (1985). These anti-idiotype antibodies may also be useful or treatment, vaccination and/or diagnosis of NANBH, as well as for an elucidation of the immunogenic regions of IICV antigens.

[0353] II.H. Diagnostic Oligonucleotide Probes and Kits

[0354] Using the disclosed portions of the isolated HCV cDNAs as a basis, including those described in Section IV.A, oligomers of approximately 8 nucleotides or more can be prepared, either by excision from recombinant polynucleotides or synthetically, which hybridize with the HCV genome and are useful in identification of the viral agent(s), further characterization of the viral genome(s), as well as in detection of the virus(es) in diseased individuals. The probes for HCV polynucleotides (natural or derived) are a length which allows the detection of unique viral sequences by hybridization. While 6-8 nucleotides may be a workable length, sequences of 10-12 nucleotides are preferred, and about 20 nucleotides appears optimal. Preferably, these sequences will derive from regions which lack heterogeneity. These probes can be prepared using routine methods, including automated oligonucleotide synthetic methods. Among useful probes, for example, are the clone 5-1-1 and the additional clones disclosed herein, as well as the various oligomers useful in probing cDNA libraries, set forth below. A complement to any unique portion of the HCV genome will be satisfactory. For use as probes, complete complementarity is desirable, though it may be unnecessary as the length of the fragment is increased.

[0355] For use of such probes as diagnostics, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids contained therein. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. The probes are usually labeled. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or kinasing, biotin, fluorescent probes, and chemiluminescent probes. The nucleic acids extracted from the sample are then treated with the labeled probe under hybridization conditions of suitable stringencies.

[0356] The probes can be made completely complementary to the HCV genome. Therefore, usually high stringency conditions are desirable in order to prevent false positives. However, as shown infra, portions of the HCV genome are variable. Therefore, conditions of high stringency should only be used if the probes are complementary to regions of the viral genome which lack heterogeneity. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for example, Maniatis, T. 1982).

[0357] Generally, it is expected that the HCV genome sequences will be present in serum of infected individuals at relatively low levels, i.e., at approximately 10^2-10^3 chimp infective doses (CID) per ml. This level may require that amplification techniques be used in hybridization assays. Such techniques are known in the art. For example, the Enzo Biochemical Corporation "Bio-Bridge" system uses terminal deoxynucleotide transferase to add unmodified 3'-poly-dT-tails to a DNA probe. The poly dT-tailed probe is hybridized to the target nucleotide sequence, and then to a biotin-modified poly-A. PCT application 84/03520 and EPA124221 describe a DNA hybridization assay in which: (1) analyte is annealed to a single-stranded DNA probe that is complementary to an enzyme-labeled oligonucleotide; and (2) the resulting tailed duplex is hybridized to an enzyme-labeled oligonucleotide. EPA 204510 describes a DNA hybridization assay in which analyte DNA is contacted with a probe that has a tail, such as a poly-dT tail, an amplifier strand that has a sequence that hybridizes to the tail of the probe, such as a poly-A sequence, and which is capable of binding a plurality of labeled strands. A particularly desirable technique may first involve amplification of the target HCV sequences in sera approximately 10,000 fold, i.e., to approximately 10^6 sequences/ml. This may be αcomplished, for example, by the polymerase chain reactions (PCR) technique described which is by Saiki et al. (1986), by Mullis, U.S. Pat. No. 4,683,195, and by Mullis et al. U.S. Pat. No. 4,683,202. The amplified sequence(s) may then be detected using a hybridization assay which is described in co-pending U.S. Serial No. 109;282 (Attorney Docket No. 2300-0171), which was filed Oct. 15, 1987, U.S. Serial No. 185,201 (filed Apr. 22, 1988), and U.S. Serial No. 252,638 (filed Sep. 30, 1988), which are assigned to the herein assignee, and are hereby incorporated herein by reference. These hybridization assays, which should detect sequences at the level of 10^6/ml, utilize nucleic acid multimers which bind to single-stranded analyte nucleic acid, and which also bind to a multiplicity of single-stranded labeled oligonucleotides. A suitable solution phase sandwich assay which may be used with labeled polynucleotide probes, and the methods for the preparation of probes is described in EPO 225,807,

published Jun. 16, 1987, which is assigned to the herein assignee, and which is hereby incorporated herein by reference.

[0358] The probes can be packaged into diagnostic kits. Diagnostic kits include the probe DNA, which may be labeled; alternatively, the probe DNA may be unlabeled and the ingredients for labeling may be included in the kit in separate containers. The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, for example, standards, as well as instructions for conducting the test.

[0359] II.I. Immunoassay and Diagnostic Kits

[0360] Both the polypeptides which react immunologically with serum containing HCV antibodies, for example, those derived from, expressed from, or encoded within the clones described in Section IV.A., and composites thereof, (see section IV.A.) and the antibodies raised against the HCV specific epitopes in these polypeptides, see for example Section IV.E, are useful in immunoassays to detect presence of HCV antibodies, or the presence of the virus and/or viral antigens, in biological samples. Design of the immunoassays is subject to a great deal of variation, and many formats are known in the art. The immunoassay will utilize at least one viral epitope derived from HCV. In one embodiment, the immunoassay uses a combination of viral epitopes derived from HVC. These epitopes may be derived from the same or from different viral polypeptides, and may be in separate recombinant or natural polypeptides, or together in the same recombinant polypeptides. An immunoassay may use, for example, a monoclonal antibody directed towards a viral epitope(s), a combination of monoclonal antibodies directed towards epitopes of one viral antigen, monoclonal antibodies directed towards epitopes of different viral antigens, polyclonal antibodies directed towards the same viral antigen, or polyclonal antibodies directed towards different viral antigens. Protocols may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, enzymatic, fluorescent chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

[0361] Typically, an immunoassay for an anti-HCV antibody(s) will involve selecting and preparing the test sample suspected of containing the antibodies, such as a biological sample, then incubating it with an antigenic (i.e., epitope-containing) HCV polypeptide(s) under conditions that allow antigen-antibody complexes to form, and then detecting the formation of such complexes. Suitable incubation conditions are well known in the art. The immunoassay may be, without limitations, in a heterogenous or in a homogeneous format, and of a standard or competitive type.

[0362] In a heterogeneous format, the polypeptide is typically bound to a solid support to facilitate separation of the sample from the polypeptide after incubation. Examples of solid supports that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidine fluoride (known as Immulon™), diazotized paper, nylon membranes, activated beads, and Protein A beads. For example, Dynatech Immulon™ 1 or Immulon™ 2 microtiter plates or 0.25 inch polysterene beads (Precision Plastic Ball) can be used in the heterogeneous format. The solid support containing the antigenic polypeptide is typically washed after separating it from the test sample, and prior to detection of bound antibodies. Both standard and competitive formats are known in the art.

[0363] In a homogeneous format, the test sample is incubated with antigen in solution. For example, it may be under conditions that will precipitate any antigen-antibody complexes which are formed. Both standard and competitive formats for these assays are known in the art.

[0364] In a standard format, the amount of HCV antibodies forming the antibody-antigen complex is directly monitored. This may be accomplished by determining whether labeled anti-xenogenic (e.g., anti-human) antibodies which recognize an epitope on anti-HCV antibodies will bind due to complex formation. In a competitive format, the amount of HCV antibodies in the sample is deduced by monitoring the competitive effect on the binding of a known amount of labeled antibody (or other competing ligand) in the complex.

[0365] Complexes formed comprising anti-HCV antibody (or, in the case of competetive assays, the amount of competing antibody) are detected by any of a number of known techniques, depending on the format. For example, unlabeled HCV antibodies in the complex may be detected using a conjugate of antixenogeneic Ig complexed with a label, (e.g., an enzyme label).

[0366] In immunoassays where HCV polypeptides are the analyte, the test sample, typically a biological sample, is incubated with anti-HCV antibodies under conditions that allow the formation of antigen-antibody complexes. Various formats can be employed. For example, a "sandwich assay" may be employed, where antibody bound to a solid support is incubated with the test sample; washed; incubated with a second, labeled antibody to the analyte, and the support is washed again. Analyte is detected by determining if the second antibody is bound to the support. In a competitive format, which can be either heterogeneous or homogeneous, a test sample is usually incubated with antibody and a labeled, competing antigen is also incubated, either sequentially or simultaneously. These and other formats are well known in the art.

[0367] The Flavivirus model for HCV allows predictions regarding the likely location of diagnostic epitopes for the virion structural proteins. The C, pre-M, M, and E domains are all likely to contain epitopes of significant potential for detecting viral antigens, and particularly for diagnosis. Similarly, domains of the nonstructural proteins are expected to contain important diagnostic epitopes (e.g., NS5 encoding a putative polymerase; and NS1 encoding a putative complement-binding antigen). Recombinant polypeptides, or viral polypeptides, which include epitopes from these specific domains may be useful for the detection of viral antibodies in infections blood donors and infected patients.

[0368] In addition, antibodies directed against the E and/or M proteins can be used in immunoassays for the detection of viral antigens in patients with HCV caused NANBH, and in

infectious blood donors. Moreover, these antibodies may be extremely useful in detecting acute-phase donors and patients.

[0369] Some of the antigenic regions of the putative polyprotein have been mapped and identified by screening the antigenicitiy of bacterial expression products of HCV cDNAs which encode portions of the polyprotein. See Section IV.B.8. Other antigenic regions of HCV may be detected by expressing the portions of the HCV cDNAs in other expression systems, including yeast systems and cellular systems derived from insects and vertebrates. In addition, studies giving rise to an antigenicity index and hydrophobicity/hydrophilicity profile give rise to information concerning the probability of a region's antigenicity.

[0370] The studies on antigenic mapping by expression of HCV cDNAs showed that a number of clones containing these cDNAs expressed polypeptides which were immunologically reactive with serum from individuals with NANBH. No single polypeptide was immunologically reactive with all sera. Five of these polypeptides were very immunogenic in that antibodies to the HCV epitopes in these polypeptides were detected in many different patient sera, although the overlap in detection was not complete. Thus, the results on the immunogenicity of the polypeptides encoded in the various clones suggest that efficient detection systems for HCV infection may include the use of panels of epitopes. The epitopes in the panel may be constructed into one or multiple polypeptides. The assays for the varying epitopes may be sequential or simultaneous.

[0371] Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the polypeptides of the invention containing HCV epitopes or antibodies directed against HCV epitopes in suitable containers, along with the remaining reagents and materials required for the conduct of the assay, as well as a suitable set of assay instructions.

[0372] II.J. Further Characterization of the HCV Genome, Virions, and Viral Antigens Using Probes Derived From cDNA to the Viral Genome

[0373] The HCV cDNA sequence information in the clones described in Section IV.A may be used to gain further information on the sequence of the HCV genome, and for identification and isolation of the HCV agent, and thus will aid in its characterization including the nature of the genome, the structure of the viral particle, and the nature of the antigens of which it is composed. This information, in turn, can lead to additional polynucleotide probes, polypeptides derived from the HCV genome, and antibodies directed against HCV epitopes which would be useful for the diagnosis and/or treatment of HCV caused NANBH.

[0374] The cDNA sequence information in the above-mentioned clones is useful for the design of probes for the isolation of additional cDNA sequences which are derived from as yet undefined regions of the HCV genome(s) from which the cDNAs in clones described in Section IV.A. are derived. For example, labeled probes containing a sequence of approximately 8 or more nucleotides, and preferably 20 or more nucleotides, which are derived from regions close to the 5'-termini or 3'-termini of the family of HCV cDNA sequences shown in **FIGS. 1,** 3, 6, 9, 14 and 62 may be used

to isolate overlapping cDNA sequences from HCV cDNA libraries. These sequences which overlap the cDNAs in the above-mentioned clones, but which also contain sequences derived from regions of the genome from which the cDNA in the above mentioned clones are not derived, may then be used to synthesize probes for identification of other overlapping fragments which do not necessarily overlap the cDNAs in the clones described in Section IV.A. Unless the HCV genome is segmented and the segments lack common sequences, it is possible to sequence the entire viral genome(s) utilizing the technique of isolation of overlapping cDNAs derived from the viral genome(s). Although it is unlikely, if the genome is a segmented genome which lacks common sequences, the sequence of the genome can be determined by serologically screening lambda-gt11 HCV cDNA libraries, as used to isolate clone 5-1-1, sequencing cDNA isolates, and using the isolated cDNAs to isolate overlapping fragments, using the technique described for the isolation and sequencing of the clones described in Section IV.A. Alternatively, characterization of the genomic segments could be from the viral genome(s) isolated from purified HCV particles. Methods for purifying HCV particles and for detecting them during the purification procedure are described herein, infra. Procedures for isolating polynucleotide genomes from viral particles are known in the art, and one procedure which may be used is shown in Example IV.A.1. The isolated genomic segments could then be cloned and sequenced. Thus, with the information provided herein, it is possible to clone and sequence the HCV genome(s) irrespective of their nature.

[0375] Methods for constructing cDNA libraries are known in the art, and are discussed supra and infra; a method for the construction of HCV cDNA libraries in lambda-gt11 is discussed infra in Section IV.A. However, cDNA libraries which are useful for screening with nucleic acid probes may also be constructed in other vectors known in the art, for example, lambda-gt10 (Huynh et al. (1985)). The HCV derived cDNA detected by the probes derived from the cDNAs described in Section IV.A, and from the probes synthesized from polynucleotides derived from these cDNAs, may be isolated from the clone by digestion of the isolated polynucleotide with the appropriate restriction enzyme(s), and sequenced. See, for example, Section IV.A.3. and IV.A.4. for the techniques used for the isolation and sequencing of HCV cDNA which overlaps HCV cDNA in clone 5-1-1, Sections IV.A.5-IV.A.7 for the isolation and sequencing of HCV cDNA which overlaps that in clone 81, and Section IV.A.8 and IV.A.9 for the isolation and sequencing of a clone which overlaps another clone (clone 36), which overlaps clone 81.

[0376] The sequence information derived from these overlapping HCV cDNAs is useful for determining areas of homology and heterogeneity within the viral genome(s), which could indicate the presence of different strains of the genome, and/or of populations of defective particles. It is also useful for the design of hybridization probes to detect HCV or HCV antigens or HCV nucleic acids in biological samples, and during the isolation of HCV (discussed infra), utilizing the techniques described in Section II.G. Moreover, the overlapping cDNAs may be used to create expression vectors for polypeptides derived from the HCV genome(s) which also encode the polypeptides encoded in clones 5-1-1, 36, 81, 91, and 1-2, and in the other clones described in Section IV.A. The techniques for the creation of these

polypeptides containing HCV epitopes, and for antibodies directed against HCV epitopes contained within them, as well as their uses, are analogous to those described for polypeptides derived from NANBV cDNA sequences contained within the HCV cDNA clones described in Section IV.A, discussed supra and infra.

[0377] Encoded within the family of cDNA sequences contained within clones 5-1-1, 32, 35, 36, 81, 91, 1-2, and the other clones described in Section IV.A. are antigen(s) containing epitopes which appear to be unique to HCV; i.e., antibodies directed against these antigens are absent from individuals infected with HAV or HBV, and from individuals not infected with HCV (see the serological data presented in Section IV.B.). Moreover, a comparison of the sequence information of these cDNAs with the sequences of HAV, HBV, HDV, and with the genomic sequences in Genebank indicates that minimal homology exists between these cDNAs and the polynucleotide sequences of those sources. Thus, antibodies directed against the antigens encoded within the cDNAs of these clones may be used to identify BB-NANBV particles isolated from infected individuals. In addition, they are also useful for the isolation of NANBH agent(s).

[0378] HCV particles may be isolated from the sera from individuals with NANBH or from cell cultures by any of the methods known in the art, including for example, techniques based on size discrimination such as sedimentation or exclusion methods, or techniques based on density such as ultracentrifugation in density gradients, or precipitation with agents such as polyethylene glycol, or chromatography on a variety of materials such as anionic or cationic exchange materials, and materials which bind due to hydrophobicity, as well as affinity columns. During the isolation procedure the presence of HCV may be detected by hybridization analysis of the extracted genome, using probes derived from the HCV cDNAs described supra, or by immunoassay (see Section II.I.) utilizing as robes antibodies directed against HCV antigens encoded within the family of cDNA sequences described in Section IV.A, and also directed against HCV antigens encoded within the overlapping HCV cDNA sequences discussed supra. The antibodies may be monoclonal, or polyclonal, and it may be desirable to purify the antibodies before their use in the immunoassay. A purification procedure for polyclonal antibodies directed against antigen(s) encoded within clone 5-1-1 is described in Section IV.E; analogous purification procedures may be utilized for antibodies directed against other HCV antigens.

[0379] Antibodies directed against HCV antigens encoded within the family of cDNAs described in Section IV.A, as well as those encoded within overlapping HCV cDNAs, which are affixed to solid supports are useful for the isolation of HCV by immunoaffinity chromatography. Techniques for immunoaffinity chromatography are known in the art, including techniques for affixing antibodies to solid supports so that they retain their immunoselective activity; the techniques may be those in which the antibodies are adsorbed to the support (see, for example, Kurstak in ENZYME IMMUNODIAGNOSIS, page 31-37), as well as those in which the antibodies are covalently linked to the support. Generally, the techniques are similar to those used for covalent linking of antigens to a solid support, which are generally described in Section II.C.; however, spacer groups may be included in

the bifunctional coupling agents so that the antigen binding site of the antibody remains accessible.

[0380] During the purification procedure the presence of HCV may be detected and/or verified by nucleic acid hybridization, utilizing as probes polynucleotides derived from the family of HCV cDNA sequences described in Section IV.A, as well as from overlapping HCV cDNA sequences, described supra. In this case, the fractions are treated under conditions which would cause the disruption of viral particles, for example, with detergents in the presence of chelating agents, and the presence of viral nucleic acid determined by hybridization techniques described in Section II.H. Further confirmation that the isolated particles are the agents which induce HCV may be obtained by infecting chimpanzees with the isolated virus particles, followed by a determination of whether the symptoms of NANBH result from the infection.

[0381] Viral particles from the purified preparations may then be further characterized. The genomic nucleic acid has been purified. Based upon its sensitivity to RNase, and not DNase I, it appears that the virus is composed of an RNA genome. See Example IV.C.2., infra. The strandedness and circularity or non-circularity can determined by techniques known in the art, including, for example, its visualization by electron microscopy, its migration in density gradients, and its sedimentation characteristics. Based upon the hybridization of the captured HCV genome to the negative strands of HCV cDNAs, it appears that HCV may be comprised of a positive stranded RNA genome (see Section IV.H.1). Techniques such as these are described in, for example, METHODS IN ENZYMOLOGY. In addition, the purified nucleic acid can be cloned and sequenced by known techniques, including reverse transcription since the genomic material is RNA. See, for example, Maniatis (1982), and Glover (1985). Utilizing the nucleic acid derived from the viral particles, it is possible to sequence the entire genome, whether or not it is segmented.

[0382] Examination of the homology of the polypeptide encoded within the continuous ORF of combined clones 14i through 39c (the ORF is shown in **FIG. 26**), suggests that a portion of the HCV polypeptide contains regions of homology with the corresponding proteins in conserved regions of flaviviruses. An example of this is described in Section IV.H.3. This evidence, in conjunction with the results which show that HCV contains a positive-stranded genome, the size of which is approximately 10,000 nucleotides, is consistent with the suggestion that HCV is distantly related to the flaviviridae. Generally, flavivirus virions and their genomes have a relatively consistent structure and organization, which are known. See Rice et al. (1986), and Brinton, M. A. (1988). Using the comparison with flaviviruses, predictions as to the location of the sequences encoding some of the HCV proteins may be made.

[0383] The structure of the HCV may also be determined and its components isolated. The morphology and size may be determined by, for example, electron microscopy. The identification and localization of specific viral polypeptide antigens such as coat or envelope antigens, or internal antigens, such as nucleic acid binding proteins, core antigens, and polynucleotide polymerase(s) may also be determined by, for example, determining whether the antigens are present as major or minor viral components, as well as by

utilizing antibodies directed against the specific antigens encoded within isolated cDNAs as probes. This information is useful in the design of vaccines; for example, it may be preferable to include an exterior antigen in a vaccine preparation. Multivalent vaccines may be comprised of, for example, an epitope derived from the genome encoding a structural protein. for example, E, as well as an epitope from another portion of the genome, for example, a nonstructural or structural polypeptide.

[0384] II.K. Cell Culture Systems and Animal Model Systems for HCV Replication

[0385] The suggestion that HCV is a flavi-like virus also provides information on methods for growing HCV. The virus is thought to be "Flavi-like" for several reasons. Based upon the HCV cDNA sequence, the genome appears to contain a large ORF which encodes a putative polyprotein. The HCV genome is positive-stranded with respect to translation of this polyprotein. The hydrophobicity profile of the HCV polyprotein resembles in particular that of putative polyproteins from known Flaviviruses. The apparent size of the genome, approximately 10,000 nucleotides in size, may be smaller than, but is close to that of known Flaviviruses. Although there is only a slight amount of amino acid homology between the HCV putative polyprotein and that of Flaviviruses, the homology is probably significant because there appear to be at three conserved motifs that have similar spacing in HCV and the Flaviviruses. Two of these conserved motifs, which are of larger size, are in the putative NS3 region. A third conserved motif is in putative NS5; the conserved sequence in this region, GDD, is usually associated with a Flavivirus polymerase. Other regions which are conserved between the Flaviviruses are also partially conserved in HCV. Methods for culturing flaviviruses are known to those of skill in the art (see, for example, the reviews by Brinton (1986) and Stollar, V. (1980)). Generally, suitable cells or cell lines for culturing HCV may include those known to support Flavivirus replication, for example, the following: monkey kidney cell lines (e.g. MK$_2$, VERO); porcine kidney cell lines (e.g. PS); baby hamster kidney cell lines (e.g. BHK); murine macrophage cell lines (e.g., P388D1, MK1, Mm1); human macrophage cell lines (e.g., U-937); human peripheral blood leukocytes; human adherent monocytes; hepatocytes or hepatocyte cell lines (e.g., HUH7, HEPG2); embryos or embryonic cells (e.g., chick embryo fibroblasts); or cell lines derived from invertebrates, preferably from insects (e.g. drosophila cell lines), or more preferably from arthropods, for example, mosquito cell lines (e.g., A. Albopictus, Aedes aegypti, Cutex tritaeniorhynchus) or tick cell lines (e.g. RML-14 Dermacentor parumapertus). it is possible that primary hepatocytes can be cultured, and then infected with HCV; or alternatively, the hepatocyte cultures could be derived from the livers of infected individuals (e.g., humans or chimpanzees). The latter case is an example of a cell which is infected in vivo being passaged in vitro. In addition, various immortalization methods can be used to obtain cell-lines derived from hepatocyte cultures. For example, primary liver cultures (before and after enrichment of the hepatocyte population) may be fused to a variety of cells to maintain stability. For example, also, cultures may be infected with transforming viruses, or transfected with transforming genes in order to create permanent or semipermanent cell lines. In addition, for example, cells in liver cultures may be fused to established cell lines (e.g., HepG2). Methods for cell fusion are

known in the art, and include, for example, the use of fusion agents such as polyethylene glycol, Sendai Virus, and Epstein-Barr virus. As discussed above, HCV is a Flavi-like virus. Therefore, it is probable that HCV infection of cell lines may be accomplished by techniques known in the art for infecting cells with Flaviviruses. These include, for example, incubating the cells with viral preparations under conditions which allow viral entry into the cell. In addition, it may be possible to obtain viral production by transfecting the cells with isolated viral polynucleotides. It is known that Togavirus and Flavivirus RNAs are infectious in a variety of vertebrate cell lines (Pfefferkorn and Shapiro (1974)), and in a mosquito cell line (Peleg (1969)). Methods for transfecting tissue culture cells with RNA duplexes, positive stranded RNAs, and DNAs (including cDNAs) are known in the art, and include, for example, techniques which use electroporation, and precipitation with DEAE-Dextran or calcium phosphate. An abundant source of HCV RNA can be obtained by performing in vitro transcription of an HCV cDNA corresponding to the complete genome. Transfection with this material, or with cloned HCV cDNA should result in viral replication and the in vitro propagation of the virus.

[0386] In addition to cultured cells, animal model systems may be used for viral replication; animal systems in which flaviviruses are known to those of skill in the art (see, for example, the review by Monath (1986)). Thus, HCV replication may occur not only in chimpanzees, but also in, for example, marmosets and suckling mice.

[0387] II.L. Screening for Anti-viral Agents for HCV

[0388] The availability of cell culture and animal model systems for HCV also makes possible screening for anti-viral agents which inhibit HCV replication, and particularly for those agents which preferentially allow cell growth and multiplication while inhibiting viral replication. These screening methods are known by those of skill in the art. Generally, the anti-viral agents are tested at a variety of concentrations, for their effect on preventing viral replication in cell culture systems which support viral replication, and then for an inhibition of infectivity or of viral pathogenicity (and a low level of toxicity) in an animal model system.

[0389] The methods and compositions provided herein for detecting HCV antigens and HCV polynucleotides are useful for screening of anti-viral agents in that they provide an alternative, and perhaps more sensitive means, for detecting the agent's effect on viral replication than the cell plaque assay or ID$_{50}$ assay. For example, the HCV-polynucleotide probes described herein may be used to quantitate the amount of viral nucleic acid produced in a cell culture. This could be accomplished, for example, by hybridization or competition hybridization of the infected cell nucleic acids with a labeled HCV-polynucleotide probe. For example, also, anti-HCV antibodies may be used to identify and quantitate HCV antigen(s) in the cell culture utilizing the immunoassays described herein. In addition, since it may be desirable to quantitate HCV antigens in the infected cell culture by a competition assay, the polypeptides encoded within the HCV cDNAs described herein are useful in these competition assays. Generally, a recombinant HCV polypeptide derived from the HCV cDNA would be labeled, and the inhibition of binding of this labeled polypeptide to an HCV polypeptide due to the antigen produced in the cell culture

system would be monitored. Moreover, these techniques are particularly useful in cases where the HCV may be able to replicate in a cell line without causing cell death.

[0390] The anti-viral agents which may be tested for efficacy by these methods are known in the art, and include, for example, those which interact with virion components and/or cellular components which are necessary for the binding and/or replication of the virus. Typical anti-viral agents may include, for example, inhibitors of virion polymerase and/or protease(s) necessary for cleavage of the precursor polypeptides. Other anti-viral agents may include those which act with nucleic acids to prevent viral replication, for example, anti-sense polynucleotides, etc.

[0391] Antisense polynucleotides molecules are comprised of a complementary nucleotide sequence which allows them to hybridize specifically to designated regions of genomes or RNAs. Antisense polynucleotides may include, for example, molecules that will block protein translation by binding to mRNA, or may be molecules which prevent replication of viral RNA by transcriptase. They may also include molecules which carry agents (non-covalently attached or covalently bound) which cause the viral RNA to be inactive by causing, for example, scissions in the viral RNA. They may also bind to cellular polynucleotides which enhance and/or are required for viral infectivity, replicative ability, or chronicity. Antisense molecules which are to hybridize to HCV derived RNAs may be designed based upon the sequence information of the HCV cDNAs provided herein. The antiviral agents based upon anti-sense polynucleotides for HCV may be designed to bind with high specificity, to be of increased solubility, to be stable, and to have low toxicity. Hence, they may be delivered in specialized systems, for example, liposomes, or by gene therapy. In addition, they may include analogs, attached proteins, substituted or altered bonding between bases, etc.

[0392] Other types of drugs may be based upon polynucleotides which "mimic" important control regions of the HCV genome, and which may be therapeutic due to their interactions with key components of the system responsible for viral infectivity or replication.

[0393] II.M. Preparation of Attenuated Strains of HCV

[0394] In addition to the above, utilizing the tissue culture systems and/or animal model systems, it may be possible to isolate attenuated strains of HCV. These strains would be suitable for vaccines, or for the isolation of viral antigens. Attenuated strains are isolatable after multiple passages in cell culture and/or an animal model. Detection of an attenuated strain in an infected cell or individual is achievable by techniques known in the art, and could include, for example, the use of antibodies to one or more epitopes encoded in HCV as a probe or the use of a polynucleotide containing an HCV sequence of at least about 8 nucleotides as a probe. Alternatively, or in addition, an attenuated strain may be constructed utilizing the genomic information of HCV provided herein, and utilizing recombinant techniques. Generally, one would attempt to delete a region of the genome encoding, for example, a polypeptide related to pathogenicity, but which allows viral replication. In addition, the genome construction would allow the expression of an epitope which gives rise to neutralizing antibodies for HCV. The altered genome could then be utilized to transform cells which allow HCV replication, and the cells grown under

conditions to allow viral replication. Attenuated HCV strains are useful not only for vaccine purposes, but also as sources for the commercial production of viral antigens, since the processing of these viruses would require less stringent protection measures for the employees involved in viral production and/or the production of viral products.

[0395] III. General Methods

[0396] The general techniques used in extracting the genome from a virus, preparing and probing a cDNA library, sequencing clones, constructing expression vectors, transforming cells, performing immunological assays such as radioimmunoassays and ELISA assays, for growing cells in culture, and the like are known in the art and laboratory manuals are available describing these techniques. However, as a general guide, the following sets forth some sources currently available for such procedures, and for materials useful in carrying them out.

[0397] III.A. Hosts and Expression Control Sequences

[0398] Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences which are compatible with the designated host are used. Among prokaryotic hosts, *E. coli* is most frequently used. Expression control sequences for prokaryotes include promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts are commonly derived from, for example, pBR322, a plasmid containing operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, which also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Commonly used prokaryotic control sequences include the Betalactamase (penicillinase) and lactose promoter systems (Chang et al. (1977)), the tryptophan (trp) promoter system (Goeddel et al. (1980)) and the lambda-derived PL promoter and N gene ribosome binding site (Shimatake et al. (1981)) and the hybrid tac promoter (De Boer et al. (1983)) derived from sequences of the trp and lac UV5 promoters. The foregoing systems are particularly compatible with *E. coli*; if desired, other prokaryotic hosts such as strains of Bacillus or Pseudomonas may be used, with corresponding control sequences.

[0399] Eukaryotic hosts include yeast and mammalian cells in culture systems. *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* are the most commonly used yeast hosts, and are convenient fungal hosts. Yeast compatible vectors carry markers which permit selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2 micron origin of replication (Broach et al. (1983)), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences which will result in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include promoters for the synthesis of glycolytic enzymes (Hess et al. (1968); Holland et al. (1978)), including the promoter for 3 phosphoglycerate kinase (Hitzeman (1980)). Terminators may also be included, such as those derived from the enolase gene (Holland (1981)). Particularly useful control systems are those which comprise the glyceraldehyde-3 phosphate dehydrogenase (GAPDH) promoter or alcohol dehydroge-

nase (ADH) regulatable promoter, terminators also derived from GAPDH, and if secretion is desired, leader sequence from yeast alpha factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably linked may be such that they are not naturally associated in the wild-type organism. These systems are described in detail in EPO 120,551, published Oct. 3, 1984; EPO 116,201, published Aug. 22, 1984; and EPO 164,556, published Dec. 18, 1985, all of which are assigned to the herein assignee, and are hereby incorporated herein by reference.

[0400] Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include viral promoters such as that from Simian Virus 40 (SV40) (Fiers (1978)), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly A addition sequences; enhancer sequences which increase expression may also be included, and sequences which cause amplification of the gene may also be desirable. These sequences are known in the art.

[0401] Vectors suitable for replication in mammalian cells are known in the art, and may include viral replicons, or sequences which insure integration of the appropriate sequences encoding NANBV epitopes into the host genome.

[0402] A vector which is used to express foreign DNA, and which may be used in vaccine preparation is Vaccinia virus. In this case the heterologous DNA is inserted into the Vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and utilize, for example, homologous recombination. The insertion of the heterologous DNA is generally into a gene which is non-essential in nature, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett et al. (1984), Chakrabarti et al. (1985); Moss (1987)). Expression of the HCV polypeptide then occurs in cells or individuals which are immunized with the live recombinant vaccinia virus.

[0403] The segment of HCV cDNA which is inserted into a vaccinia vector may be derived from the "x" region of the HCV genome (see **FIG. 69**); for example, it may encode a polypeptide comprised of amino acid numbers 406-661. The polypeptide encoding sequence may be attached to a leader sequence. The leader sequence may be that for tissue plasminogen activator (tPA), or from another source, e.g., that for beta-globin. The heterologous polynucleotide may be inserted into a vaccinia vector which is a modified version of pSC11, due to the addition of a polylinker sequence which contains a cloning site.

[0404] The segment of HCV cDNA which is inserted into the vaccinia vector may also be derived from the "x" region of the HCV genome, but it may encode a larger polypeptide, i.e., one comprised of amino acid numbers 347-906. The polypeptide encoding sequence, which may or may not be

attached to an upstream heterologous leader sequence, may be inserted into the modified pSC11 vaccinia vector described above.

[0405] In order to detect whether or not the HCV polypeptide is expressed from the vaccinia vector, BSC1 cells may be infected with the recombinant vector and grown on microscope slides under conditions which allow expression. The cells may then be acetone-fixed, and immunofluorescence assays performed using serum which is known to contain anti-HCV antibodies to a polypeptide(s) encoded in the region of the HCV genome from which the HCV segment in the recombinant expression vector was derived.

[0406] Other systems for expression of eukaryotic or viral genomes include insect cells and vectors suitable for use in these cells. These systems are known in the art, and include, for example, insect expression transfer vectors derived from the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV), which is a helper-independent, viral expression vector. Expression vectors derived from this system usually use the strong viral polyhedrin gene promoter to drive expression of heterologous genes. Currently the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373 (**FIG. 70**). Many other vectors, known to those of skill in the art, have also been designed for improved expression. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; See Luckow and Summers (1989)). AcNPV transfer vectors for high level expression of nonfused foreign proteins are shown in **FIG. 70**. In the figure, the numbers shown refer to positions wihtin the native gene, where the A of the ATG codon is +1. **FIG. 70** also shows a restriction endonuclease map of the transfer vector pAc373. The map shows that a unique BamHI site is located following position –8 with respect to the translation initiation codon ATG of the polyhedrin gene. There are no cleavage sites for SmaI, PstI, BglII, XbaI or SstI. Good expression of nonfused foreign proteins usually requires foreign genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. The plasmid also contains the polyhedrin polyadenylation signal and the ampicillin-resistance (amp) gene and origin of replication for selection and propagation in *E. coli*.

[0407] Methods for the introduction of heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summer and Smith, Texas Agricultural Experiment Station Bulletin No. 1555; Ju et al. (1987); Smith et al. (1983); and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. The inserted sequences may be those which encode all or varying segments of the polyprotein, or other orfs which encode viral polypeptides. For example, the insert could encode the following numbers of amino acid segments from the polyprotein: amino acids 1-1078; amino acids 332-662; amino acids 406-662; amino acids 156-328, and amino acids 199-328.

[0408] The signals for posttranslational modifications, such as signal peptide cleavage, proteolytic cleavage, and phosphorylation, appear to be recognized by insect cells.

The signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells. Examples of the signal sequences from vertebrate cells which are effective in invertebrate cells are known in the art, for example, the human interleukin 2 signal (IL2$_s$) which is a signal for transport out of the cell, is recognized and properly removed in insect cells.

[0409] III.B. Transformations

[0410] Transformation may be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus, and by direct uptake of the polynucleotide. The transformation procedure used depends upon the host to be transformed. For example, transformation of the *E. coli* host cells with lambda-gt11 containing BB-NANBV sequences is discussed in the Example section, infra. Bacterial transformation by direct uptake generally employs treatment with calcium or rubidium chloride (Cohen (1972); Maniatis (1982)). Yeast transformation by direct uptake may be carried out using the method of Hinnen et al. (1978). Mammalian transformations by direct uptake may be conducted using the calcium phosphate precipitation method of Graham and Van der Eb (1978), or the various known modifications thereof. Other methods for the introduction of recombinant polynucleotides into cells, particularly into mammalian cells, which are known in the art include dextran-mediated transfection, calcium phosphate mediated transfection, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the polynucleotides into nuclei.

[0411] III.C. Vector Construction

[0412] Vector construction employs techniques which are known in the art. Site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions which generally are specified by the manufacturer of these commercially available enzymes. In general, about 1 microgram of plasmid or DNA sequence is cleaved by 1 unit of enzyme in about 20 microliters buffer solution by incubation of 1-2 hr at 37° C. After incubation with the restriction enzyme, protein is removed by phenol/chloroform extraction and the DNA recovered by precipitation with ethanol. The cleaved fragments may be separated using polyacrylamide or agarose gel electrophoresis techniques, according to the general procedures found in Methods in Enzymology (1980) 65:499-560.

[0413] Sticky ended cleavage fragments may be blunt ended using *E. coli* DNA polymerase I (Klenow) in the presence of the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with S1 nuclease may also be used, resulting in the hydrolysis of any single stranded DNA portions.

[0414] Ligations are carried out using standard buffer and temperature conditions using T4 DNA ligase and ATP; sticky end ligations require less ATP and less ligase than blunt end ligations. When vector fragments are used as part of a ligation mixture, the vector fragment is often treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase to remove the 5'-phosphate and thus prevent religation of the vector; alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation.

[0415] Ligation mixtures are transformed into suitable cloning hosts, such as *E. coli*, and successful transformants selected by, for example, antibiotic resistance, and screened for the correct construction.

[0416] III.D. Construction of Desired DNA Sequences

[0417] Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer as described by Warner (1984). If desired the synthetic strands may be labeled with ^{32}P by treatment with polynucleotide kinase in the presence of ^{32}P-ATP, using standard conditions for the reaction.

[0418] DNA sequences, including those isolated from cDNA libraries, may be modified by known techniques, including, for example site directed mutagenesis, as described by Zoller (1982). Briefly, the DNA to be modified is packaged into phage as a single stranded sequence, and converted to a double stranded DNA with DNA polymerase using, as a primer, a synthetic oligonucleotide complementary to the portion of the DNA to be modified, having the desired modification included in its own sequence. The resulting double stranded DNA is transformed into a phage supporting host bacterium. Cultures of the transformed bacteria, which contain replications of each strand of the phage, are plated in agar to obtain plaques. Theoretically, 50% of the new plaques contain phage having the mutated sequence, and the remaining 50% have the original sequence. Replicates of the plaques are hybridized to labeled synthetic probe at temperatures and conditions which permit hybridization with the correct strand, but not with the unmodified sequence. The sequences which have been identified by hybridization are recovered and cloned.

[0419] III.E. Hybridization with Probe

[0420] DNA libraries may be probed using the procedure of Grunstein and Hogness (1975). Briefly, in this procedure, the DNA to be probed is immobilized on nitrocellulose filters, denatured, and prehybridized with a buffer containing 0-50% formamide, 0.75 M NaCl, 75 mM Na citrate, 0.02% (wt/v) each of bovine serum albumin, polyvinyl pyrollidone, and Ficoll, 50 mM Na Phosphate (pH 6.5), 0.1% SDS, and 100 micrograms/ml carrier denatured DNA. The percentage of formamide in the buffer, as well as the time and temperature conditions of the prehybridization and subsequent hybridization steps depends on the stringency required. Oligomeric probes which require lower stringency conditions are generally used with low percentages of formamide, lower temperatures, and longer hybridization times. Probes containing more than 30 or 40 nucleotides such as those derived from cDNA or genomic sequences generally employ higher temperatures, e.g., about 40-42° C., and a high percentage, e.g., 50%, formamide. Following prehybridization, 5'-^{32}P-labeled oligonucleotide probe is added to the buffer, and the filters are incubated in this mixture under hybridization conditions. After washing, the treated filters are subjected to autoradiography to show the location of the hybridized probe; DNA in corresponding locations on the original agar plates is used as the source of the desired DNA.

[0421] III.F. Verification of Construction and Sequencing

[0422] For routine vector constructions, ligation mixtures are transformed into *E. coli* strain HB101 or other suitable host, and successful transformants selected by antibiotic resistance or other markers. Plasmids from the transformants

are then prepared according to the method of Clewell et al. (1969), usually following chloramphenicol amplification (Clewell (1972)). The DNA is isolated and analyzed, usually by restriction enzyme analysis and/or sequencing. Sequencing may be by the dideoxy method of Sanger et al. (1977) as further described by Messing et al. (1981), or by the method of Maxam et al. (1980). Problems with band compression, which are sometimes observed in GC rich regions, were overcome by use of T-deazoguanosine according to Barr et al. (1986).

[0423] III.G. Enzyme Linked Immunosorbent Assay

[0424] The enzyme-linked immunosorbent assay (ELISA) can be used to measure either antigen or antibody concentrations. This method depends upon conjugation of an enzyme to either an antigen or an antibody, and uses the bound enzyme activity as a quantitative label. To measure antibody, the known antigen is fixed to a solid phase (e.g., a microplate or plastic cup), incubated with test serum dilutions, washed, incubated with anti-immunoglobulin labeled with an enzyme, and washed again. Enzymes suitable for labeling are known in the art, and include, for example, horseradish peroxidase. Enzyme activity bound to the solid phase is measured by adding the specific substrate, and determining product formation or substrate utilization calorimetrically. The enzyme activity bound is a direct function of the amount of antibody bound.

[0425] To measure antigen, a known specific antibody is fixed to the solid phase, the test material containing antigen is added, after an incubation the solid phase is washed, and a second enzyme-labeled antibody is added. After washing, substrate is added, and enzyme activity is estimated calorimetrically, and related to antigen concentration.

IV. EXAMPLES

[0426] Described below are examples of the present invention which are provided only for illustrative purposes, and not to limit the scope of the present invention. In light of the present disclosure, numerous embodiments within the scope of the claims will be apparent to those of ordinary skill in the art. The procedures set forth, for example, in Sections IV.A. may, if desired, be repeated but need not be, as techniques are available for construction of the desired nucleotide sequences based on the information provided by the invention. Expression is exemplified in E. coli; however, other systems are available as set forth more fully in Section III.A. Additional epitopes derived from the genomic structure may also be produced, and used to generate antibodies as set forth below.

[0427] IV.A. Preparation, Isolation and Sequencing of HCV cDNA

[0428] IV.A.1. Preparation of HCV cDNA

[0429] The source of NANB agent was a plasma pool derived from a chimpanzee with chronic NANBH. The chimpanzee had been experimentally infected with blood from another chimpanzee with chronic NANBH resulting from infection with HCV in a contaminated batch of factor 8 concentrate derived from pooled human sera. The chimpanzee plasma pool was made by combining many individual plasma samples containing high levels of alanine aminotransferase activity; this activity results from hepatic injury due to the HCV infection. Since 1 ml of a 10^{-6}

dilution of this pooled serum given i.v. caused NANBH in another chimpanzee, its CID was at least 10^6/ml, i.e., it had a high infectious virus titer.

[0430] A cDNA library from the high titer plasma pool was generated as follows. First, viral particles were isolated from the plasma; a 90 ml aliquot was diluted with 310 ml of a solution containing 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl. Debris was removed by centrifugation for 20 min at 15,000×g at 20° C. Viral particles in the resulting supernatant were then pelleted by centrifugation in a Beckman SW28 rotor at 28,000 rpm for 5 hours at 20° C. To release the viral genome, the particles were disrupted by suspending the pellets in 15 ml solution containing 1% sodium dodecyl sulfate (SDS), 10 mM EDTA, 10 mM Tris-HCl, pH 7.5, also containing 2 mg/ml proteinase k, followed by incubation at 45° C. for 90 min. Nucleic acids were isolated by adding 0.8 micrograms MS2 bacteriophage RNA as carrier, and extracting the mixture four times with a 1:1 mixture of phenol:chloroform (phenol saturated with 0.05M Tris-HCl, pH 7.5, 0.1% (v/v) beta-mercaptoethanol, 0.1% (w/v) hydroxyquinolone, followed by extraction two times with chloroform. The aqueous phase was concentrated with 1-butanol prior to precipitation with 2.5 volumes absolute ethanol overnight at −20° C. Nucleic acid was recovered by centrifugation in a Beckman SW41 rotor at 40,000 rpm for 90 min at 4° C., and dissolved in water that had been treated with 0.05% (v/v) diethylpyrocarbonate and autoclaved.

[0431] Nucleic acid obtained by the above procedure (<2 micrograms) was denatured with 17.5 mM CH_3HgOH; cDNA was synthesized using this denatured nucleic acid as template, and was cloned into the EcoRI site of phage lambda-gt11 using methods described by Huynh (1985), except that random primers replaced oligo(dT) 12-18 during the synthesis of the first cDNA strand by reverse transcriptase (Taylor et al. (1976)). The resulting double stranded cDNAs were fractionated according to size on a Sepharose CL-4B column; elated material of approximate mean size 400, 300, 200, and 100 base-pairs were pooled into cDNA pools 1, 2, 3, and 4, respectively. The lambda-gt11 cDNA library was generated from the cDNA in pool 3.

[0432] The lambda-gt11 cDNA library generated from pool 3 was screened for epitopes that could bind specifically with serum derived from a patient who had previously experienced NANBH. About 10^6 phage were screened with patient sera using the methods of Huynh et al. (1985), except that bound human antibody was detected with sheep anti-human Ig antisera that had been radio-labeled with ^{125}I. Five positive phages were identified and purified. The five positive phages were then tested for specificity of binding to sera from 8 different humans previously infected with the NANBH agent, using the same method. Four of the phage encoded a polypeptide that reacted immunologically with only one human serum, i.e., the one that was used for primary screening of the phage library. The fifth phage (5-1-1) encoded a polypeptide that reacted immunologically with 5 of 8 of the sera tested. Moreover, this polypeptide did not react immunologically with sera from 7 normal blood donors. Therefore, it appears that clone 5-1-1 encodes a polypeptide which is specifically recognized immunologically by sera from NANB patients.

[0433] IV.A.2. Sequences of the HCV cDNA in Recombinant Phage 5-1-1, and of the Polypeptide Encoded Within the Sequence.

[0434] The cDNA in recombinant phage 5-1-1 was sequenced by the method of Sanger et al. (1977). Essentially, the cDNA was excised with EcoRI, isolated by size fractionation using gel electrophoresis. The EcoRI restriction fragments were subcloned into the M13 vectors, mp18 and mp19 (Messing (1983)) and sequenced using the dideoxy-chain termination method of Sanger et al. (1977). The sequence obtained is shown in **FIG. 1**.

[0435] The polypeptide encoded in **FIG. 1** that is encoded in the HCV cDNA is in the same translational frame as the N-terminal beta-galactosidase moiety to which it is fused. As shown in Section IV.A., the translational open reading frame (ORF) of 5-1-1 encodes epitope(s) specifically recognized by sera from patients and chimpanzees with NANBH infections.

[0436] IV.A.3. Isolation of Overlapping HCV cDNA to cDNA in Clone 5-1-1.

[0437] Overlapping HCV cDNA to the cDNA in clone 5-1-1 was obtained by screening the same lambda-gt11 library, created as described in Section IV.A.1., with a synthetic polynucleotide derived from the sequence of the HCV cDNA in clones 5-1-1, as shown in **FIG. 1**. The sequence of the polynucleotide used for screening was:

[0438] 5'-TCC CTT GCT CGA TGT ACG GTA AGT GCT GAG AGC ACT CTT CCA TCT CAT CGA ACT CTC GGT AGA GGA CTT CCC TGT CAG GT-3'.

[0439] The lambda-gt11 library was screened with this probe, using the method described in Huynh (1985). Approximately 1 in 50,000 clones hybridized with the probe. Three clones which contained cDNAs which hybridized with the synthetic probe have been numbered 81, 1-2, and 91.

[0440] IV.A.4. Nucleotide Sequences of Overlapping HCV cDNAs to cDNA in Clone 5-1-1.

[0441] The nucleotide sequences of the three cDNAs in clones 81, 1-2, and 91 were determined essentially as in Section IV.A.2. The sequences of these clones relative to the HCV cDNA sequence in phage 5-1-1 is shown in **FIG. 2**, which shows the strand encoding the detected HCV epitope, and where the homologies in the nucleotide sequences are indicated by vertical lines between the sequences.

[0442] The sequences of the cloned HCV cDNAs are highly homologous in the overlapping regions (see **FIG. 2**). However, there are differences in two regions. Nucleotide 67 in clone 1-2 is a thymidine, whereas the other three clones contain a cytidine residue in this position. It should be noted, however, that the same amino acid is encoded when either C or T occupies this position.

[0443] The second difference is that clone 5-1-1 contains 28 base pairs which are not present in the other three clones. These base pairs occur at the start of the cDNA sequence in 5-1-1, and are indicated by small letters. The 28 bp region is probably a terminal artifact in clone 5-1-1 which enhances its antigenicity.

[0444] The sequences of small letters in the nucleotide sequence of clones 81 and 91 simply indicate that these sequences have not been found in other cDNAs because cDNAs overlapping these regions were not yet isolated.

[0445] A composite HCV cDNA sequence derived from overlapping cDNAs in clones 5-1-1, 81, 1-2 and 91 is shown in **FIG. 3**. However, in this figure the unique 28 base pairs of clone 5-1-1 are omitted. The figure also shows the sequence of the polypeptide encoded within the ORF of the composite HCV cDNA.

[0446] IV.A.5. Isolation of Overlapping HCV cDNAs to cDNA in Clone 81.

[0447] The isolation of HCV cDNA sequences upstream of, and which overlap those in clone 81 cDNA was accomplished as follows. The lambda-gt11 cDNA library prepared as described in Section IV.A.1. was screened by hybridization with a synthetic polynucleotide probe which was homologous to a 5' terminal sequence of clone 81. The sequence of clone 81 is presented in **FIG. 4**. The sequence of the synthetic polynucleotide used for screening was:

[0448] 5'CTG TCA GGT ATG ATT GCC GGC TTC CCG GAC 3'.

[0449] The methods were essentially-as described in Huynh (1985), except that the library filters were given two washes under stringent conditions, i.e., the washes were in 5×SSC, 0.1% SDS at 55° C. for 30 minutes each. Approximately 1 in 50,000 clones hybridized with the probe. A positive recombinant phage which contained cDNA which hybridized with the sequence was isolated and purified. This phage has been numbered clone 36.

[0450] Downstream cDNA sequences, which overlaps the carboxyl-end sequences in clone 81 cDNA. were isolated using a procedure similar to that for the isolation of upstream cDNA sequences, except that a synthetic oligonucleotide probe was prepared which is homologous to a 3' terminal sequence of clone 81. The sequence of the synthetic polynucleotide used for screening was:

[0451] 5' TTT GGC TAG TGG TTA GTG GGC TGG TGA CAG 3'

[0452] A positive recombinant phage, which contained cDNA which hybridized with this latter sequence was isolated and purified, and has been numbered clone 32.

[0453] IV.A.6. Nucleotide Sequence of HCV cDNA in Clone 36.

[0454] The nucleotide sequence of the cDNA in clone 36 was determined essentially as described in Section IV.A.2. The double-stranded sequence of this cDNA, its region of overlap with the HCV cDNA in clone 81, and the polypeptide encoded by the ORF are shown in **FIG. 5**.

[0455] The ORF in clone 36 is in the same translational frame as the HCV antigen encoded in clone 81. Thus, in combination, the ORFs in clones 36 and 81 encode a polypeptide that represents part of a large HCV antigen. The sequence of this putative HCV polypeptide and the double stranded DNA sequence encoding it, which is derived from the combined ORFs of the HCV cDNAs of clones 36 and 81, is shown in **FIG. 6**.

[0456] IV.A.7 Nucleotide Sequences of HCV cDNA in Clone 32

[0457] The nucleotide sequence of the cDNA in clone 32 was determined essentially as was that described in Section IV.A.2 for the sequence of clone 5-1-1. The sequence data indicated that the cDNA in clone 32 recombinant phage was derived from two different sources. One fragment of the cDNA was comprised of 418 nucleotides derived from the HCV genome; the other fragment was comprised of 172 nucleotides derived from the bacteriophage MS2 genome, which had been used as a carrier during the preparation of the lambda gt11 plasma cDNA library.

[0458] The sequence of the cDNA in clone 32 corresponding to that of the HCV genome is shown in FIG. 7. The region of the sequences that overlaps that of clone 81, and the polypeptide encoded by the ORF are also indicated in the figure. This sequence contains one continuous ORF that is in the same translational frame as the HCV antigen encoded by clone 81.

[0459] IV.A.8 Isolation of Overlapping HCV cDNA to cDNA in Clone 36

[0460] The isolation of HCV cDNA sequences upstream of, and which overlap those in clone 36 cDNA was accomplished as described in Section IV.A.5, for those which overlap clone 81 cDNA, except that the synthetic polynucleotide was based on the 5'-region of clone 36. The sequence of the synthetic polynucleotide used for screening was:

[0461] 5' AAG CCA CCG TGT GCG CTA GGG CTC AAG CCC 3'

[0462] Approximately 1 in 50,000 clones hybridized with the probe. The isolated, purified clone of recombinant phage which contained cDNA which hybridized to this sequence was named clone 35.

[0463] IV.A.9 Nucleotide Sequence of HCV cDNA in Clone 35

[0464] The nucleotide sequence of the cDNA in clone 35 was determined essentially as described in Section IV.A.2. The sequence, its region of overlap with that of the cDNA in clone 36, and the putative polypeptide encoded therein, are shown in FIG. 8.

[0465] Clone 35 apparently contains a single, continuous ORF that encodes a polypeptide in the same translational frame as that encoded by clone 36, clone 81, and clone 32. FIG. 9 shows the sequence of the long continuous ORF that extends through clones 35, 36, 81, and 32, along with the putative HCV polypeptide encoded therein. This combined sequence has been confirmed using other independent cDNA clones derived from the same lambda gt11 cDNA library.

[0466] IV.A.10. Isolation of Overlapping HCV cDNA to cDNA in Clone 35

[0467] The isolation of HCV cDNA sequences upstream of, and which overlap those in clone 35 cDNA was accomplished as described in Section IV.A.8, for those which overlap clone 36 cDNA, except that the synthetic polynucleotide was based on the 5'-region of clone 35. The sequence of the synthetic polynucleotide used for screening was:

[0468] 5'CAG GAT GCT GTC TCC CGC ACT CAA CGT 3'

[0469] Approximately 1 in 50,000 clones hybridized with the probe. The isolated, purified clone of recombinant phage which contained cDNA which hybridized to this sequence was named clone 37b.

[0470] IV.A.11. Nucleotide Sequence of HCV in Clone 37b

[0471] The nucleotide sequence of the cDNA in clone 37b was determined essentially as described in Section IV.A.2. The sequence, its region of overlap with that of the cDNA in clone 35, and the putative polypeptide encoded therein, are shown in FIG. 10.

[0472] The 5'-terminal nucleotide of clone 35 is a T, whereas the corresponding nucleotide in clone 37b is an A. The cDNAs from three other independent clones which were isolated during the procedure in which clone 37b was isolated, described in Section IV.A.10, have also been sequenced. The cDNAs from these clones also contain an A in this position. Thus, the 5'-terminal T in clone 35 may be an artefact of the cloning procedure. It is known that artefacts often arise at the 5'-termini of cDNA molecules.

[0473] Clone 37b apparently contains one continuous ORF which encodes a polypeptide which is a continuation of the polypeptide encoded in the ORF which extends through the overlapping clones 35, 36, 81 and 32.

[0474] IV.A.12 Isolation of Overlapping HCV cDNA to cDNA in Clone 32

[0475] The isolation of HCV cDNA sequences downstream of clone 32 was accomplished as follows. First, clone cla was isolated utilizing a synthetic hybridization probe which was based on the nucleotide sequence of the HCV cDNA sequence in clone 32. The method was essentially that described in Section IV.A.5, except that the sequence of the synthetic probe was:

[0476] 5' AGT GCA GTG GAT GAA CCG GCT GAT AGC CTT 3'.

[0477] Utilizing the nucleotide sequence from clone cla, another synthetic nucleotide was synthesized which had the sequence:

[0478] 5' TCC TGA GGC GAC TGC ACC AGT GGA TAA GCT 3'.

[0479] Screening of the lambda gt11 library using the clone cla derived sequence as probe yielded approximately 1 in 50,000 positive colonies. An isolated, purified clone which hybridized with this probe was named clone 33b.

[0480] IV.A.13 Nucleotide Sequence of HCV cDNA in Clone 33b

[0481] The nucleotide sequence of the cDNA in clone 33b was determined essentially as described in Section IV.A.2. The sequence, its region of overlap with that of the cDNA in clone 32, and the putative polypeptide encoded therein, are shown in FIG. 11.

[0482] Clone 33b apparently contains one continuous ORF which is an extension of the ORFs in overlapping clones 37b, 35, 36, 81 and 32. The polypeptide encoded in clone 33b is in the same translational frame as that encoded in the extended ORF of these overlapping clones.

[0483] IV.A.14 Isolation of Overlapping HCV cDNAs to cDNA Clone 37b and to cDNA in Clone 33b

[0484] In order to isolate HCV cDNAs which overlap the cDNAs in clone 37b and in clone 33b, the following synthetic oligonucleotide probes, which were derived from the cDNAs in those clones, were used to screen the lambda gt11 library, using essentially the method described in Section IV.A.3. The probes used were:

[0485] 5'CAG GAT GCT GTC TCC CGC ACT CAA CGT C 3'and

[0486] 5' TCC TGA GGC GAC TGC ACC AGT GGA TAA GCT 3'

[0487] to detect colonies containing HCV cDNA sequences which overlap those in clones 37b and 33b, respectively. Approximately 1 in 50,000 colonies were detected with each probe. A clone which contained cDNA which was upstream of, and which overlapped the cDNA in clone 37b, was named clone 40b. A clone which contained cDNA which was downstream of, and which overlapped the cDNA in clone 33b was named clone 25c.

[0488] IV.A.15 Nucleotide Sequences of HCV cDNA in clone 40b and in clone 25c

[0489] The nucleotide sequences of the cDNAs in clone 40b and in clone 25c were determined essentially as described in Section IV.A.2. The sequences of 40b and 25c, their regions of overlap with the cDNAs in clones 37b and 33b, and the putative polypeptides encoded therein, are shown in **FIG. 12** (clone 40b) and **FIG. 13** (clone 25c).

[0490] The 5'-terminal nucleotide of clone 40b is a G. However, the cDNAs from five other independent clones which were isolated during the procedure in which clone 40b was isolated, described in Section IV.A.14, have also been sequenced. The cDNAs from these clones also contain a T in this position. Thus, the G may represent a cloning artifact (see the discussion in Section IV.A.11).

[0491] The 5'-terminus of clone 25c is ACT, but the sequence of this region in clone cla (sequence not shown), in clone 33b is TCA. This difference may also represent a cloning artifact, as may the 28 extra 5'-terminal nucleotides in clone 5-1-1.

[0492] Clones 40b and 25c each apparently contain an ORF which is an extension of the continuous ORF in the previously sequenced clones. The nucleotide sequence of the ORF extending through clones 40b, 37b, 35, 36, 81, 32, 33b, and 25c, and the amino acid sequence of the putative polypeptide encoded therein, are shown in **FIG. 14**. In the figure, the potential artifacts have been omitted from the sequence, and instead, the corresponding sequences in non-5'-terminal regions of multiple overlapping clones are shown.

[0493] IV.A.16. Preparation of a Composite HCV cDNA from the cDNAs in Clones 36, 81, and 32

[0494] The composite HCV cDNA, C100, was constructed as follows. First the cDNAs from the clones 36, 81, and 32 were excised with EcoRI. The EcoRI fragment of cDNA from each clone was cloned individually into the EcoRI site of the vector pGEM3-blue (Promega Biotec) The resulting recombinant vectors which contained the cDNAs from clones 36, 81, and 32 were named pGEM3-blue/36,

pGEM3-blue/81, and pGEM3-blue/32, respectively. The appropriately oriented recombinant of pGEM3-blue/81 was digested with NaeI and NarI, and the large (~2850 bp) fragment was purified and ligated with the small (~570 bp) NaeI/NarI purified restriction fragment from pGEM3-blue/36. This composite of the cDNAs from clones 36 and 81 was used to generate another pGEM3-blue vector containing the continuous HCV ORF contained within the overlapping cDNA within these clones. This new plasmid was then digested with PvuII and EcoRI to release a fragment of approximately 680 bp, which was then ligated with the small (580 bp) PvuII/EcoRI fragment isolated from the appropriately oriented pGEM3-blue/32 plasmid, and the composite cDNA from clones 36, 81, and 32 was ligated into the EcoRI linearized vector pSODcf1, which is described in Section IV.B.1, and which was used to express clone 5-1-1 in bacteria. Recombinants containing the ~1270 bp EcoRI fragment of composite HCV cDNA (C100) were selected, and the cDNA from the plasmids was excised with EcoRI and purified.

[0495] IV.A.17. Isolation and Nucleotide Sequences of HCV cDNAs in Clones 14i, 11b, 7f, 7e, 8h, 33c, 14c, 8f, 33f, 33g, and 39c

[0496] The HCV cDNAs in clones 14i, 11b, 7f, 7e, 8h, 33c, 14c, 8f, 33f, 33g, and 39c were isolated by the technique of isolating overlapping cDNA fragments from the lambda gt11 library of HCV cDNAs described in Section IV.A.1. The technique used was essentially as described in Section IV.A.3., except that the probes used were designed from the nucleotide sequence of the last isolated clones from the 5' and the 3' end of the combined HCV sequences. The frequency of clones which hybridized with the probes described below was approximately 1 in 50,000 in each case.

[0497] The nucleotide sequences of the HCV cDNAs in clones 14i, 7f, 7e, 8h, 33c, 14c, 8f, 33f, 33g, and 39c were determined essentially as described in Section IV.A.2., except that the cDNA excised from these phages were substituted for the cDNA isolated from clone 5-1-1.

[0498] Clone 33c was isolated using a hybridization probe based on the sequence of nucleotides in clone 40b. The nucleotide sequence of clone 40b is presented in **FIG. 12**. The nucleotide sequence of the probe used to isolate 33c was:

[0499] 5' ATC AGG ACC GGG GTG AGA ACA ATT ACC ACT 3'

[0500] The sequence of the HCV cDNA in clone 33c, and the overlap with that in clone 40b, is shown in **FIG. 15**, which also shows the amino acids encoded therein.

[0501] Clone 8h was isolated using a probe based on the sequence of nucleotides in clone 33c. The nucleotide sequence of the probe was

[0502] 5' AGA GAC AAC CAT GAG GTC CCC GGT GTT C 3'.

[0503] The sequence of the HCV cDNA in clone 8h, and the overlap with that in clone 33c, and the amino acids encoded therein, are shown in **FIG. 16**.

[0504] Clone 7e was isolated using a probe based on the sequence of nucleotides in clone 8h. The nucleotide sequence of the probe was

[0505] 5' TCG CAC CTT TAC CTG GTC ACG AGG CAC 3'.

[0506] The sequence of HCV cDNA in clone 7e, the overlap with clone 8h, and the amino acids encoded therein, are shown in **FIG. 17**.

[0507] Clone 14c was isolated with a probe based on the sequence of nucleotides in clone 25c. The sequence of clone 25c is shown in **FIG. 13**. The probe in the isolation of clone 14c had the sequence

[0508] 5' ACC TTC CCC ATT AAT GCC TAC ACC ACG GGC 3'.

[0509] The sequence of HCV cDNA in clone 14c, its overlap with that in clone 25c, and the amino acids encoded therein are shown in **FIG. 18**.

[0510] Clone 8f was isolated using a probe based on the sequence of nucleotides in clone 14c. The nucleotide sequence of the probe was

[0511] 5' TCC ATC TCT CAA GGC AAC TTG CAC CGC TAA 3'.

[0512] The sequence of HCV cDNA in clone 8f, its overlap with that in clone 14c, and the amino acids encoded therein are shown in **FIG. 19**.

[0513] Clone 33f was isolated using a probe based on the nucleotide sequence present in clone 8f. The nucleotide sequence of the probe was

[0514] 5' TCC ATG GCT GTC CGC TTC CAC CTC CAA AGT 3'.

[0515] The sequence of HCV cDNA in clone 33f, its overlap with that in clone 8f, and the amino acids encoded therein are shown in **FIG. 20**.

[0516] Clone 33g was isolated using a probe based on the sequence of nucleotides in clone 33f. The nucleotide sequence of the probe was

[0517] 5' GCG ACA ATA CGA CAA CAT CCT CTG AGC CCG 3'.

[0518] The sequence of HCV cDNA in clone 33g, its overlap with that in clone 33f, and the amino acids encoded therein are shown in **FIG. 21**.

[0519] Clone 7f was isolated using a probe based on the sequence of nucleotides in clone 7e. The nucleotide sequence of the probe was

[0520] 5' AGC AGA CAA GGG GCC TCC TAG GGT GCA TAA T 3'.

[0521] The sequence of HCV cDNA in clone 7f, its overlap with clone 7e, and the amino acids encoded therein are shown in **FIG. 22**.

[0522] Clone 11b was isolated using a probe based on the sequence of clone 7f. The nucleotide sequence of the probe was

[0523] 5'CAC CTA TGT TTA TAA CCA TCT CAC TCC TCT 3'.

[0524] The sequence of HCV cDNA in clone 11b, its overlap with clone 7f, and the amino acids encoded therein are shown in **FIG. 23**.

[0525] Clone 14i was isolated using a probe based on the sequence of nucleotides in clone 11b. The nucleotide sequence of the probe was

[0526] 5'CTC TGT CAC CAT ATT ACA AGC GCT ATA TCA 3'.

[0527] The sequence of HCV cDNA in clone 14i, its overlap with 11b, and the amino acids encoded therein are shown in **FIG. 24**.

[0528] Clone 39c was isolated using a probe based on the sequence of nucleotides in clone 33g. The nucleotide sequence of the probe was

[0529] 5'CTC GTT GCT ACG TCA CCA CAA TTT GGT GTA 3'

[0530] The sequence of HCV cDNA in clone 39c, its overlap with clone 33g, and the amino acids encoded therein are shown in **FIG. 25**.

[0531] IV.A.18. The Composite HCV cDNA Sequence Derived from Isolated Clones Containing HCV cDNA

[0532] The HCV cDNA sequences in the isolated clones described supra have been aligned to create a composite HCV cDNA sequence. The isolated clones, aligned in the 5' to 3' direction are; 14i, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, and 39c.

[0533] A composite HCV cDNA sequence derived from the isolated clones, and the amino acids encoded therein, is shown in **FIG. 26**.

[0534] In creating the composite sequence the following sequence heterogeneities have been considered. Clone 33c contains an HCV cDNA of 800 base pairs, which overlaps the cDNAs in clones 40b and 37c. In clone 33c, as well as in 5 other overlapping clones, nucleotide #789 is a G. However, in clone 37b (see Section IV.A.11), the corresponding nucleotide is an A. This sequence difference creates an apparent heterogeneity in the amino acids encoded therein, which would be either CYS or TYR, for G or A, respectively. This heterogeneity may have important ramifications in terms of protein folding.

[0535] Nucleotide residue #2 in clone 8h HCV cDNA is a T. However, as shown infra, the corresponding residue in clone 7e is an A; moreover, an A in this position is also found in 3 other isolated overlapping clones. Thus, the T residue in clone 8h may represent a cloning artifact. Therefore, in **FIG. 26**, the residue in this position is designated as an A.

[0536] The 3'-terminal nucleotide in clone 8f HCV cDNA is a G. However, the corresponding residue in clone 33f, and in 2 other overlapping clones is a T. Therefore, in **FIG. 26**, the residue in this position is designated as a The 3'-terminal sequence in clone 33f HCV cDNA is TTGC. However, the corresponding sequence in clone 33g and in 2 other overlapping clones is ATTC. Therefore, in **FIG. 26**, the corresponding region is represented as ATTC.

[0537] Nucleotide residue #4 in clone 33g HCV cDNA is a T. However, in clone 33f and in 2 other overlapping clones the corresponding residue is an A. Therefore, in **FIG. 26**, the corresponding residue is designated as an A.

[0538] The 3'-terminus of clone 14i is an AA, whereas the corresponding dinucleotide in clone 11b, and in three other clones, is TA. Therefore, in **FIG. 26**, the TA residue is depicted.

[0539] The resolution of other sequence heterogeneities is discussed supra.

[0540] An examination of the composite HCV cDNA indicates that it contains one large ORF. This suggests that the viral genome is translated into a large polypeptide which is processed concomitant with, or subsequent to translation.

[0541] IV.A.19. Isolation and Nucleotide Sequences of HCV cDNAs in Clones 12f, 35f, 19g, 26g, and 15e

[0542] The HCV cDNAs in clones 12f, 35f, 19g, 26g, and 15e were isolated essentially by the technique described in Section IV.A.17, except that the probes were as indicated below. The frequency of clones which hybridized with the probes was approximately 1 in 50,000 in each case. The nucleotide sequences of the HCV cDNAs in these clones were determined essentially as described in Section IV.A.2., except that the cDNA from the indicated clones were substituted for the cDNA isolated from clone 5-1-1.

[0543] The isolation of clone 12f, which contains cDNA upstream of the HCV cDNA in **FIG. 26**, was accomplished using a hybridization probe based on the sequence of nucleotides in clone 14i. The nucleotide sequence of the probe was

[0544] 5' TGC TTG TGG ATG ATG CTA CTC ATA TCC CAA 3'.

[0545] The HCV cDNA sequence of clone 12f, its overlap with clone 14i, and the amino acids encoded therein are shown in **FIG. 27**.

[0546] The isolation of clone 35f, which contains cDNA downstream of the HCV cDNA in **FIG. 26**, was accomplished using a hybridization probe based on the sequence of nucleotides in clone 39c. The nucleotide sequence of the probe was

[0547] 5' AGC AGC GGC GTC AAA AGT GAA GGC TAA CTT 3'.

[0548] The sequence of clone 35f, its overlap with the sequence in clone 39c, and the amino acids encoded therein are shown in **FIG. 28**. The isolation of clone 19g was accomplished using a hybridization probe based on the 3' sequence of clone 35f. The nucleotide sequence of the probe was

[0549] 5' TTC TCG TAT GAT ACC CGC TGC TTT GAC TCC 3'.

[0550] The HCV cDNA sequence of clone 19g, its overlap with clone 35f, and the amino acids encoded therein are shown in **FIG. 29**.

[0551] The isolation of clone 26g was accomplished using a hybridization probe based on the 3' sequence of clone 19g. The nucleotide sequence of the probe was

[0552] 5' TGT GTG GCG ACG ACT TAG TCG TTA TCT GTG 3'.

[0553] The HCV cDNA sequence of clone 26g, its overlap with the sequence in clone 19g, and the amino acids encoded therein are shown in **FIG. 30**.

[0554] Clone 15e was isolated using a hybridization probe based on the 3' sequence of clone 26g. The nucleotide sequence of the probe was

[0555] 5'CAC ACT CCA GTC AAT TCC TGG CTA GGC AAC 3'.

[0556] The HCV cDNA sequence of clone 15e, its overlap with the sequence in clone 26g, and the amino acids encoded therein are shown in **FIG. 31**.

[0557] The HCV cDNA sequences in the isolated clones described supra. have been aligned to create a composite HCV cDNA sequence. The isolated clones, aligned in the 5' to 3' direction are: 12f, 14i, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f 33f, 33g, 39c, 35f, 19g, 26g, and 15e.

[0558] A composite HCV cDNA sequence derived from the isolated clones, and the amino acids encoded therein, is shown in **FIG. 32**.

[0559] IV.A.20. Alternative Method of Isolating cDNA Sequences Upstream of the HCV cDNA Sequence in Clone 12f

[0560] Based on the most 5' HCV sequence in **FIG. 32**, which is derived from the HCV cDNA in clone 12f, small synthetic oligonucleotide primers of reverse transcriptase are synthesized and used to bind to the corresponding sequence in HCV genomic RNA, to prime reverse transcription of the upstream sequences. The primer sequences are proximal to the known 5'-terminal sequence of clone 12f, but sufficiently downstream to allow the design of probe sequences upstream of the primer sequences. Known standard methods of priming and cloning are used. The resulting cDNA libraries are screened with sequences upstream of the priming sites (as deduced from the elucidated sequence in clone 12f). The HCV genomic RNA is obtained from either plasma or liver samples from chimpanzees with NANBH, or from analogous samples from humans with NANBH.

[0561] IV.A.21. Alternative method Utilizing Tailing to Isolate Sequences from the 5'-Terminal Region of the HCV Genome

[0562] In order to isolate the extreme 5'-terminal sequences of the HCV RNA genome, the cDNA product of the first round of reverse transcription, which is duplexed with the template RNA, is tailed with oligo C. This is accomplished by incubating the product with terminal transferase in the presence of CTP. The second round of cDNA synthesis, which yields the complement of the first strand of cDNA, is accomplished utilizing oligo G as a primer for the reverse transcriptase reaction. The sources of genomic HCV RNA are as described in Section IV.A.20. The methods for tailing with terminal transferase, and for the reverse transcriptase reactions are as in Maniatis et al. (1982). The cDNA products are then cloned, screened, and sequenced.

[0563] IV.A.22. Alternative Method Utilizing Tailing to Isolate Sequences from the 3'-Terminal Region of the HCV Genome

[0564] This method is based on previously used methods for cloning cDNAs of Flavivirus RNA. In this method, the RNA is subjected to denaturing conditions to remove secondary structures at the 3'-terminus, and is then tailed with Poly A polymerase using rATP as a substrate. Reverse transcription of the poly A tailed RNA is catalyzed by reverse transcriptase, utilizing oligo dT as a primer. The second strands of cDNA are synthesized, the cDNA products are cloned, screened, and sequenced.

[0565] IV.A.23 Creation of Lambda-gt11 HCV cDNA Libraries Containing Larger cDNA Inserts

[0566] The method used to create and screen the Lambda gt11 libraries are essentially as described in Section IV.A.1., except that the library is generated from a pool of larger size cDNAs eluted from the Sepharose CL-4B column.

[0567] IV.A.24. Creation of HCV cDNA Libraries Using Synthetic Oligomers as Primers

[0568] New HCV cDNA libraries have been prepared from the RNA derived from the infectious chimpanzee plasma pool described in Section IV.A.1., and from the poly A+ RNA fraction derived from the liver of this infected animal. The cDNA was constructed essentially as described by Gubler and Hoffman (1983), except that the primers for the first cDNA strand synthesis were two synthetic oligomers based on the sequence of the HCV genome described supra. Primers based on the sequence of clone 11 b and 7e were, respectively,

[0569] 5'CTG GCT TGA AGA ATC 3'and

[0570] 5' AGT TAG GCT GGT GAT TAT GC 3'.

[0571] The resulting cDNAs were cloned into lambda bacteriophage vectors, and screened with various other synthetic oligomers, whose sequences were based on the HCV sequence in FIG. 32.

[0572] IV.A.25. Creation of HCV cDNA Library From Liver of a Chimpanzee with Infectious NANBH

[0573] An HCV cDNA library was created from liver from the chimpanzee from which the HCV cDNA library in Section IV.A.1. was created. The technique for creating the library was similar to that in Section IV.A.24, except for this different source of the RNA, and that a primer based on the sequence of HCV cDNA in clone 11b was used. The sequence of the primer was

[0574] 5'CTG GCT TGA AGA ATC 3'.

[0575] IV.A.26. Isolation and Nucleotide Sequence of Overlapping HCV cDNA in Clone k9-1 to cDNA in Clone 11b

[0576] Clone k9-1 was isolated from the HCV cDNA library created from the liver of an NANBH infected chimpanzee, as described in Section IV.A.25. The library was screened for clones which overlap the sequence in clone 11b, by using a clone which overlaps clone 11b at the 5'-terminus, clone 11e. The sequence of clone 11b is shown in FIG. 23. Positive clones were isolated with a frequency of 1 in 500,000. One isolated clone, k9-1, was subjected to further study. The overlapping nature-of the HCV cDNA in clone k9-1 to the 5'-end of the HCV-cDNA sequence in FIG. 26 was confirmed by probing the clone with clone Alex46; this latter clone contains an HCV cDNA sequence of 30 base pairs which corresponds to those base pairs at the 5'terminus of the HCV cDNA in clone 14i, described supra.

[0577] The nucleotide sequence of the HCV cDNA isolated from clone k9-1 was determined using the techniques described supra. The sequence of the HCV cDNA in clone k9-1, the overlap with the HCV cDNA in FIG. 26 (indicated by the dotted line), and the amino acids encoded therein are shown in FIG. 46.

[0578] The HCV cDNA sequence in clone k9-1 has been aligned with those of the clones described in Section IV.A.19. to create a composite HCV cDNA sequence, with the k9-1 sequence being placed upstream of the sequence shown in FIG. 32. The composite HCV cDNA which includes the k9-1 sequence, and the amino acids encoded therein, is shown in FIG. 47.

[0579] IV.A.27. Isolation and Sequence of Overlapping HCV cDNA Clones 13i, 26j, CA59a, CA84a, CA156e and CA167b

[0580] The clones 13i, 26j, CA59a, CA84a, CA156e and CA167b were isolated from the lambda-gt11 library described in Section IV.A.1. The frequencies with which positive clones appeared with the respective probes was about 1 in 50,000.

[0581] The isolation of clone 13i was accomplished using a synthetic probe derived from the sequence of clone 12f. The sequence of the probe was:

[0582] 5' GAA CGT TGC GAT CTG GAA GAC AGG GAC AGG 3'.

[0583] The isolation of clone 26j was accomplished using a probe derived from the 5'-region of clone K9-1. The sequence of the probe was:

[0584] 5' TAT CAG TTA TGC CAA CGG AAG CGG CCC CGA 3'.

[0585] The HCV cDNA sequences of clones 13i and 26j, are shown in FIGS. 48 and 49, respectively. Also shown are the amino acids encoded therein, as well as the overlap of clone 13i with clone 12f, and the overlap of clone 26j with clone 13i. The sequences for these clones confirmed the sequence of clone K9-1, which had been isolated from a different HCV cDNA library (see Section IV:A.26).

[0586] Clone CA59a was isolated utilizing a probe based upon the sequence of the 5'-region of clone 26j. The sequence of this probe was:

[0587] 5'CTG GTT AGC AGG GCT TTT CTA TCA CCA CAA 3'.

[0588] A probe derived from the sequence of clone CA59a was used to isolate clone CA84a. The sequence of the probe used for this isolation was:

[0589] 5' AAG GTC CTG GTA GTG CTG CTG CTA TTT GCC 3'.

[0590] Clone CA156e was isolated using a probe derived from the sequence of clone CA84a. The sequence of the probe was:

[0591] 5' ACT GGA CGA CGC AAG GTT GCA ATT GCT CTA 3'.

[0592] Clone CA167b was isolated using a probe derived from the sequence of clone CA 156e. The sequence of the probe was:

[0593] 5' TTC GAC GTC ACA TCG ATC TGC TTG TCG GGA 3'.

[0594] The nucleotide sequences of the HCV cDNAs in clones CA59a, CA84a, CA156e, and CA167b, are shown FIGS. 50, 51, 52, and 53, respectively. The amino acids

encoded therein, as well as the overlap with the sequences of relevant clones, are also shown in the FIGS.

[0595] IV.A.28. Creation of "pi" HCV cDNA Library

[0596] A library of HCV cDNA, the "pi" library, was constructed from the same batch of infectious chimpanzee plasma used to construct the lambda-gt11 described in Section IV.A.1, and utilizing essentially the same techniques. However, construction of the pi library utilized a primer-extension method, in which the primer for reverse transcriptase was based on the sequence of clone CA59A. The sequence of the primer was:

[0597] 5' GGT GAC GTG GGT TTC 3'.

[0598] IV.A.29. Isolation and Sequence of Clone pi14a

[0599] Screening of the "pi" HCV cDNA library described in Section IV.A.28 with the probe used to isolate clone CA167b (see Section IV.A.27.) yielded clone pi14a. The clone contains about 800 base pairs of cDNA which overlaps clones CA16i7b, CA156e, CA84a and CA59a (which were isolated from the HCV cDNA library described in Section IV.A.1.). In addition, pi14a also contains about 250 base pairs of DNA which are upstream of the HCV cDNA in clone CA167b.

[0600] The combined ORF derived from the HCV cDNA sequences in clones pi14a, CA167b, CA156e, CA84a, CA59a, K9-1, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, and 15e is shown in FIG. 54; also shown are the amino acids encoded therein.

[0601] IV.A.30. Isolation and Sequence of Clones CA216a, CA290a and ag30a

[0602] Based on the sequence of clone CA167b (see Section IV.A.27 and FIG. 53), a synthetic probe was made having the following sequence:

[0603] 5' GGC TTT ACC ACG TCA CCA ATG ATT GCC CTA 3'

[0604] The above probe was used to screen the lambda-gt11 library described in Section IV.A.1, which yielded clone CA216a, whose HCV sequences are shown in FIG. 56.

[0605] Another probe was made based on the sequence of clone CA216a having the following sequence:

[0606] 5' TTT GGG TAA GGT CAT CGA TAC CCT TAC GTG 3'

[0607] Screening the above library with this probe yielded clone CA290a, the HCV sequences therein being shown in FIG. 57.

[0608] In a parallel approach, a primer-extension cDNA library was made using nucleic acid extracted from the same infectious plasma used in the original lambda-gt11 cDNA library described above. The primer used was based on the sequence of clones CA216a and CA290a:

[0609] 5' GAA GCC GCA CGT AAG 3'

[0610] The cDNA library was made using methods similar to those described previously for libraries used in the isolation of clones pi14a and k9-1 (see Sections IV.A.26 and

IV.A.29). The probe used to screen this library was based on the sequence of clone CA290a:

[0611] 5'CCG GCG TAG GTC GCG CAA TTT GGG TAA 3'

[0612] Clone ag30a was isolated from the new library with the above probe, and contained about 670 basepairs of HCV sequence. See FIG. 58. Part of this sequence overlaps the HCV sequence of clones CA216a and CA290a. About 300 base-pairs of the ag30a sequence, however, is upstream of the sequence from clone CA290a. The non-overlapping sequence shows a start codon (*) and stop codons that may indicate the start of the HCV ORF. Also indicated in FIG. 58 are putative small encoded peptides (#) which may play a role in regulating translation, as well as the putative first amino acid of the putative polypeptide (/), and downstream amino acids encoded therein.

[0613] IV.1%.31. Isolation and Sequence of Clone CA205a

[0614] Clone CA205a was isolated from the original lambda gt-11 library, using a synthetic probe derived from the HCV sequence in clone CA290a (FIG. 57). The sequence of the probe was:

[0615] 5'TCA GAT CGT TGG TGG AGT TTA CTT GTT GCC 3'.

[0616] The sequence of the HCV cDNA in CA205a, shown in FIG. 59, overlaps with the cDNA sequences in both clones ag30a and CA290a. The overlap of the sequence with that of CA290a is shown by the dotted line above the sequence (the figure also shows the putative amino acids encoded in this fragment).

[0617] As observed from the HCV cDNA sequences in clones CA205a and ag30a, the putative HCV polyprotein appears to begin at the ATG start codon; the HCV sequences in both clones contain an in-frame, contiguous double stop codon (TGATAG) forty two nucleotides upstream from this ATG. The HCV ORF appears to begin after these stop codons, and to extend for at least 8907 nucleotides (see the composite HCV cDNA shown in FIG. 62).

[0618] IV.A.32. Isolation and Sequence of Clone 18g

[0619] Based on the sequence of clone ag30a (see IV.A.30 and FIG. 58) and of an overlapping clone from the original lambda gt-11 library, CA230a, a synthetic probe was made having the following sequence:

[0620] 5'CCA TAG TGG TCT GCG GAA CCG GTG AGT ACA 3'.

[0621] Screening of the original lambda-gt11 HCV cDNA library (described in Section IV.A.1.) with the probe yielded clone 18g, the HCV cDNA sequence of which is shown in FIG. 60. Also shown in the figure are the overlap with clone ag30a, and putative polypeptides encoded within the HCV cDNA.

[0622] The cDNA in clone 18g (C18g or 18g) overlaps that in clones ag30a and CA205a, described in Section IV.A.32. The sequence of C18g also contains the double stop codon region observed in clone ag30a. The polynucleotide region upstream of these stop codons presumably represents part of the 5'-region of the HCV genome, which may contain short ORFS, and which can be confirmed by direct sequenc-

ing of the purified HCV genome. These putative small encoded peptides may play a regulatory role in translation. The region of the HCV genome upstream of that represented by C18g can be isolated for sequence analysis using essentially the technique described in Section IC.A.20., except that that the primers of reverse transcriptase are based upon the sequence of C18g. Since HCV appears to be a Flavi-like virus, it is probable that the 5'-terminus of the genome will be modified with a "cap" structure. It is known that Flavivirus genomes contain 5'-terminal "cap" structures. (Yellow Fever virus, Rice et al. (1988); Dengue virus, Hahn et al (1988); Japanese Encephalitis Virus (1987)).

[0623] IV.A.33. Isolation and Sequence of Clones from the beta-HCV cDNA Library

[0624] Clones containing cDNA representative of the 3'-terminal region of the HCV genome were isolated from a cDNA library constructed from the original infectious chimpanzee plasma pool which was used for the creation of the HCV cDNA lambda-gt11 library described in Section IV-A.1. In order to create the DNA library, RNA extracted from the plasma was "tailed" with poly rA using poly (rA) polymerase, and cDNA was synthesized using oligo(dT)$_{1218}$ as a primer for reverse transcriptase. The resulting RNA:cDNA hybrid was digested with RNAase H, and converted to double stranded HCV cDNA. The resulting HCV cDNA was cloned into lambda-gt10, using essentially the technique described in Huynh (1985), yielding the beta (or b) HCV cDNA library. The procedures used were as follows.

[0625] An aliquot (12 ml) of the plasma was treated with proteinase K, and extracted with an equal volume of phenol saturated with 0.05M Tris-Cl, pH 7.5, 0.05% (v/v) beta-mercaptoethanol, 0.1% (w/v) hydroxyquinolone, 1 mM EDTA. The resulting aqueous phase was re-extracted with the phenol mixture, followed by 3 extractions with a 1:1 mixture containing phenol and chloroform:isoamyl alcohol (24:1), followed by 2 extractions with a mixture of chloroform and isoamyl alcohol (1:1). Subsequent to adjustment of the aqueous phase to 200 mM with respect to NaCl, nucleic acids in the aqueous phase were precipitated overnight at −20° C., with 2.5 volumes of cold absolute ethanol. The precipitates were collected by centrifugation at 10,000 RPM for 40 min., washed with 70% ethanol containing 20 mM NaCl, and with 100% cold ethanol, dried for 5 min. in a dessicator, and dissolved in water.

[0626] The isolated nucleic acids from the infectious chimpanzee plasma pool were tailed with poly rA utilizing poly-A polymerase in the presence of human placenta ribonuclease inhibitor (HPR1) (purchased from Amersham Corp.), utilizing MS2 RNA as carrier. Isolated nucleic acids equivalent to that in 2 ml of plasma were incubated in a solution containing THN (50 mM Tris HCl, pH 7.9, 10 mm MgCl$_2$, 250 mM NaCl, 2.5 mM MnCl$_2$, 2 mM dithiothreitol (DTT)), 40 micromolar alpha-[^{32}P] ATP, 20 units HPR1 (Amersham Corp.), and about 9 to 10 units of RNase free poly-A polymerase (BRL). Incubation was for 10 min. at 37° C., and the reactions were stopped with EDTA (final concentration about 250 mM). The solution was extracted with an equal volume of phenol-chloroform, and with an equal volume of chloroform, and nucleic acids were precipitated overnight at −20° C. with 2.5 volumes of ethanol in the presence of 200 mM NaCl.

[0627] IV.A.33.a. Isolation of Clone b5a

[0628] The beta HCV cDNA library was screened by hybridization using a synthetic probe, which had a sequence based upon the HCV cDNA sequence in clone 15e. The sequence of the probe was:

[0629] 5' ATT GCG AGA TCT ACG GGG CCT GCT ACT CCA 3'.

[0630] Screening of the library yielded clone beta-5a (b5a), which contains an HCV cDNA region of approximately 1000 base pairs. The 5'-region of this cDNA overlaps clones 35f, 19g, 26g, and 15e (these clones are described supra). The region between the 3'-terminal poly-A sequence and the 3'-sequence which overlaps clone 15e, contains approximately 200 base pairs. This clone allows the identification of a region of the 3'-terminal sequence the HCV genome.

[0631] The sequence of b5a is contained within the sequence of the HCV cDNA in clone 16jh (described infra). Moreover, the sequence is also present in CC34a, isolated from the original lambda-gt11 library. (The original lambda-gt11 library is referred to herein as the "C" library).

[0632] IV.A.34. Isolation and Sequence of Clones Generated by PCR Amplification of the 3'-Region of the HCV Genome

[0633] Multiple cDNA clones have been generated which contain nucleotide sequences derived from the 3'-region of the HCV genome. This was accomplished by amplifying a targeted region of the genome by a polymerase chain reaction technique described in Saiki et al. (1986), and in Saiki et al. (1988), which was modified as described below. The HCV RNA which was amplified was obtained from the original infectious chimpanzee plasma pool which was used for the creation of the HCV cDNA lambda-gt11 library described in Section IV.A.1. Isolation of the HCV RNA was as described in Section IV.A.33. The isolated RNA was tailed at the 3'-end with ATP by E. coli poly-A polymerase as described in Sippel (1973), except that the nucleic acids isolated from chimp serum were substituted for the nucleic acid substrate. The tailed RNA was then reverse transcribed into cDNA by reverse transcriptase, using an oligo dT-primer adapter, essentially as described by Han (1987), except that the components and sequence of the primer-adapter were:

Stuffer	NotI	SP6Promoter	Primer
AATTC	GCGGCCGC	CATACGATTTAGGTGACACTATAGAA	T$_{15}$

[0634] The resultant cDNA was subjected to amplification by PCR using two primers:

Primer	Sequence
JH32 (30mer)	ATAGCGGCCGCCCTCGATTGCGAGATCTAC
JH11 (20mer)	AATTCGGGCGGCCGCCATACGA

[0635] The JH32 primer contained 20 nucleotide sequences hybridizable to the 5'-end of the target region in the cDNA, with an estimated T$_m$ of 66° C. The JH11 was

derived from a portion of the oligo dT-primer adapter; thus, it is specific to the 3'-end of the cDNA with a T_m of 64° C. Both primers were designed to have a recognition site for the restriction enzyme, NotI, at the 5'-end, for use in subsequent cloning of the amplified HCV cDNA.

[0636] The PCR reaction was carried out by suspending the cDNA and the primers in 100 microliters of reaction mixture containing the four deoxynucleoside triphosphates, buffer salts and metal ions, and a thermostable DNA polymerase isolated from Thermus aguaticus (Taq polymerase), which are in a Perkin Elmer Cetus PCR kit (NBDI-0043 or N801-0055). The PCR reaction was performed for 35 cycles in a Perkin Elmer Cetus DNA thermal cycler. Each cycle consisted of a 1.5 min denaturation step at 94° C., an annealing step at 60° C. for 2 min, and a primer extension step at 72° C. for 3 min. The PCR products were subjected to Southern blot analysis using a 30 nucleotide probe, JH34, the sequence of which was based upon that of the 3'-terminal region of clone 15e. The sequence of JH34 is:

[0637]   5 CTT GAT CTA CCT CCA ATC ATT CAA AGA CTC 3'.

[0638] The PCR products detected by the HCV cDNA probe ranged in size from about 50 to about 400 base pairs.

[0639] In order to clone the amplified HCV cDNA, the PCR products were cleaved with NotI and size selected by polyacrylamide gel electrophoresis. DNA larger than 300 base pairs was cloned into the NotI site of pUC18S The vector pUC18S is constructed by including a NotI polylinker cloned between the EcoRI and SalI sites of pUC18. The clones were screened for HCV cDNA using the JH34 probe. A number of positive clones were obtained and sequenced. The nucleotide sequence of the HCV cDNA insert in one of these clones, 16jh, and the amino acids encoded therein, are shown in **FIG. 61**. A nucleotide heterogeneity, detected in the sequence of the HCV cDNA in clone 16jh as compared to another clone of this region, is indicated in the figure.

[0640]   IV.A.35 Compiled HCV cDNA Sequence

[0641] The HCV cDNA sequence compiled from a series of overlapping clones derived from the various HCV cDNA libraries described supra. and infra is shown in **FIG. 62**. The clones from which the sequence was derived are b114a, 18g, ag30a, CA205a, CA290a, CA216a, p114a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1),26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, and 16jh. In the figure the three dashes above the sequence indicate the position of the putative initiator methionine codon.

[0642] Clone b114a was obtained using the cloning procedure described for clone b5a, supra. except that the probe was the synthetic probe used to detect clone 18g, supra. Clone b114a overlaps with clones 18g, ag30a, and CA205a, except that clone b114a contains an extra two nucleotides upstream of the sequence in clone 18g (i.e., 5'-CA). These extra two nucleotides have been included in the HCV genomic sequence shown in **FIG. 62**.

[0643] It should be noted that although several of the clones described supra. have been obtained from libraries other than the original HCV cDNA lambda-gt11 library described in Section IV.A.1., these clones contain HCV cDNA sequences which overlap HCV cDNA sequences in the original library. Thus, essentially all of the HCV sequence is derivable from the original lambda-gt11 library which was used to isolate the first clone (5-1-1).

[0644]   IV.A.36 Isolation and Sequence of Clone 6k

[0645] Based on the sequence of clone 16jh and clone b5a (see IV.A.34. and **FIG. 61**), a synthetic probe was made having the following sequence:

[0646]   5' TCT TCA ACT GGG CAG TAA GAA CAA AGC TCA 3'.

[0647] Screening of the original lambda-gt11 HCV cDNA library (described in Section IV.A.1.) with the probe yielded clones with a frequency of approximately 1 in 10^6; one of these was called clone 6k (also called C6k), the HCV cDNA sequence of which is shown in **FIG. 71**. Also shown in the figure are the overlap with clone 16jh, and putative polypeptides encoded within the HCV cDNA. Sequence information on the HCV cDNA in clone 6k was obtained from only one strand. Information on the deposit of this clone is provided infra, wherein the clone is listed as Lambda gt11 C6k. Confirmation of the C6K sequence as part of an ORF encoding HCV1 polypeptide has been obtained by sequencing other overlapping clones.

[0648] A composite of the HCV cDNA sequence derived from overlapping clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, 16jh, and 16k is shown in **FIG. 72**. The figure also shows the amino acids encoded in the sense strand of the cDNA, which is the equivalent of the genomic RNA.

[0649]   IV.A.36 Construction of pS3-56$_{C100m}$

[0650] The vector pS3-56$_{C100}$ contains a construct which encodes the fusion polypeptide SOD-C100 (see Section IV.B.4.). In addition, this vector contains an ms2 phage sequence, which was removed from the HCV C100 encoding sequence by digestion of pS3-56$_{C100}$ with XmaI and SalI, followed by isolation of the large fragment. The afore-mentioned digestion, however, removes some of the HCV sequence. The latter was recreated by ligation of the fragment with the following linkers, which also introduced a SalI site and a stop codon. The linker sequences, annealed to each other, are shown in **FIG. 73**.

[0651] The resulting vector is called pS3-56$_{100m}$ (also called pS356$_{C100m}$).

[0652]   IV.A.37. Construction of Composite Sequence C200

[0653] An HCV-cDNA sequence, C200, which is a composite of HCV sequences derived from clones 33c, 31, and 35, was constructed. Clones 33c and 35 are described in Section IV.A.17. and IV.A.8., respectively. Clone 31 is from the C library, and has one difference from the confirmed sequence of HCV-1 in **FIG. 62**. The sequence of clone 31 is shown in **FIG. 74**, which also shows the amino acids encoded therein, and the location of restriction enzyme sites within the HCV cDNA. A C200 cassette was constructed by ligating together a 718 bp fragment obtained by digestion of clone 33c DNA with EcoRI and HinfI, a 179 bp fragment obtained by digestion of clone 31 DNA with HinfI and BglI,

and a 377 bp fragment obtained by digestion of clone 35 DNA with BglI and EcoRI. The construct of ligated fragments were inserted into the EcoRI site of pBR322, yielding the plasmid pBR322-C200.

[0654] IV.A.38. Isolation and Sequence of Clone p131jh.

[0655] A clone containing sequence from the 3'-region of the HCV genome, and which contains an in-frame stop codon, was isolated essentially as described in Section IV.A.34, except that HCV1 RNA was converted to cDNA using the oligonucleotide

    5' AAT TCG CGG CCG CCA TAC GAT TTA GGT GAC
              ACT ATA GAA T₁₅ 3'.

[0656] The cDNA was then amplified by the PCR reaction using the primers:

[0657] 5' TTC GCG GCC GCT ACA GCG GGG GAG ACA T 3'and

[0658] 5' AAT TCG CGG CCG CCA TAC GA 3'.

[0659] After amplification, the PCR products were precipitated with spermine, digested with NotI, and extracted with phenol. The purified products were cloned into the NotI site of pUC18S, and HCV positive clones were selected using the oligonucleotide:

[0660] 5'CGA TGA AGG TTG GGG TAA ACA CTC CGG CCT 3'.

[0661] The HCV cDNA in one clone, designated p131jh, is shown in FIG. 75. This clone contains an in-frame stop codon for the large ORF contained in the HCV genome.

[0662] IV.B. Expression and Purification of Polypeptides Encoded Within HCV cDNAs and Identification of the Expressed Products as HCV Induced Antigens.

[0663] IV.B.1. Expression of the Polypeptide Encoded in Clone 5-1-1.

[0664] The HCV polypeptide encoded within clone 5-1-1 (see Section IV.A.2., supra) was expressed as a fusion polypeptide with superoxide dismutase (SOD). This was accomplished by subcloning the clone 5-1-1 cDNA insert into the expression vector pSODcf1 (Steimer et al. (1986)) as follows.

[0665] First, DNA isolated from pSODcf1 was treated with BamHI and EcoRI, and the following linker was ligated into the linear DNA created by the restriction enzymes:

    5' GAT CCT GGA ATT CTG ATA AGA
              CCT TAA GAC TAT TTT AA 3'

[0666] After cloning, the plasmid containing the insert was isolated.

[0667] Plasmid containing the insert was restricted with EcoRI. The HCV cDNA insert in clone 5-1-1 was excised with EcoRI, and ligated into this EcoRI linearized plasmid DNA. The DNA mixture was used to transform E. coli strain D1210 (Sadler et al. (1980)). Recombinants with the 5-1-1 cDNA in the correct orientation for expression of the ORF shown in FIG. 1 were identified by restriction mapping and nucleotide sequencing.

[0668] Recombinant bacteria from one clone were induced to express the SOD-NANB₅₋₁₋₁ polypeptide by growing the bacteria in the presence of IPTG.

[0669] IV.B.2. Expression of the Polypeptide Encoded in Clone 81.

[0670] The HCV cDNA contained within clone 81 was expressed as a SOD-NANB₈₁ fusion polypeptide. The method for preparing the vector encoding this fusion polypeptide was analogous to that used for the creation of the vector encoding SOD-NANB₅₋₁₋₁ except that the source of the HCV cDNA was clone 81, which was isolated as described in Section IV.A.3, and for which the cDNA sequence was determined as described in Section IV.A.4. The nucleotide sequence of the HCV cDNA in clone 81, and the putative amino acid sequence of the polypeptide encoded therein are shown in FIG. 4.

[0671] The HCV cDNA insert in clone 81 was excised with EcoRI, and ligated into the pSODcf1 which contained the linker (see IV.B.1.) and which was linearized by treatment with EcoR1. The DNA mixture was used to transform E. coli strain D1210. Recombinants with the clone 81 HCV cDNA in the correct orientation for expression of the ORF shown in FIG. 4 were identified by restriction mapping and nucleotide sequencing.

[0672] Recombinant bacteria from one clone were induced to express the SOD-NANB₈₁ polypeptide by growing the bacteria in the presence of IPTG.

[0673] IV.B.3. Identification of the Polypeptide Encoded Within Clone 5-1-1 as an HCV and NANBH Associated Antigen.

[0674] The polypeptide encoded within the HCV cDNA of clone 5-1-1 was identified as a NANBH associated antigen by demonstrating that sera of chimpanzees and humans infected with NANBH reacted immunologically with the fusion polypeptide, SOD-NANB₅₋₁₋₁, which is comprised of superoxide dismutase at its N-terminus and the in-frame 5-1-1 antigen at its C-terminus. This was accomplished by "Western" blotting (Towbin et al. (1979)) as follows.

[0675] A recombinant strain of bacteria transformed with an expression vector encoding the SOD-NANB₅₋₁₋₁ polypeptide, described in Section IV.B.I., was induced to express the fusion polypeptide by growth in the presence of IPTG. Total bacterial lysate was subjected to electrophoresis through polyacrylamide gels in the presence of SDS according to Laemnli (1970). The separated polypeptides were transferred onto nitrocellulose filters (Towbin et al. (1979)). The filters were then cut into thin strips, and the strips were incubated individually with the different chimpanzee and human sera. Bound antibodies were detected by further incubation with ¹²⁵I-labeled sheep anti-human Ig, as described in Section IV.A.1.

[0676] The characterization of the chimpanzee sera used for the Western blots and the results, shown in the photograph of the autoradiographed strips, are presented in FIG. 33. Nitrocellulose strips containing polypeptides were incubated with sera derived from chimpanzees at different times during acute NANBH (Hutchinson strain) infections (lanes 1-16), hepatitis A infections (lanes 17-24, and 26-33), and hepatitis B infections (lanes 34-44). Lanes 25 and 45 show positive controls in which the immunoblots were incubated

with serum from the patient used to identify the recombinant clone 5-1-1 in the original screening of the lambda-gt11 cDNA library (see Section IV.A.1.).

[0677] The band visible in the control lanes, 25 and 45, in **FIG. 23** reflects the binding of antibodies to the NANB$_{5-1-1}$ moiety of the SOD fusion polypeptide. These antibodies do not exhibit binding to SOD alone, since this has also been included as a negative control in these samples, and would have appeared as a band migrating significantly faster than the SOD-NANB$_{5-1-1}$ fusion polypeptide.

[0678] Lanes 1-16 of **FIG. 33** show the binding of antibodies in sera samples of 4 chimpanzees; the sera were obtained just prior to infection with NANBH, and sequentially during acute infection. As seen from the figure, whereas antibodies which reacted immunologically with the SOD-NANB$_{5-1-1}$ polypeptide were absent in sera samples obtained before administration of infectious HCV inoculum and during the early acute phase of infection, all 4 animals eventually induced circulating antibodies to this polypeptide during the late part of, or following the acute phase. Additional bands observed on the immunoblots in the cases of chimps numbers 3 and 4 were due to background binding to host bacterial proteins.

[0679] In contrast to the results obtained with sera from chimps infected with NANBH, the development of antibodies to the NANB$_{5-1-1}$ moiety of the fusion polypeptide was not observed in 4 chimpanzees infected with HAV or 3 chimpanzees infected with HBV. The only binding in these cases was background binding to the host bacterial proteins, which also occurred in the HCV infected samples.

[0680] The characterization of the human sera used for the Western blots, and the results, which are shown in the photograph of the autoradiographed strips, are presented in **FIG. 34**. Nitrocellulose strips containing polypeptides were incubated with sera derived from humans at different times during infections with NANBH (lanes 1-21), HAV (lanes 33-40), and HBV (lanes 41-49). Lanes 25 and 50 show positive controls in which the immunoblots were incubated with serum from patient used in the original screening of the lambda-gt11 library, described supra. Lanes 22-24 and 26-32 show "non-infected" controls in which the sera was from "normal" blood donors.

[0681] As seen in **FIG. 34**, sera from nine NANBH patients, including the serum used for screening the lambda-gt11 library, contained antibodies to the NANB$_{5-1-1}$ moiety of the fusion polypeptide. Sera from three patients with NANBH did not contain these antibodies. It is possible that the anti-NANB$_{5-1-1}$ antibodies will develop at a future date in these patients. It is also possible that this lack of reaction resulted from a different NANBV agent being causative of the disease in the individuals from which the non-responding serum was taken.

[0682] **FIG. 34** also shows that sera from many patients infected with HAV and HBV did not contain anti-NANB$_{5-1-1}$ antibodies, and that these antibodies were also not present in the sera from "normal" controls. Although one HAV patient (lane 36) appears to contain anti-NANB$_{5-1-1}$ antibodies, it is possible that this patient had been previously infected with HCV, since the incidence of NANBH is very high and since it is often subclinical.

[0683] These serological studies indicate that the cDNA in clone 5-1-1 encodes epitopes which are recognized specifically by sera from patients and animals infected with BB-NANBV. In addition, the cDNA does not appear to be derived from the primate genome. A hybridization probe made from clone 5-1-1 or from clone 81 did not hybridize to "Southern" blots of control human and chimpanzee genomic DNA from uninfected individuals under conditions where unique, single-copy genes are detectable. These probes also did not hybridize to Southern blots of control bovine genomic DNA.

[0684] IV.B.4. Expression of the Polypeptide Encoded in a Composite of the HCV cDNAs in Clones 36, 81 and 32

[0685] The HCV polypeptide which is encoded in the ORF which extends through clones 36, 81 and 32 was expressed as a fusion polypeptide with SOD. This was accomplished by inserting the composite cDNA, C100, into an expression cassette which contains the human superoxide dismutase gene, inserting the expression cassette into a yeast expression vector, and expressing the polypeptide in yeast.

[0686] An expression cassette containing the composite C100 cDNA derived from clones 36, 81, and 32, was constructed by inserting the ~1270 bp EcoRI fragment into the EcoRI site of the vector-pS3-56 (also called pS356), yielding the plasmid pS3-56$_{C100}$ The construction of C100 is described in Section IV.A.16, supra.

[0687] The vector pS3-56, which is a pBR322 derivative, contains an expression cassette which is comprised of the ADH2/GAPDH hybrid yeast promoter upstream of the human superoxide dismutase gene, and a downstream alpha factor transcription terminator. A similar cassette, which contains these control elements and the superoxide dismutase gene has been described in Cousens et al. (1987), and in copending application EPO 196,056, published Oct. 1, 1986, which is commonly owned by the herein assignee. The cassette in pS3-56, however, differs from that in Cousens et al. (1987) in that the heterologous proinsulin gene and the immunoglobulin hinge are deleted, and in that the gln$_{154}$ of the superoxide dismutase is followed by an adaptor sequence which contains an EcoRI site. The sequence of the adaptor is:

```
        5'-AAT TTG GGA ATT CCA TAA TGA
        GAC CCT TAA GGT ATT ACT GAG CT 3'.
```

[0688] The EcoRI site allows the insertion of heterologous sequences which, when expressed from a vector containing the cassette, yield polypeptides which are fused to superoxide dismutase via an oligopeptide linker containing thd amino acid sequence:

[0689] -asn-leu-gly-ile-arg-.

[0690] After recombinants containing the C100 cDNA insert in the correct orientation were isolated, the expression cassette containing the C100 cDNA was excised from pS3-56$_{C100}$ with BamHI, and a fragment of ~3400 bp which contains the cassette was isolated and purified. This fragment was then inserted into the BamHI site of the yeast vector pAB24.

[0691] Plasmid pAB24, the significant features of which are shown in **FIG. 35**, is a yeast shuttle vector which contains the complete 2 micron sequence for replication [Broach (1981)] and pBR322 sequences. It also contains the

yeast URA3 gene derived from plasmid YEp24 [Botstein et al. (1979)], and the yeast LEU^{2d} gene derived from plasmid pC1/1. EPO Pub. No. 116,201. Plasmid pAB24 was constructed by digesting YEp24 with EcoRI and religating the vector to remove the partial 2 micron sequences. The resulting plasmid, YEP24deltaRI, was linearized by digestion with ClaI and ligated with the complete 2 micron plasmid which had been linearized with ClaI. The resulting plasmid, pCBou, was then digested with XbaI and the 8605 bp vector fragment was gel isolated. This isolated XbaI fragment was ligated with a 4460 bp XbaI fragment containing the LEU^{2d} gene isolated from pC1/1; the orientation of the LEU^{2d} gene is in the same direction as the URA3 gene. Insertion of the expression was in the unique BamHI site of the pBR322 sequence, thus interrupting the gene for bacterial resistance to tetracycline.

[0692] The recombinant plasmid which contained the SOD-C100 expression cassette, pAB24C100-3, was transformed into yeast strain JSC 308, as well as into other yeast strains. The cells were transformed as described by Hinnen et al. (1978), and plateo onto ura-selective plates. Single colonies were inoculated into leu-selective media and grown to saturation. The culture was induced to express the SOD-C100 polypeptide (called C100-3) by growth in YEP containing 1% glucose.

[0693] Strain JSC 308 is of the genotype MAT @, leu2, ura3(del) DM15 (GAP/ADR1) integrated at the ADR1 locus. In JSC 308, over-expression of the positive activator gene product, ADR1, results in hyperderepression (relative to an ADR1 wild type control) and significantly higher yields of expressed heterologous proteins when such proteins are synthesized via an ADH2 UAS regulatory system. The construction of the yeast strain JSC 308 is disclosed in copending application, U.S. Serial No. 190,868, filed concurrently herewith, and which is hereby incorporated herein by reference.

[0694] The complete C100-3 fusion polypeptide encoded in pAB24C100-3 should contain 154 amino acids of human SOD at the amino-terminus, 5 amino acid residues derived from the synthetic adaptor containing the EcoRI site, 363 amino acid residues derived from C100 cDNA, and 5 carboxy-terminal amino acids derived from the MS2 nucleotide sequence adjoining the HCV cDNA sequence in clone 32. (see Section IV.A.7.) The putative amino acid sequence of the carboxy-terminus of this polypeptide, beginning at the penultimate Ala residue of SOD, is shown in **FIG. 36**; also shown is the nucleotide sequence encoding this portion of the polypeptide.

[0695] IV.B.4.b. Contents of Selective Media For Yeast Auxotrophs

Leu- Medium		
Yeast Minimal Media	850 ml	
Leu- Supplements (10x)	100 ml	
50% Glucose (in MILLI Q)	40 ml	

[0696] (If the Leu- medium is to be used for growth of yeast in flasks, Ura- supplements are also included in the medium.

Yeast Minimal Medium		
Yeast Nitrogen Base w/o amino acids	6.7 g	
MILLI Q water	q.s. to 850 ml	
Leu- Supplements		
Adenine	0.8 g	
Uridine	0.6 g	
L-Tryptophan	0.4 g	
L-Histidine	0.4 g	
L-Arginine	0.4 g	
L-Methionine	0.4 g	
L-Tyrosine	0.6 g	
L-Lysine	0.6 g	
L-Phenylalanine	0.96 g	

[0697] Add all components to a coffee grinder and grind until the powder is homogenous. The powder may be added to a solution or the mix can be autoclaved as a 10x concentrated solution by adding 2L of MILLI Q water.

Ura-/Sorbitol Plates		
Ura-/Sorbitol Medium for Plates	500 ml	
50% Glucose	20 ml	
20% Casamino acids	12.5 ml	

[0698]

1% Adenine	2.5 ml	
1% Tryptophan	2.5 ml	

[0699] Stir gently and pour into plates. Flame plates.

[0700] Ura- Sorbitol Medium

Ura- Sorbitol Medium		
D-Sorbitol	91 g	
Agar	10 g	
Yeast Nitrogen Base without amino acids	3.35 g	
Water	q.s. to 450 ml	

[0701] Yeast Nitrogen Base without amino acids 3.35 g Water q.s. to 450 ml

[0702] Autoclave for 30 minutes

YEP		
Peptone	20 g	
Yeast Extract	10 g	
MILLI Q Water	q.s. to 1000 ml	

[0703] Autoclave at 121° C. for 30 minutes. The solution is good for six months when stored at 15 to 30° C.

Leu- Plates	
Leu- Plate Medium	950 ml
50% Glucose	40 ml
5% Threonine	4 ml
1% Adenine	1 ml

[0704]   Leu- Medium for Plates

Leu- Medium for Plates	
Agar	20 g
Leu- Supplements	0.25 g
10x Basal Salts w/o amino acids	100 ml
MILLI Q	q.s. to 950 ml

[0705]   10×Basal Salts

10x Basal Salts	
Yeast Nitrogen Base w/o Amino Acids	66.8 g
Succinic Acid	100 g
NaOH	60 g
MILLI Q Water	q.s. to 1000 ml

[0706]   Filter Sterilize

Leu-, Ura- plates	
Leu-, Ura- Plate media	840 ml
50% Glucose	160 ml
5% Threonine	8 ml

[0707]   Leu-, Ura- Plate Medium

Leu-, Ura- Plate Medium	
Yeast Nitrogen Base without amino acids	6.7 g
Bacto Agar	20 g
Leu-, Ura- supplements	0.5 g

(Note:
the Leu-, Ura- supplement recipe is the same as the Leu- supplement recipe, except that uridine is not added.)

[0708]   (Note: the Leu-, Ura- supplement recipe is the same as the Leu- supplement recipe, except that uridine is not added.)

[0709]   IV.B.5. Identification of the Polypeptide Encoded within C100 as an NANBH Associated Antigen

[0710]   The C100-3 fusion polypeptide expressed from plasmid pAB24C100-3 in yeast strain JSC 308 was characterized with respect to size, and the polypeptide encoded within C100 was identified as an NANBH-associated antigen by its immunological reactivity with serum from a human with chronic NANBH.

[0711]   The C100-3 polypeptide, which was expressed as described in Section IV.B.4., was analyzed as follows. Yeast JSC 308 cells were transformed with pAB24, or with pAB24C100-3, and were induced to express the heterologous plasmid encoded polypeptide. The induced yeast cells in 1 ml of culture (OD_{650} nm ~20) were pelleted by centrifugation at 10,000 rpm for 1 minute, and were lysed by vortexing them vigorously (10×1 min) with 2 volumes of solution and 1 volume of glass beads (0.2 millimicron diameter). The solution contained 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM phenylmethylsulphonyl fluoride (PMSF), and 1 microgram/ml pepstatin. Insoluble material in the lysate, which includes the C100-3 polypeptide, was collected by centrifugation (10,000 rpm for 5 minutes), and was dissolved by boiling for 5 minutes in Laemmli SDS sample buffer. [See Laemmli (1970)]. An amount of polypeptides equivalent to that in 0.3 ml of the induced yeast culture was subjected to electrophoresis through 10% polyacrylamide gels in the presence of SDS according to Laemmli (1970). Protein standards were co-electrophoresed on the gels. Gels containing the expressed polypeptides were either stained with Coomassie brilliant blue, or were subjected to "Western" blotting as described in Section IV.B.2., using serum from a patient with chronic NANBH to determine the immunological reactivity of the polypeptides expressed from pAB24 and from pAB24C100-3.

[0712]   The results are shown in FIG. 37. In FIG. 37A the polypeptides were stained with Coomassie brilliant blue. The insoluble polypeptide(s) from JSC 308 transformed with pAB24 and from two different colonies of JSC transformed with pAB24C100-3 are shown in lane 1 (pAB24), and lanes 2 and 3, respectively. A comparison of lanes 2 and 3 with lane 1 shows the induced expression of a polypeptide corresponding to a molecular weight of ~54,000 daltons from JSC 308 transformed with pAB24C100-3, which is not induced in JSC 308 transformed with pAB24. This polypeptide is indicated by the arrow.

[0713]   FIG. 37B shows the results of the Western blots of the insoluble polypeptides expressed in JSC 308 transformed with pAB24 (lane 1), or with pAB24C100-3 (lane 2). The polypeptides expressed from pAB24 were not immunologically reactive with serum from a human with NANBH. However, as indicated by the arrow, JSC 308 transformed with pAB24C100-3 expressed a polypeptide of ~54,000 dalton molecular weight which did react immunologically with the human NANBH serum. The other immunologically reactive polypeptides in lane 2 may be degradation and/or aggregation products of this ~54,000 dalton polypeptide.

[0714]   IV.B.6. Purification of Fusion Polypeptide C100-3

[0715]   The fusion polypeptide, C100-3, comprised of SOD at the N-terminus and in-frame C100 HCV-polypeptide at the C-terminus was purified by differential extraction of the insoluble fraction of the extracted host yeast cells in which the polypeptide was expressed.

[0716]   The fusion polypeptide, C100-3, was expressed in yeast strain JSC 308 transformed with pAB24C100-3, as described in Section IV.B.4. The yeast cells were then lysed by homogenization, the insoluble material in the lysate was extracted at pH 12.0, and C100-3 in the remaining insoluble fraction was solubilized in buffer containing SDS.

[0717]   The yeast lysate was prepared essentially according to Nagahuma et al. (1984). A yeast cell suspension was

prepared which was 33% cells (v/v) suspended in a solution (Buffer A) containing 20 mM Tris HCl, pH 8.0, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonylfluoride (PMSF). An aliquot of the suspension (15 ml) was mixed with an equal volume of glass beads (0.45-0.50 mm diameter), and the mixture was vortexed at top speed on a Super Mixer (Lab Line Instruments, Inc.) for 8 min. The homogenate and glass beads were separated, and the glass beads were washed 3 times with the same volume of Buffer A as the original packed cells. After combining the washes and homogenate, the insoluble material in the lysate was obtained by centrifuging the homogenate at 7,000×g for 15 minutes at 4° C., resuspending the pellets in Buffer A equal to twice the volume of original packed cells, and re-pelleting the material by centrifugation at 7,000×g for 15 min. This washing procedure was repeated 3 times.

[0718] The insoluble material from the lysate was extracted at pH 12.0 as follows. The pellet was suspended in buffer containing 0.5 M NaCl, 1 mM EDTA, where the suspending volume was equal to 1.8 times the original packed cells. The pH of the suspension was adjusted by adding 0.2 volumes of 0.4 M Na phosphate buffer, pH 12.0. After mixing, the suspension was centrifuged at 7,000×g for 15 min at 4° C., and the supernatant removed. The extraction was repeated 2 times. The extracted pellets were washed by suspending them in 0.5 M NaCl, 1 mM EDTA, using a suspension volume equal to two volumes of the original packed cells, followed by centrifugation at 7,000×g for 15 min at 4° C.

[0719] The C100-3 polypeptide in the extracted pellet was solubilized by treatment with SDS. The pellets were suspended in Buffer A equal to 0.9 volumes of the original packed cell volume, and 0.1 volumes of 2% SDS was added. After the suspension was mixed, it was centrifuged at 0 7,000×g for 15 min at 4° C. The resulting pellet was extracted 3 more times with SDS. The resulting supernatants, which contained C100-3 were pooled.

[0720] This procedure purifies C100-3 more than 10-fold from the insoluble fraction of the yeast homogenate, and the recovery of the polypeptide is greater than 50%.,

[0721] The purified preparation of fusion polypeptide was analyzed by polyacrylamide gel electrophoresis according to Laemmli (1970). Based upon this analysis, the polypeptide was greater than 80% pure, and had an apparent molecular weight of ˜54,000 daltons.

[0722] IV.B.7.a. Purification of Fusion Polypeptide C100-3 (Alternate method 1)

[0723] The fusion polypeptide, C100-3 (HCV c100-3), expressed in yeast strain JSC 308 transformed with pAb24C100-3, may be purified by an alternative method. In this method the antigen is precipitated from the crude cell lysate with acetone; the acetone precipitated antigen is then subjected to ion-exchange chromatography, and further purified by gel filtration.

[0724] The transformed yeast are grown under conditions which allow expression (see Section IV.B.4). A cell lysate is prepared by suspending the cells in Buffer A (20 mM Tris HCl, pH 8.0, 1 mM EDTA, 1 mM PMSF. The cells are broken by grinding with glass beads in a Dynomill type homogenizer or its equivalent. The extent of cell breakage is monitored by counting cells under a microscope with phase

optics. Broken cells appear dark, while viable cells are light-colored. The percentage of broken cells is determined.

[0725] When the percentage of broken cells is approximately 90% or greater, the broken cell debris is separated from the glass beads by centrifugation, and the glass beads are washed with Buffer A. After combining the washes and homogenate, the insoluble material in the lysate is obtained by centrifugation. The material in the pellet is washed to remove soluble proteins by suspension in Buffer B (50 mM glycine, pH 12.0, 1 mM DTT, 500 mM NaCl), followed by Buffer C (50 mM glycine, pH 10.0, 1 mM DTT). The insoluble material is recovered by centrifagation, and solubilized by suspension in Buffer C containing SDS. The extract solution may be heated in the presence of beta-mercaptoethanol and concentrated by ultrafiltration. The HCV c100-3 in the extract is precipitated with cold acetone. If desired, the precipitate may be stored at temperatures at about or below −15° C.

[0726] Prior to ion exchange chromatography, the acetone precipitated material is recovered by centrifugation, and may be dried under nitrogen. The precipitate is suspended in Buffer D (50 mM glycine, pH 10.0, 1 mM DTT, 7 M urea), and centrifuged to pellet insoluble material. The supernatant material is applied to an anion exchange column previously equilibrated with Buffer D. Fractions are collected and analyzed by ultraviolet absorbance or gel electrophoresis on SDS polyacrylamide gels. Those fractions containing the HCV c100-3 polypeptide are pooled.

[0727] In order to purify the HCV c100-3 polypeptide by gel filtration, the pooled fractions from the ion-exchange column are heated in the presence of beta-mercaptoethanol and SDS, and the eluate is concentrated by ultrafiltration. The concentrate is applied to a gel filtration column previously equilibrated with Buffer E (20 mM Tris HCl, pH 7.0, 1 mM DTT, 0.1% SDS). The presence of HCV c100-3 in the eluted fractions, as well as the presence of impurities, are determined by gel electrophoresis on polyacrylamide gels in the presence of SDS and visualization of the polypeptides. Those fractions containing purified HCV c100-3 are pooled. Fractions high in HCV c100-3 may be further purified by repeating the gel filtration process. If the removal of particulate material is desired, the HCV c100-3 containing material may be filtered through a 0.22 micron filter.

[0728] IV.B.7.b. Expression and Purification of Fusion Polypeptide C100-3 (Alternate Method 2)

[0729] The fusion polypeptide C100-3 (also called HCV c100-3), is expressed in yeast strain JSC308 transformed with pAB24C100-3. The expression of the C100-3 coding region is under control of the ADH2 upstream activator sequences (UAS) and GAP promoter sequences. This system is repressed when glucose is present in the medium. In the fermentation process, the inocula cultures (Inoculum 1 and 2) are prepared in selective medium containing a high amount of glucose to repress synthesis of the C100-3 polypeptide. Inoculum 2 is then diluted into complete medium containing an initial concentration of glucose sufficient to allow substantial mass increase before being metabolically exhausted by fermentation growth of the culture. After the glucose has been exhausted from this expression medium, the cells derepress the ADH-2 regulated system as they begin to grow by respiration. In this method, the majority of the mass increase of the cell culture is

functionally uncoupled from the production of the HCV C100-3 polypeptide. The expressed C100-3 polypeptide thus expressed is purified by isolation of an insoluble cell fraction, extraction of this fraction with SDS followed by acetone precipitation, solubilization of the acetone precipitate, followed by chromatography on Q-Sepharose and on gel filtration columns.

[0730] Preparation and isolation of transformants is as follows. Yeast strain JSC308 (MATa, ura3-delta 1, leu2-04 [cir°],.;DM15 (G_{418}^R) is transformed with plasmid pAB24c100-3, using the lithium transformation procedure described by Ito et al. (1984). The transformation mix is plated onto selective ura$^-$ agar plates, and the plates are incubated at 30° C. for 2 to 4 days. Next, transformants having high plasmid copy numbers are selected by streaking ura$^+$ colonies to leucine selective plates. Transformants are selected on their ability to express the C100-3 polypeptide, as described below.

[0731] In order to test expression of C100-3, single transformant colonies are transferred into leu$^-$, 2% glucose medium and grown at 30° C. until saturation. Under these conditions, expression of C100-3 is repressed due to the high glucose concentration in the medium. Expression is induced by a ½s dilution of the saturated culture into YEP/1% glucose; the diluted cells are grown to saturation. The cells are harvested, lysed by grinding with glass beads in TE buffer containing NaCl. The insoluble fractions are collected by centrifugation, and solubilized by resuspension in SDS sample buffer and boiling. The solubilized fraction is examined by fractionation on standard 10% denaturing acrylamide gels (Laemmli (1970)). The polypeptides on the gel are visualized by staining with Coomassie blue. Evidence of expression is initially determined by appearance of a new polypeptide in extracts of transformants harboring pAB24c100-3 as compared with control extracts (cells transformed with pAB24 vector lacking the C100-3 coding region). A protein band of about 53 Kd was clearly seen in extracts of cells harboring the C100-3 expression plasmid; this band was absent from the control extract.

[0732] A stock is prepared from a single transformant colony by streaking onto a leu selective agar plate, and incubating at 30° C. for 1~4 days. Single colonies are picked, and individually inoculated into about 5 ml of leu$^-$, 2% glucose medium, and grown to saturation at 30° C. One ml. is aseptically removed from each tube, and is transferred to a flask containing 500 ml of leu$^-$ medium, 2% glucose. The flask is incubated at 30° C. with agitation, for approximately 29 hours, after which time the flask is removed from incubation, an O.D. 650 value is determined, and the viability of the sample is determined. If the sample is viable, glycerol is added to a final concentration of 15%, and the sample is stored in 1 ml aliquots at ≦60° C.

[0733] A working seed stock is prepared. An aliquot of the frozen stock is plated onto leu$^-$ selective plates. An isolated small colony is picked, inoculated into 1-5 ml of selective culture (described above), and incubated at 30° C. for one day. One ml from this culture is used to inoculate a larger leu$^-$ selective media culture (500-1000 ml). After 30-60 hours of incubation, the O.D.$_{.650}$ value and viability is determined. Glycerol is added to a final concentration of 15% to the viable culture. The culture is stored at ≦60° C. in 1 ml aliquots

[0734] The stocks of transformed cells are analyzed. Viability of the stocks is equal to or greater than $5×10^5$ viable cells per ml of culture. Phenotypic analysis is for the chromosomal markers MATa and ::DM15(G418^R). MATa is tested by a plate assay that detects secretion of mating factor when the test cells are patched onto a lawn of cells carrying the opposite mating type. Opposite mating types of the lawn and patched cells produce a clear halo around the patch. DM15 (G418^R) is tested by patching cells onto YEPD plates with and without geneticin. The presence of the latter marker allows for growth of the cells in geneticin plates.

[0735] The plasmids from the cells are also analyzed by restriction map and nucleotide sequence confirmation for the expression cassette.

[0736] In order to prepare inoculum 1, the transformed yeast cells from the working stock are incubated in sterile selective medium (leu$^-$, 2% glucose); incubation is at 30±2° C. at 250-350 rpm for 30 to 36 hours.

[0737] In order to prepare inoculum 2, sterile selective medium at pH 5.9±0.1 containing approximately 10 grams of yeast nitrogen base without amino acids (DIFCO) per liter, 0.5 grams of Leu$^-$ supplements per liter, antifoam (approximately 0.1 ml/L), and 200 grams of dextrose per liter, is prepared in a fermentor. Inoculum 1 is transferred aseptically to the fermentor, and is incubated at 30±2° C. at an agitation speed of 400±50 rpm with an air flow of 10±2 liters per minute. Incubation is for 24±6 hours.

[0738] Expression of C100-3 is accomplished by transferring inoculum 2 to a fresh batch of sterile YEP medium with 2% dextrose, and incubating the cells in a fermentor for 53±7 hours at 30±2° C., with an agitation speed of 200±20 rpm and an air flow 200+20 liters per minute. After the incubation, the fermentor culture is cooled to <20° C., and harvested by continuous flow centrifugation. The supernatant is discarded, and the yeast slurry is collected.

[0739] The C100-3 polypeptide is partially purified by removal of the soluble yeast fraction. The yeast slurry is adjusted to 50 mM Tris HCl (using 1.0 M Tris HCl, pH 8.0), 0.15 M NaCl, 2 mM EDTA, and 1 mM PMSF. The yeast is mechanically disrupted using a continuous flow Dynomill glass bead mill with 0.5 mm nominal diameter glass beads. Breakage is continued until >90% of the yeast cells are broken. The resulting lysate is then diluted to achieve a 10% (v/v) solids concentration based on pre-lysis volume and packed cell volume by adding the same lysis buffer containing PMSF. Gross cellular debris is removed by continuous flow centrifgation in a Westfalia Model SA-1 centrifuge, and is discarded. The partially clarified, diluted lysate is further centrifuged at a higher relative G-force to recover the C100-3 polypeptide. This step is accomplished by continuous centrifugation of the lysate in a CEPA LE centrifuge at full speed (flow rate, 100 to 200±20 ml/minute). The supernatant is discarded, and the CEPA pellets are collected and stored at ≦60° C.

[0740] Further purification of the C100-3 polypeptide from the CEPA pelleted material is accomplished by acetone precipitation of an SDS solubilized fraction, followed by ion-exchange chromatography of the precipitated material, and then by gel filtration chromatography of the eluate from the ion-exchange column.

[0741] The CEPA pelleted material is resuspended in a Tris/EDTA buffer (20 mM Tris HCL, pH 8.0, 1.0 mM

EDTA, 1.0 mM PMSF), and insoluble material is collected by centrifugation at 17,000×G for 60 minutes at approximately 4° C. The pellet is washed twice with a glycine/DTT/NaCl buffer (50 mM glycine, 1.0 mM DTT, 50 mM NaCl, pH 12) and once with glycine/DTT buffer (50 mM glycine, 1.0 mM DTT, pH 10.0) before it is extracted by suspension in the same buffer containing 0.5% SDS (w/v). The pellet is recovered by centrifugation (17,000×G, 30 min, about 4° C.), and the SDS extraction is repeated. The two extracts are combined, and heated to 80-85° C. to solubilize the C100-3 polypeptide. After solubilization, the extract is cooled and BME is added to a concentration of 1% (w/v), and the extract solution is precipitated with cold acetone to remove excess SDS; this material may be stored at <−15° C. for up to five weeks. The acetone precipitate is recovered by centrifugation (5,000×G).

[0742] In order to further purify the material in the acetone precipitate by chromatography, the precipitate is suspended in glycine/urea buffer (50 mM glycine, pH 10, 7 mM urea, 10 mM DTT), is heated to 80-85° C., then cooled. The extract is then applied to a Q-Sepharose anion exchange column (2.5 L Q Sepharose, 25 cm diameter column) which was previously equilibrated against the glycine/urea buffer; the flow rate is ˜50 ml per minute, and the temperature is 2 to 8° C. The fractions are collected, and those containing C100-3 polypeptide, as determined by absorbance at 280 nm, are pooled.

[0743] The material which passes through the ion exchange column is further purified by gel filtration. The pool of fractions from the ion-exchange column is adjusted to 0.5% (w/v) SDS, and is concentrated two-fold with an ultrafiltration unit using a 30K molecular weight cut-off membrane. The concentrated fraction is then adjusted to 2% (v/v) BME, heated, the protein concentration is measured, and the fraction is cooled. The cooled eluate is then applied to Sephacryl S-300 HR gel filtration columns which were previously equilibrated with an SDS/Tris buffer (0.1% SDS (w/v), 20 mM Tris HCl, pH 7.0, 10 mM DTT). The gel filtration columns are 55 cm high by 25 cm diameter; the filtration is over two of these units in series; the operating flow is 100 ml per minute. Fraction collection is started immediately after loading. The eluted fractions are analyzed by electrophoresis on polyacrylamide gels containing SDS, in order to determine which of the fractions should be pooled. Prior to electrophoresis, the test samples and a reference sample are prepared by boiling in a buffer containing BME and SDS. Following electrophoresis, the gels are stained with Coomassie blue for visualization of the protein bands. The determination of which fractions to pool is based on the following analysis. The fraction containing the highest and purest amount of polypeptide is called "peak fraction". This fraction together with fractions which elute earlier are pooled up to, and including the first fraction exhibiting a decrease of approximately ⅓ the amount of C100-3 polypeptide band relative to the adjacent fraction, and a decrease of approximately ⅔ relative to the peak fraction. The decrease is observed in the relative thickness of the C100-3 bands. Fractions which elute later than the C100-3 peak are pooled up to, but not including the first fraction exhibiting a visible band at the molecular weight of about 18,000 relative to a molecular weight marker, and including the the last fraction exhibiting a decrease of approximately ⅔ of the polypeptide band relative to the peak fraction.

[0744] The pooled fractions are further purified by repeating the gel filtration process. The fractions from the second gel filtration column are analyzed as described above, and are further analyzed by HPLC to determine pooling. Analysis by HPLC uses a TSK-400 gel filtration HPLC column, equipped with a computerized integrator. All samples are prepared in a buffer containing 20 mM DTT to prevent oxidation of the C100-3 polypeptide. Pooling based on HPLC analysis is as follows. Using the HPLC chromatograms, the ratio of peak height to peak area for the C100-3 peak in each of the fractions is calculated. The ratio values follow a trend, increasing to a maximum value and then decreasing. Those fractions with a ratio equal to or greater than 85% of the maximum value and which meet the criteria in gel electrophoresis are pooled. The total volume of the pool of fractions is measured, the protein concentration is determined by the Lowry method, and the concentration of the final pool is adjusted to 0.5 to 1.0 mg/ml with the same buffer used for the gel filtration columns.

[0745] IV.B.8. Expression and Antigenicity of Polypeptides Encoded in HCV cDNA

[0746] IV.B.8.a. polypeptides Expressed in *E. coli*

[0747] The polypeptides encoded in a number of HCV cDNAS which span the HCV genomic ORF were expressed in *E. coli*, and tested for their antigenicity using serum obtained from a variety of individuals with NANBH. The clones from which the HCV cDNAs were isolated, as well as their relative relationships, and antigenicity, are shown in **FIG. 63**. Also indicated in the figure are the putative polypeptides encoded in the ORF of the HCV genome, based upon the Flavivirus model and the hydropathic character of the putative encoded polypeptides. However, the hydrophobicity profiles (described infra), indicate that HCV diverges from the Flavivirus model, particularly with respect to the region upstream of NS2. Moreover, the boundaries indicated are not intended to show firm demarcations between the putative polypeptides.

[0748] Possible protein domains of the encoded HCV polyprotein, as well as the approximate boundaries, are the following:

[0749] Putative Domain Approximate Boundary (amino acid nos.)

Putative Domain	Approximate Boundary (amino acid nos.)
C (nucleocapsid protein)	1–120
E (Virion envelope protein(s) and possibly matrix (M) proteins	120–400
NS1 (complement fixation antigen?)	400–660
NS2 (unknown function)	660–1050
NS3 (protease?)	1050–1640
NS4 (unknown function)	1640–2000
NS5 (polymerase)	2000–? end

[0750] These domains are, however, extremely tentative, since they are based upon the Flavivirus model, and recent evidence suggests that the relationship between HCV and the flaviviridae may be distant.

[0751] The expression vectors containing the cloned HCV cDNAs were constructed from pSODcf1, which is described in Section IV.B.1. In order to be certain that a correct reading frame would be achieved, three separate expression vectors, pcf1AB, pcf1CD, and pcf1EF were created by ligating three new linkers, AB, CD, and EF to a BamHI-EcoRI fragment derived by digesting to completion the vector pSODcf1 with EcoRI and BamHI, followed by treatment with alkaline phosphatase. The linkers were created from six oligomers, A, B, C, D, E, and F. Each oligomer was phosphorylated by treatment with kinase in the presence of ATP prior to annealing to its complementary oligomer. The sequences of the synthetic linkers were the following.

Name	DNA Sequence (5' to 3')
A	GATC CTG AAT TCC TGA TAA
B	GAC TTA AGG ACT ATT TTA A
C	GATC CGA ATT CTG TGA TAA
D	GCT TAA GAC ACT ATT TTA A
E	GATC CTG GAA TTC TGA TAA
F	GAC CTT AAG ACT ATT TTA A

[0752] Each of the three linkers destroys the original EcoRI site, and creates a new EcoRI site within the linker, but within a different reading frame. Hence, the HCV cDNA EcoRI fragments isolated from the clones when inserted into the expression vector, were in three different reading frames.

[0753] The HCV cDNA fragments in the designated lambda-gt11 clones (indicated in **FIG. 63**) were excised by digestion with EcoRI; each fragment was inserted into pcf1AB, pcf1CD, and pcf1EF. These expression constructs were then transformed into D1210 *E. coli* cells, the transformants were cloned, and polypeptides were expressed as described in Section IV.B.2.

[0754] Expression products of the indicated HCV cDNAs were tested for antigenicity by direct immunological screening of the colonies, using a modification of the method described in Helfman et al. (1983). Briefly, as shown in **FIG. 64**, the bacteria were plated onto nitrocellulose filters overlaid on ampicillin plates to give approximately 1,000 colonies per filter. Colonies were replica plated onto nitrocellulose filters, and the replicas were regrown overnight in the presence of 2 mM IPTG and ampicillin. The bacterial colonies were lysed by suspending the nitrocellulose filters for about 15 to 20 min in an atmosphere saturated with CHCl$_3$ vapor. Each filter then was placed in an individual 100 mm Petri dish containing 10 ml of 50 mM Tris HCl, pH 7.5, 150 mM NaCl, 5 MM MgCl$_2$, 3% (w/v) BSA, 40 micrograms/ml lysozyme, and 0.1 microgram/ml DNase. The plates were agitated gently for at least 8 hours at room temperature. The filters were rinsed in TBST (50 mM Tris HCl, pH 8.0, 150 mM NaCl, 0.005% Tween 20). After incubation, the cell residues were rinsed and incubated in TBS (TBST without Tween) containing 10% sheep serum; incubation was for 1 hour. The filters were then incubated with pretreated sera in TBS from individuals with NANBH, which included: 3 chimpanzees; 8 patients with chronic NANBH whose sera were positive with respect to antibodies to HCV C100-3 polypeptide (described in Sections IV.B.6. and IV.B.7.) (also called C100); 8 patients with chronic

NANBH whose sera were negative for anti-C100 antibodies; a convalescent patient whose serum was negative for anti-C100 antibodies; and 6 patients with community acquired NANBH, including one whose sera was strongly positive with respect to anti-C100 antibodies, and one whose sera was marginally positive with respect to anti-C100 antibodies. The sera, diluted in TBS, was pretreated by preabsorption with hSOD. Incubation of the filters with the sera was for at least two hours. After incubation, the filters were washed two times for 30 min with TBST. Labeling of expressed proteins to which antibodies in the sera bound was accomplished by incubation for 2 hours with ^{125}I-labeled sheep anti-human antibody. After washing, the filters were washed twice for 30 min with TBST, dried, and autoradiographed.

[0755] As seen from the results shown in **FIG. 65, a** number of clones expressed polypeptides containing HCV epitopes which were immunologically reactive with serum from individuals with NANBH. Five of these polypeptides were very immunogenic in that antibodies to HCV epitopes in these polypeptides were detected in many different patient sera. The clones encoding these polypeptides, and the location of the polypeptide in the putative HCV polyprotein (wherein the amino acid numbers begin with the putative initiator codon) are the following: clone 5-1-1, amino acids 1694-1735; clone C100, amino acids 1569-1931; clone 33c, amino acids 1192-1457; clone CA279a, amino acids 1-84; and clone CA290a amino acids 9-177. The location of the immunogenic polypeptides within the putative HCV polyprotein are shown immediately below.

Clones Encoding Polypeptides of Proven Reactivity with Sera from NANBH Patients

[0756]

Clone	Location within the HCV polyprotein (amino acid no. beginning with putative initiator methionine)
CA279a	1–84
CA74a	437–582
13i	511–690
CA290a	9–177
33c	1192–1457
40b	1266–1428
5-1-1	1694–1735
81	1689–1805
33b	1916–2021
25c	1949–2124
14c	2054–2223
8f	2200–3325
33f	2287–2385
33g	2348–2464
39c	2371–2502
15e	2796–2886
C100	1569–1931

[0757] The results on the immunogenicity of the polypeptides encoded in the various clones examined suggest efficient detection and immunization systems may include panels of HCV polypeptides/epitopes.

[0758] IV.B.8.b. Expression of HCV Epitopes in Yeast

[0759] Three different yeast expression vectors which allow the insertion of HCV cDNA into three different

reading frames are constructed. The construction of one of the vectors is described in Section IV.B.4., except that HCV cDNA from the clones listed in Section IV.B.8.a. are substituted for the C100 HCV cDNA. The construction of the other vectors replaces the adaptor described in Section IV.B.4. with one of the-following adaptors:

```
Adaptor 1
ATT TTG AAT TCC TAA TGA G
    AC TTA AGG ATT ACT CAG CT

Adaptor 2
AAT TTG GPA TTC TAA TGA G
    AC CTT AAG ATT ACT CAG CT.
```

[0760] The inserted HCV cDNA is expressed in yeast transformed with the vectors, using the expression conditions described in Section IV.B.4. The resulting polypeptides are screened using the sera from individuals with NANBH, described in Section IV.B.8.a.

[0761] IV.B.9. Expression and Purification of Fusion Polypeptide SOD-C33c

[0762] A fusion polypeptide comprised of SOD at the N-terminus and in-frame C33c HCV-polypeptide at the C-terminus (SOD-C33c), is encoded in clone pCF1EF/C33c (see Section IV.B.8.). This polypeptide was expressed in *E. coli*, and purified therefrom.

[0763] Expression was accomplished by inoculating 1500 ml of Luria broth containing ampicillin (100 micrograms/ml) with 15 ml of an overnight culture of *E. coli* D1210 transformed with clone pCF1EF/C33c. The cells were grown to an O.D. of 0.3, IPTG was added to yield a final concentration of 2 mM, and growth continued until the cells attained a density of 1 O.D., at which time they were harvested by centrifugation at 3,000×g at 4° C. for 20 minutes. The packed cells can be stored at −80° C. for several months.

[0764] In order to purify the SOD-C33c polypeptide the bacterial cells in which the polypeptide was expressed were subjected to osmotic shock and mechanical disruption, the insoluble fraction containing SOD—C33c was isolated and subjected to differential extraction with an alkaline-NaCl solution, and the fusion polypeptide in the extract purified by chromatography on columns of S-Sepharose and Q-Sepharose.

[0765] The crude extract resulting from osmotic shock and mechanical disruption was prepared by the following procedure. One gram of the packed cells were suspended in 10 ml of a solution containing 0.02 M Tris HCl, pH 7.5, 10 mM EDTA, 20% sucrose, and incubated for 10 minutes on ice. The cells were then pelleted by centrifugation at 4,000×g for 15 min at 4° C. After the supernatant was removed, the cell pellets were resuspended in 10 ml of Buffer Al (0.01M Tris HCl, pH 7.5, 1 mM EDTA, 14 mM beta-mercaptoethanol [BME]), and incubated on ice for 10 minutes. The cells were again pelleted at 4000×g for 15 minutes at 4° C. After removal of the clear supernatant (periplasmic fraction I), the cell pellets were resuspended in Buffer Al, incubated on ice for 10 minutes, and again centrifuged at 4,000×g for 15 minutes at 4° C. The clear supernatant (periplasmic fraction II) was removed, and the cell pellet resuspended in 5 ml of Buffer A2 (0.02 M Tris HCl, pH 7.5, 14 mM BME, 1 mM

EDTA, 1 mM PMSF). In order to disrupt the cells, the suspension (5 ml) and 7.5 ml of Dyno-mill lead-free acid washed glass beads (0.10-0.15 mm diameter)(obtained from Glen-Mills, Inc.) were placed in a Falcon tube, and vortexed at top speed for two minutes, followed by cooling for at least 2 min on ice; the vortexing-cooling procedure was repeated another four times. After vortexing, the slurry was filtered through a scintered glass funnel using low suction; the glass beads were washed two times with Buffer A2, and the filtrate and washes combined.

[0766] The insoluble fraction of the crude extract was collected by centrifugation at 20,000×g for 15 min at 4° C., washed twice with 10 ml Buffer A2, and resuspended in 5 ml of MILLI-Q water.

[0767] A fraction containing SOD-C33c was isolated from the insoluble material by adding to the suspension NaOH (2 M) and NaCl (2 M) to yield a final concentration of 20 mM each, vortexing the mixture for 1 minute, centrifuging it 20,000×g for 20 min at 4° C., and retaining the supernatant.

[0768] In order to purify SOD-C33c on S-Sepharose, the supernatant fraction was adjusted to a final concentration of 6M urea, 0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA. This fraction was then applied to a column of S-Sepharose Fast Flow (1.5×10 cm) which had been equilibrated with Buffer B (0.05M Tris HCl, pH 7.5, 14 mM BME, 1 mM EDTA). After application, the column was washed with two column volumes of Buffer B. The flow through and wash fractions were collected. The flow rate of application and wash, was 1 ml/min; and collected fractions were 1 ml. In order to identify fractions containing SOD-C33c, aliquots of the fractions were analyzed by electrophoresis on 10% polyacrylamide gels containing SDS followed by staining with Coomassie blue. The fractions are also analyzable by western blots using an antibody directed against SOD. Fractions containing SOD-C33c were pooled.

[0769] Further purification of SOD-C33c was on a Q-Sepharose column (1.5×5 cm) which was equilibrated with Buffer B. The pooled fractions containing SOD-C33c obtained from chromatography on S-Sepharose was applied to the column. The column was then washed with Buffer B, and eluted with 60 ml of a gradient of 0.0 to 0.4 M NaCl in Buffer B. The flow rate for application, wash, and elution was 1 ml/min; collected fractions were 1 ml. All fractions from the Q-Sepharose column were analyzed as described for the S-Sepharose column. The peak of SOD-C33c eluted from the column at about 0.2 M NaCl.

[0770] The SOD-C33c obtained from the Q-Sepharose column was greater than about 90% pure, as judged by analysis on the polyacrylamide SDS gels and immunoblot using a monoclonal antibody directed against human SOD.

[0771] IV.B.10. Expression in Yeast of Fusion Polypeptide SOD-C200-C100

[0772] IV.B.10.a. Construction of an Expression Vector Comprised of Expression Cassette C200-C100

[0773] An expression cassette containing the ADH2/GAP promoter, a sequence encoding SOD, the composite HCV sequences C200 and C100, and the alpha-factor terminator terminator was constructed. The C200 sequence overlaps the C100 sequence. Thus, the C200-C100 construct was designed to express the segment of the continuous ORF

contained in clones 33c/31/35/36/81/32. **FIG. 79** shows the sequence of the HCV cDNA in the construct, the polypeptides encoded therein, and the putative restriction enzyme sites encoded therein.

[0774] The C200-C100 construct was formed by ligating together a ˜1.29 Kbp fragment obtained by digestion of plasmid pBR322-C200 with EcoRI and NcoI, and a ˜950 bp fragment. obtained by digestion of plasmid pS3-56$_{C100m}$ with NcoI and SalI. The construction of plasmids pBR322-C200 and of pS3-56$_{C100m}$ are described in Sections IV.A.37 and IV.A.36., respectively.

[0775] In order to join the ADH2/GAP promoter, and the sequence encoding SOD to the 5'-terminus of the construct, and the alpha-factor terminator to the 3'-terminus of the construct, the C200-C100 construct was inserted into the vector pS3-34, which had been digested with EcoRI and SalI.

[0776] The vector pS3-34 was created from the vector pYSI3 by deletion of the insulin sequences encoded therein, and by insertion of two linkers, C and D. These linkers allow the SOD encoding sequence and-the HCV C200-C100 sequences to be in correct reading frame. Deletion of the insulin sequences was accomplished by digestion with EcoRI and SalI, followed by purification of the large vector fragment. The sequences of the inserted linkers are:

```
Linker C:   5' AAT TTG GAA TTC TAA TTA ATT AAG 3'

Linker D:        AC CTT AAG ATT AAT TAA TTCAGCT
```

[0777] The underlined sequences, CTTAAG and CAGCT, indicate an EcoRI site and a SalI site, respectively.

[0778] The plasmid pYSI3, described briefly in Table 1 of the U.S. Pat. No. 4,751,180, was used as a convenient way to attach the C100 and C200-C100m sequences to the ADH2/GAP hybrid promoter, the alpha-factor terminator and the SOD sequences. Previously, pYSI3 was used to express insulin; therefore, the vector contains a BamHI cassette (2.4 Kbp) which comprises the ADH2/GAP hybrid yeast promoter upstream of the hSOD gene (see U.S. Pat. No. 4,751,180) which in turn is fused to a hinge region and an insulin gene. Downstream of the insulin sequence is the yeast alpha-factor transcription terminator which is a 270 bp, SalI to EcoRI fragment, from the *Saccharomyces cerevisiae* alpha-factor gene described in Singh et al., *Nucleic Acids Research* (1983) 11(12):4049-4063. The insulin and hinge sequences were removed from the vector by digestion with EcoRI and SalI and the appropriate synthetic adapters were inserted between these two restriction sites to yield plasmid pS3-56 and pS3-34.

[0779] An expression vector containing the C200-C100 expression cassette was constructed by insertion of the cassette into the yeast expression vector pAB24; the vector pAB24 is described in Section IV.B.4.. The insertion was accomplished by excising a 4349 bp BamHI/BamHI cassette from the pS3-34/C200-C100 cloning vector and inserting the fragment into the BamHI site of the expression vector pAB24. The resulting vector, which is called pAB24/C200-C100, was transformed into yeast strain JSC308. Yeast strain JSC308 is described in commonly owned U.S. Ser. No. 190,868, and is on deposit with the American Type Culture Collection under Accession No. 20879.

[0780] IV.B.10.b. Expression of Fusion Polypeptide SOD/C200-C100

[0781] Expression of fusion polypeptide SOD/C200-C100 encoded in pAB24/C200-C100 was in yeast. strain JSC308, which had been transformed with the isolated expression vector. The yeast transformants were grown in an inoculum culture for 24 to 30 hours in Leu⁻ medium containing 2% glucose; growth was at 30° C. at an agitation rate of 300 rpm. Expression of the fusion polypeptide was obtained by inoculating 500 ml of YEP containing 2% glucose with 25 ml of the inoculum culture, followed by incubation at 30° C. for 48 hours at an agitation rate of about 300 rpm. Growth was in a 2.8 L Fernbach flask.

[0782] Alternatively, 500 ml of inoculum was added to 10 L of YEP containing 4% glucose, and growth was in a Braun Biotech Model Biostat E fermentor at an agitation rate of about 400±20 rpm at a temperature of about 30°±2° C. and an air flow rate of 10±slpm; the cells were harvested 50 hours after inoculation.

[0783] IV.B.11. Expression of HCV Polypeptide C100 in Yeast

[0784] The composite HCV cDNA C100 (see Section IV.A.16) was fused directly to the ADH2/GAP promoter, and expressed in yeast.

[0785] IV.B.11.a. Construction of Yeast Expression Vector pC100⁻ d#3

[0786] The construction of a yeast expression vector in which the HCV cDNA C100 sequence (described in Sections IV.A.16.) was fused directly to the ADH2/GAP promoter was accomplished by a protocol which included amplification of the C100 sequence using a PCR method, followed by ligation of the amplified sequence into a cloning vector. After cloning, the C100 sequence was excised, and with a sequence which contained the ADH2/GAP promoter, was ligated to a large fragment of a yeast vector to yield a yeast expression vector. A flow chart of the construction of the yeast expression vector is shown in **FIG. 76**.

[0787] The PCR amplification of C100 was performed using as template the vector pS3-56$_{C100}$ (see Section IV.A.36.), which had been linearized by digestion with SalI.

[0788] The oligonucleotide primers used for the amplification were designed to facilitate cloning into the expression vector, and to introduce a translation termination codon. Specifically, novel 5'-HindIII and 3'-SalI sites were generated with the PCR oligonucleotides. The oligonucleotide containing the SalI site also encodes the double termination codons, TAA and TGA. The oliogonucleotide containing the HindIII site also contains an untranslated leader sequence derived from the pgap63 gene, situated immediately upstream of the AUG codon. The pEco63GAPDH gene is described by Holland and Holland (1980) and by Kniskern et al. (1986). The PCR primer sequences used for the direct expression of C100m were:

```
5' GAG TGC TCA AGC TTC AAA ACA AAA TGG CTC
   ACT TTC TAT CCC AGA CAA AGC AGA GT 3'
and
```

-continued

```
5' GAG TGC TCG TCG ACT CAT TAG GGG GAA
   ACA TGG TTC CCC CGG GAG GCG AA 3'.
```

[0789] Amplification by PCR, utilizing the primers, and template, was with a Cetus-Perkin-Elmer PCR kit, and was performed according to the manufacturer's directions. The PCR conditions were 29 cycles of 94° C. for a minute, 37° C. for 2 minutes, 72° C. for 3 minutes; and the final incubation was at 72° C. for 10 minutes. The DNA can be stored at 4° C. or –20° C. overnight.

[0790] After amplification, the PCR products were digested with HindIII and SalI. The major product of 1.1 kb was purified by electrophoresis on a gel, and the eluted purified product was ligated with a large SalI-HindIII fragment of pBR322. In order to isolate correct recombinants, competent HB101 cells were transformed with the recombinant vectors, and after cloning, the desired recombinants were identified on the basis of the predicted size of HindIII-SalI fragments excised from the clones. One of the clones which contained the a HindIII-SalI fragment of the correct size was named pBR322/C100 d. Confirmation that this clone contained amplified C100 was by direct sequence analysis of the HindIII-SalI fragment.

[0791] The expression vector containing C100 was constructed by ligating the HindIII-SalI fragment from pBR322/C100⁻d to a 13.1 kb BamHI-SalI fragment of pBS24.1, and a 1369 bm BamHI-HindIII fragment containing the ADH2/GAP promoter. (The latter fragment is described in EPO 164,556). The pBS24.1 vector is described in commonly owned U.S. Ser. No. 382,805 filed Jul. 19, 1989. The ADH2/GAP promoter fragment was obtained by digestion of the vector pPGAP/AG/HindIII with HindIII and BamHI, followed by purification of the 1369 bp fragment on a gel.

[0792] Competent HB101 cells were transformed with the recombinant vectors; and correct recombinants were identified by the generation of a 2464 bp fragment and a 13.1 kb fragment generated by BamHI and SalI digestion of the cloned vectors. One of the cloned correct recombinant vectors was named pC100⁻d#3.

[0793] IV.B.11.b. Expression of C100 from pC100⁻d#3

[0794] In order to express C100, competent cells of *Saccharomyces cerevisiae* strain AB122 (MATa leu2 ura3-53 prb 1-1122 pep4-3 prcl-407[cir-0]) were transformed with the expression vector pC100 d3. The transformed cells were plated on URA-sorbitol, and individual transformants were then streaked on Leu⁻ plates.

[0795] Individual clones were cultured in Leu⁻, ura⁻ medium with 2% glucose at 30° C. for 24-36 hours. One liter of Yeast Extract Peptone Medium (YEP) containing 2% glucose was inoculated with 10 ml of the overnight culture, and the resulting culture was grown at 30° C. at an agitation rate of 400 rpm and an aeration late of 1 L of air per 1 L of medium per minute (i.e., 1 vvm) for 48 hours. The pH of the medium was not controlled. The culture was grown in a BioFlo II fermentor manufactured by New Brunswick Science Corp. Following fermentation, the cells were isolated and analyzed for C100 expression.

[0796] Analysis for expressed C100 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels, and by western blots. The Western blots were probed with rabbit polyclonal antibodies directed against the SOD-C100 polypeptide expressed in yeast. The expected size of the C100 polypeptide is 364 amino acids. By gel analysis the expressed polypeptide has a MW$_r$ of 39.9K.

[0797] Both analytical methods demonstrated that the expressed C100 polypeptide was present in total cell lysates, but was absent from crude extracts. These results suggest that the expressed C100 polypeptide may be insoluble.

[0798] IV.B.12 Expression of HCV Polypeptide S2 in Yeast

[0799] An 52 polypeptide encoded in the HCV cDNA shown in **FIG. 72** contains amino acids 199 to 328 encoded in the ORF. The clone, pi14a, described supra., contains an HCV cDNA which encodes these amino acids.

[0800] The protocol for the construction of the expression vector encoding the S2 polypeptide and for its expression in yeast was analagous to that used for the expression of the C100 polypeptide, described in Sections IV.B.11, IV.B.11a and IV.B.11b, except for the following. (A flow chart of the construction of the yeast expression vector encoding S2 is shown in **FIG. 77**.)

[0801] The template for the PCR reaction was the vector pBR322/Pi14a, which had been linearized by digestion with HindIII.

[0802] The oligonucleotides used as primers for the amplification by PCR of the S2 encoding sequence were the following.

[0803] For the 5'-region of the S2 sequence:

```
5' GAG TGC TCA AGC TTC AAA ACA AAA TGG GGC TCT
   ACC ACG TCA CCA ATG ATT GCC CTA AC 3';
```

[0804] and

[0805] for the 3'-region of the S2 sequence:

```
5' GAG TGC TCG TCG ACT CAT TAA GGG GAC CAG TTC
   ATC ATC ATA TCC CAT GCC AT 3'.
```

[0806] The primer for the 5'-region introducies a HindIII site and an ATG start codon into the amplified product. The primer for the 3' region introduces translation stop codons and a SalI site into the amplified product.

[0807] The PCR conditions were 29 cycles of 94° C. for a minute, 37° C. for 2 minutes, 72° C. for 3 minutes, and the final incubation was at 72° C. for 10 minutes.

[0808] The main product of the PCR reaction was a 413 bp fragment, which was gel purified. The purified fragment was ligated to the large fragment obtained from pBR322 digested with HindIII and SalI fragment, yielding the plasmid pBR322/S2d.

[0809] Ligation of the 413 bp HindIII-SalI S2 fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant

vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.77 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding S2 fused directly to the ADH2/GAP promoter is identified as pS2d#9.

[0810] Analysis for expressed 52 polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The expected size of the S2 polypeptide is 130 amino acids. By gel analysis, the expressed polypeptide has a MW^r of 16 Kd. The expressed S2 was detected in the total lysates, and not in the crude extracts, suggesting that the expressed S2 may be insoluble.

[0811] IV.B.13. Expression of HCV C Polypeptide in Yeast

[0812] A polypeptide encoded in the HCV cDNA shown in **FIG. 72**, and which contains amino acids numbers 1 to 122 encoded in the ORF, is named herein the C polypeptide.

[0813] The protocol for the construction of the expression vector encoding the C polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described in Sections IV.B.11, IV.B.11.a and IV.B.11.b, except for the following. (A flow chart of the construction of the yeast expression vector encoding the C polypeptide is shown in **FIG. 92**.)

[0814] The template for the PCR reaction was pBR322/Ag30a which had been linearized with HindIII. The HCV cDNA in clone Ag30a is described in Section IV.A.30. The oligonucleotides used as primers for the amplification by PCR of the C encoding sequence were the following.

[0815] For the 5'-region of the C sequence:

```
5' GAG TGC TCG TCG ACT CAT TAA CCC AAA TTG CGC
GAC CTA CGC CGG GGG TCT GT 3'.
```

[0816] and

[0817] for the 3'-region of the C sequence:

```
5' GAG TGC AGC TTC AAA ACA AAA TGA GCA CGA
ATC CTA AAC CTC AAA AAA AAA AC 3',
```

[0818] The primer for the 5'-region introduces a HindIII site into the amplified product, and the primer for the 3'-region introduces translation stop codons and a SalI site. The PCR was run for 29 cycles of 94° C. for a minute, 37° C. for 2 minutes, 72° C. for 3 minutes, and the final incubation was at 72° C. for 10 minutes.

[0819] The major product of PCR amplification is a 381 bp polynucleotide. Ligation of this fragment with the SalI-HindIII large SalI-HindIII fragment of pBR322 yielded the plasmid pBR322/C2.

[0820] Ligation of the 381 bp HindIII-SalI C coding fragment excised from pBR322/C2 with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector

pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the presence of a 1.74 kb fragment after digestion with BamHI and SalI. An expression vector constructed from the amplified sequence, and containing the sequence encoding C fused directly to the ADH2/GAP promoter is identified as pC22.

[0821] Analysis for expressed C polypeptide by the transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels. The C polypeptide is expected to have 122 amino acids and by gel analysis the expressed polypeptide has a MW_r of approximately 13.6 Kd.

[0822] IV.B.14. Expression of a Polypeptide Which Contains Amino Acid Numbers 404-661 of the HCV Polyprotein

[0823] A polypeptide encoded in the HCV cDNA shown in **FIG. 72**, and which contains amino acids numbers 404 to 661 encoded in a region of the HCV ORF designated as "x" is named herein the NS1 polypeptide. It should be noted that this designation is not meant to connote that the characteristics of this polypeptide are equivalent to that of the NS1 polypeptide of the flaviviruses, for the reasons discussed supra.

[0824] IV.B.14.a. Expression of NS1 in Yeast

[0825] The protocol for the construction of the expression vector encoding the NS1 polypeptide and for its expression in yeast was analogous to that used for the expression of the C100 polypeptide, described in Sections IV.B.11, IV.B.11.a, and IV.B.11.b, except for the following. (A flow chart of the construction of the yeast expression vector encoding NS1 is shown in **FIG. 78**)

[0826] The template for the PCR amplification of the NS1 encoding sequence was pBR K-9-1/59a (also called pBR K/9-1/59a), which had been linearized by digestion with HindIII. The clone CA59a, described in Example IV.A.27, was inserted into the EcoRI site of pBR322 and the clone K-9-1, described in Example IV.A.26, was inserted into the BglII site of pPGAP. A PstI to NheI fragment from the pBR322/CA59a vector was ligated to a PstI to NheI fragment from the pPGAP3/K-9-1 to create the vector pBR K-9-1/59a, from which the HCV cDNA can be released by digestion with BglII and EcoRI. The vector pPGAP3 is a PBR322 derivative with the GAP promoter and terminator, described in U.S. Patent Application No. 468,589, filed on Feb. 22, 1983.

[0827] The oligonucleotides used as primers for PCR amplification of the NS1 coding sequence were designed to not only to generate novel 5' HindIII site, and a 3'-SalI site and double termination codons; in addition, the 3'-oligonucleotide primer also included an XhoI restriction site. The sequences of the oligonucleotide primers is the following.

[0828] For the 5'-region of the NS1 sequence:

[0829] 5' GAG TGC TCA AGC TTA CAA AAC AAA ATG GCA CCA GGC GCC AAG CAG AAC GTC CAG CTG ATC 3'; and

[0830] For the 3'-region of the NS1 sequence:

```
5' GAG TGC TCC TCG AGG TCG ACT CAT TAC TCG GAC
CTG TCC CTA TCT TCC AGA TCG CAA CG 3'.
```

[0831] For cloning NS1 into a yeast experssion vector, the PCR conditions were 15 cycles of 94° C. for 1.5 minutes, 60° C. for 2 minutes, and 72° C. for 3 minutes. After 15 cycles, the last incubation was at 72° C. for 10 minutes.

[0832] After amplification by PCR, the products were digested with HindIII and XhoI, and an 809 bp product isolated after gel electrophoresis. This product was ligated with the large HindIII-SalI fragment of pBR322. (This construction results in the loss of 3'-XhoI site from the NS1 coding sequence, and the SalI site of pBR322). After cloning in HB101, plasmids containing the correct insert were identified by the presence of an 800 bp HindIII-SalI fragment, after digestion of the DNA with HindIII and partial digestion with SalI. A plasmid containing the correct insert was named pBR322/NS11d/13.

[0833] An 800 bp fragment which encodes NS1 was excised from pBR322/NS11d/13 by digestion with HindIII and partial digestion with SalI, and was isolated by gel electrophoresis. Ligation of this fragment with the 1.36 kb BamHI-HindIII fragment containing the ADH2/GAP promoter, and with the large BamHI-SalI fragment of the yeast vector pBS24.1 yielded recombinant vectors, which were cloned. Correct recombinant vectors were identified by the liberation of 2008 bp and 165 bp fragments in addition to a 13.1 kb fragment after digestion with HindIII and SalI. A recombinant vector containing the desired insert is named pNS11d/13 (also named pNS11/13-15).

[0834] Analysis for expressed NS1 polypeptide by the pNS11d/13 transformed cells was performed on total cell lysates and crude extracts prepared from single yeast colonies obtained from the Leu⁻ plates. The cell lysates and crude extracts were analyzed by electrophoresis on SDS polyacrylamide gels and by Western blots. A human serum sample was identified by the experiment described in Example IV.B.8.a to contain antibodies against the NS1 region. This serum was used as a primary antibody in the western blotting. The NS1 polypeptide is expected to have 258 amino acids, and by gel analysis the expressed polypeptide has a MW_r of approximately 29 K. Both methods of analysis indicated the presence of expressed NS1 polypeptide in the total cell lysates, but not in crude extracts. These results suggest that the expressed NS1 polypeptide is insoluble.

[0835] IV.B.14.b. Expression of NS1 in Mammalian Cells

[0836] A vector for expression of NS1 in mammalian cells was constructed using a 795 bp fragment obtained by PCR amplification of the NS1 ORF which is contained in plasmid pPGAT/K9-1/59a, described in Section IV.B.14.a. The PCR reaction was performed using the following oligonucleotide sequences as primers.

[0837] The primer for the 5'-region of NS1 was:

```
5' GGA TCC GCT AGC GCC CCC AAG CAG AAC GTC
CAG CTG ATC AAC ACC 3';
```

[0838] where the underlined sequence encodes an NheI site.

[0839] The primer for the 3'-region of NS1 was:

```
5' GGA TCC AAG CTT TTA CTC GGA CCT GTC CCT
ATC TTC CAG ATC GCA ACG 3';
```

[0840] where the first and second underlined sequences encode a HindIII site and a stop codon, respectively.

[0841] A polyacrylamide gel purified 795 bp fragment was digested with NheI and HindIII. The resulting 777 bp NheI-HindIII fragment, which encodes NS1, was ligated to the 3.98 Kbp fragment obtained by digestion of pCMV6a120 with NheI, and partial digestion with HindIII. A resulting plasmid containing the correct insert was designated ptpa-NS1.

[0842] The vector pCMV6a120, which is described in U.S. Ser. No. 138,894 filed Dec. 24, 1987 (which is incorporated by reference, along with any foreign filed counterparts), is a mammalian cell expression vector which encodes gp120 of human immunodeficiency virus (HIV). The gp120 encoding sequence was excised by the digestion with NheI and the partial digestion with HindIII.

[0843] Transient expression studies were performed in COS-7 cells (Gluzman (1981)), which had been transfected with ptpa-NS1. Transfection was accomplished by lipofection using techniques described by Felgner et al. (1987). In order to perform immunofluorescence studies, the transfected cells were subcultured into 2-chamber plastic slide wells (Lab-Tek). The COS-7 cells were fixed with acetone at 72 hours following transfection, and cells producing NS1 were identified using indirect immunofluorescence methods (Pachl et al. (1987)). In the immunofluorescence studies, the source of primary antibodies was an HCV positive human antiserum which was immunoreactive with bacterially expressed NS1. The secondary antibody was FITC-conjugated goat anti-human IgG (Tago, Inc., Burlingame, Calif.), which had been diluted 1:200. Immunofluorescence on the slides was observed using a Leitz Dialux 20 EB fluorescent microscope. The cells which were transfected with ptpa-NS1 exhibited a diffuse cytoplasmic immunofluorescent staining pattern. Mock controls were also run. Positive controls included cells transfected with plasmids expresing CMV glycoprotein B, including plasmids pXgB8, the pXgB23c1v1-4 series, and the pXgB24c1v1-3 series.

[0844] IV.B.14.c. Expression of NS1 in Mammalian Cells Using a Vector with a Selectable Marker

[0845] In order to physically link the NS1 encoding sequence to a sequence encoding a selectable marker, i.e., the DHFR gene, the NS1 ORF DNA sequence was subcloned into two mammalian cell expression vectors, pCMVAdhfr and pMCMVAdhfr. The vector pCWVAdhfr contains the human CMV major immediate early (MIE) promoter, and also contains the mouse dhfr gene linked to the adenovirus major late promoter (Stuve et al. (1987)). The vector pMCMVAdhfr is colinear to pCMVAdhfr, except that murine CMV (MCMV) MIE promoter is substituted for the human CMV MIE promoter. The MCMV MIE promoter is a HpaI-PstI fragment, which was cloned from pON402 (Manning and Mocarski (1988)).

[0846] In order to subclone the NS1 ORF DNA, it was excised from ptpa-NS1 by partial digestion with SalI. The 962 bp fragment was then ligated to SalI digested pCM-VAdhfr, or to SalI digested pMCMVAdhfr. Each of these vectors contains a unique SalI site.

[0847] The recombinant dhfr vectors comprised of the NS1 sequence were used to transfect dhfr− CHO cells in order to generate stable cell lines expressing this ORF. Transfection was by the polybrene transfection procedure of Chaney et al. (1986).

[0848] IV.C. Identification of RNA in Infected Individuals Which Hybridizes to HCV cDNA.

[0849] IV.C.1. Identification of RNA in the Liver of a Chimpanzee With NANBH Which Hybridizes to HCV cDNA.

[0850] RNA from the liver of a chimpanzee which had NANBH was shown to contain a species of RNA which hybridized to the HCV cDNA contained within clone 81 by Northern blotting, as follows.

[0851] RNA was isolated from a liver biopsy of the chimpanzee from which the high titer plasma was derived (see Section IV.A.1.) using techniques described in Maniatis et al. (1982) for the isolation of total RNA from mammalian cells, and for its separation into poly A and poly A− fractions. These RNA fractions were subjected to electrophoresis on a formaldehyde/agarose gel (1% w/v), and transferred to nitrocellulose. (Maniatis et al. (1982)). The nitrocellulose filters were hybridized with radiolabeled HCV cDNA from clone 81 (see **FIG. 4** for the nucleotide sequence of the insert.) To prepare the radiolabeled probe, the HCV cDNA insert isolated from clone 81 was radiolabeled with ^{32}P by nick translation using DNA Polymerase I (Maniatis et al. (1982)). Hybridization was for 18 hours at 42° C. in a solution containing 10% (w/v) Dextran sulphate, 50% (w/v) deionized formamide, 750 mM NaCl, 75 mM Na citrate, 20 mM Na$_2$HPO$_4$, pH 6.5, 0.1% SDS, 0.02% (w/v) bovine serum albumin (BSA), 0.02% (w/v) Ficoll-400, 0.02% (w/v) polyvinylpyrrolidone, 100 micrograms/ml salmon sperm DNA which had been sheared by sonication and denatured, and 10^6 CPM/ml of the nick-translated cDNA probe.

[0852] An autoradiograph of the probed filter is shown in **FIG. 38**. Lane 1 contains ^{32}P-labeled restriction fragment markers. Lanes 2-4 contain chimpanzee liver RNA as follows: lane 2 contains 30 micrograms of total RNA; lane 3 contains 30 micrograms of poly A− RNA; and lane 4 contains 20 micrograms of poly A+ RNA. As shown in **FIG. 38**, the liver of the chimpanzee with NANBH contains a heterogeneous population of related poly A+ RNA molecules which hybridizes to the HCV cDNA probe, and which appears to be from about 5000 nucleotides to about 11,000 nucleotides in size. This RNA, which hybridizes to the HCV cDNA, could represent viral genomes and/or specific transcripts of the viral genome.

[0853] The experiment described in Section IV.C.2., infra, is consistent with the suggestion that HCV contains an RNA genome.

[0854] IV.C.2. Identification of HCV Derived RNA in Serum from Infected Individuals.

[0855] Nucleic acids were extracted from particles isolated from high titer chimpanzee NANBH plasma as described in Section IV.A.1. Aliquots (equivalent to 1 ml of original plasma) of the isolated nucleic acids were resuspended in 20 microliters 50 mM Hepes, pH 7.5, 1 mm EDTA and 16 micrograms/ml yeast soluble RNA. The samples were denatured by boiling for 5 minutes followed by immediate freezing, and were treated with RNase A (5 microliters containing 0.1 mg/ml RNase A in 25 mM EDTA, 40 mM Hepes, pH 7.5) or with DNase I (5 microliters containing 1 unit DNase I in 10 mM MgCl$_2$, 25 mM Hepes, pH 7.5); control samples were incubated without enzyme. Following incubation, 230 microliters of ice-cold 2×SSC containing 2 micrograms/ml yeast soluble RNA was added, and the samples were filtered on a nitrocellulose filter. The filters were hybridized with a cDNA probe from clone 81, which had been P-labeled by nick-translation. **FIG. 39** shows an autoradiograph of the filter. Hybridization signals were detected in the DNase treated and control samples (lanes 2 and 1, respectively), but were not detected in the RNase treated sample (lane 3). Thus, since RNase A treatment destroyed the nucleic acids isolated from the particles, and DNase I treatment had no effect, the evidence strongly suggests that the HCV genome is composed of RNA.

[0856] IV.C.3. Detection of Amplified HCV Nucleic Acid Sequences derived from HCV Nucleic Acid Sequences in Liver and Plasma Specimens from Chimpanzees with NANBH

[0857] HCV nucleic acids present in liver and plasma of chimpanzees with NANBH, and in control chimpanzees, were amplified using essentially the polymerase chain reaction (PCR) technique described by Saiki et al. (1986). The primer oligonucleotides were derived from the HCV cDNA sequences in clone 81, or clones 36 and 37. The amplified sequences were detected by gel electrophoresis and Southern blotting, using as probes the appropriate cDNA oligomer with a sequence from the region between, but not including, the two primers.

[0858] Samples of RNA containing HCV sequences to be examined by the amplification system were isolated from liver biopsies of three chimpanzees with NANBH, and from two control chimpanzees. The isolation of the RNA fraction was by the guanidinium thiocyanate procedure described in Section IV.C.1.

[0859] Samples of RNA which were to be examined by the amplification system were also isolated from the plasmas of two chimpanzees with NANBH, and from one control chimpanzee, as well as from a pool of plasmas from control chimpanzees. One infected chimpanzee had a CID/ml equal to or greater than 10^6, and the other infected chimpanzee had a CID/ml equal to or greater than 10^5.

[0860] The nucleic acids were extracted from the plasma as follows. Either 0.1 ml or 0.01 ml of plasma was diluted to a final volume of 1.0 ml, with a TENB/proteinase K/SDS solution (0.05 M Tris-HCL, pH 8.0, 0.001 M EDTA, 0.1 M NaCl, 1 mg/ml Proteinase K, and 0.5% SDS) containing 10 micrograms/ml polyadenylic acid, and incubated at 37° C. for 60 minutes. After this proteinase K digestion, the resultant plasma fractions were deproteinized by extraction with TE (10.0 mM Tris-HCl, pH 8.0, 1 mM EDTA) saturated phenol. The phenol phase was separated by centrifugation, and was reextracted with TENB containing 0.1% SDS. The resulting aqueous phases from each extraction were pooled, and extracted twice with an equal volume of phenol/chlo-

roform/isoamyl alcohol [1:1(99:2)], and then twice with an equal volume of a 99:1 mixture of chloroform/isoamyl alcohol. Following phase separation by centrifugation, the aqueous phase was brought to a final concentration of 0.2 M Na Acetate, and the nucleic acids were precipitated by the addition of two volumes of ethanol. The precipitated nucleic acids were recovered by ultracentrifugation in a SW 41 rotor at 38 K, for 60 minutes at 4° C.

[0861] In addition to the above, the high titer chimpanzee plasma and the pooled control plasma alternatively were extracted with 50 micrograms of poly A carrier by the procedure of Chomcyzski and Sacchi (1987). This procedure uses an acid guanidinium thiocyanate extraction. RNA was recovered by centrifugation at 10,000 RPM for 10 minutes at 4° C. in an Eppendorf microfuge.

[0862] On two occasions, prior to the synthesis of cDNA in the PCR reaction, the nucleic acids extracted from plasma by the proteinase K/SDS/phenol method were further purified by binding to and elution from S and S Elutip-R Columns. The procedure followed was according to the manufacturer's directions.

[0863] The cDNA used as a template for the PCR reaction was derived from the nucleic acids (either total nucleic acids or RNA) prepared as described above. Following ethanol precipitation, the precipitated nucleic acids were dried, and resuspended in DEPC treated distilled water. Secondary structures in the nucleic acids were disrupted by heating at 65° C. for 10 minutes, and the samples were immediately cooled on ice. cDNA was synthesized using 1 to 3 micrograms of total chimpanzee RNA from liver, or from nucleic acids (or RNA) extracted from 10 to 100 microliters of plasma. The synthesis utilized reverse transcriptase, and was in a 25 microliter reaction, using the protocol specified by the manufacturer, BRL. The primers for cDNA synthesis were those also utilized in the PCR reaction, described below. All reaction mixtures for cDNA synthesis contained 23 units of the RNAase inhibitor, RNASIN™ (Fisher/Promega). Following cDNA synthesis, the reaction mixtures were diluted with water, boiled for 10 minutes, and quickly chilled on ice.

[0864] The PCR reactions were performed essentially according to the manufacturer's directions (Cetus-Perkin-Elmer), except for the addition of 1 microgram of RNase A. The reactions were carried out in a final volume of 100 microliters. The PCR was performed for 35 cycles, utilizing a regimen of 37° C., 72° C., and 94° C.

[0865] The primers for cDNA synthesis and for the PCR reactions were derived from the HCV cDNA sequences in either clone 81, clone 36, or clone 37b. (The HCV cDNA sequences of clones 81, 36, and 37b are shown in FIGS. 4, 5, and 10, respectively.) The sequences of the two 16-mer primers derived from clone 81 were:

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5' CAA TCA TAC CTG ACA G 3'
and
5' GAT AAC CTC TGC CTG A 3'.
```

[0866] The sequence of the primer from clone 36 was:

[0867] 5' GCA TGT CAT GAT GTA T 3'.

[0868] The sequence of the primer from clone 37b was:

[0869] 5' ACA ATA CGT GTG TCA C 3'.

[0870] In the PCR reactions, the primer pairs consisted of either the two 16-mers derived from clone 81, or the 16-mer from clone 36 and the 16-mer from clone 37b.

[0871] The PCR reaction products were analyzed by separation of the products by alkaline gel electrophoresis, followed by Southern blotting, and detection of the amplified HCV-cDNA sequences with a ^{32}P-labeled internal oligonucleotide probe derived from a region of the HCV cDNA which does not overlap the primers. The PCR reaction mixtures were extracted with phenol/chloroform, and the nucleic acids precipitated from the aqueous phase with salt and ethanol. The precipitated nucleic acids were collected by centrifugation, and dissolved in distilled water. Aliquots of the samples were subjected to electrophoresis on 1.8% alkaline agarose gels. Single stranded DNA of 60, 108, and 161 nucleotide lengths were co-electrophoresed on the gels as molecular weight markers. After electrophoresis, the DNAs in the gel were transferred onto Biorad Zeta Probe™ paper. Prehybridization and hybridization, and wash conditions were those specified by the manufacturer (Biorad).

[0872] The probes used for the hybridization-detection of amplified HCV cDNA sequences were the following. When the pair of PCR primers were derived from clone 81, the probe was an 108-mer with a sequence corresponding to that which is located in the region between the sequences of the two primers. When the pair of PCR primers were derived from clones 36 and 37b, the probe was the nick-translated HCV cDNA insert derived from clone 35. The primers are derived from nucleotides 155-170 of the clone 37b insert, and 206-268 of the clone 36 insert. The 3'-end of the HCV cDNA insert in clone 35 overlaps nucleotides 1-186 of the insert in clone 36; and the 5'-end of clone 35 insert overlaps nucleotides 207-269 of the insert in clone 37b. (Compare FIGS. 5, 8 and 10.) Thus, the cDNA insert in clone 35 spans part of the region between the sequences of the clone 36 and 37b derived primers, and is useful as a probe for the amplified sequences which include these primers.

[0873] Analysis of the RNA from the liver specimens was according to the above procedure utilizing both sets of primers and probes. The RNA from the liver of the three chimpanzees with NANBH yielded positive hybridization results for amplification sequences of the expected size (161 and 586 nucleotides for 81 and 36 and 37b, respectively), while the control chimpanzees yielded negative hybridization results. The same results were achieved when the experiment was repeated three times.

[0874] Analysis of the nucleic acids and RNA from plasma was also according to the above procedure utilizing the primers and probe from clone 81. The plasmas were from two chimpanzees with NANBH, from a control chimpanzee, and pooled plasmas from control chimpanzees. Both of the NANBH plasmas contained nucleic acids/RNA which yielded positive results in the PCR amplified assay, while both of the control plasmas yielded negative results. These results have been repeatably obtained several times.

[0875] Defective viruses have been known to occur in RNA viruses. By using PCR technology it is possible to design primers to amplify sequences of the HCV genome. By analysis of the amplified products, it is expected to be able to identify both defective versions of the viral genome

as well as wild-type viral species. Accordingly, using two primers based on known HCV sequence, one can predict accurately the expected size of the PCR product. Any larger species observed by gel electrophoresis and hybridization analysis could represent potential wild-type genomes. Alternatively, any smaller species observed in this fashion might represent defective agents. Analyses of these types would be useful in confirming the exact origin of the known HCV sequence, whether it is indeed a wild-type viral sequence or a defective genome. Techniques and methods for these analyses are well known in the art and have been previously described. This methodology will enable one skilled in the art to obtain related (wild-type or defective) forms of the viral genome.

[0876] IV.D. Radioimmunoassay for Detecting HCV Antibodies in Serum from Infected Individuals

[0877] Solid phase radioimmunoassays to detect antibodies to HCV antigens were developed based upon Tsu and Herzenberg (1980). Generally, microtiter plates (Immulon 2, Removawell strips) are coated with purified polypeptides containing HCV epitopes. The coated plates are incubated with either human serum samples suspected of containing antibodies to the HCV epitopes, or to appropriate controls. During incubation, antibody, if present, is immunologically bound to the solid phase antigen. After removal of the unbound material and washing of the microtiter plates, complexes of human antibody-NANBV antigen are detected by incubation with ^{125}I-labeled sheep anti-human immunoglobulin. Unbound labeled antibody is removed by aspiration, and the plates are washed. The radioactivity in individual wells is determined; the amount of bound human anti-HCV antibody is proportional to the radioactivity in the well.

[0878] The detection in serum of antibodies to HCV epitopes present in fusion polypeptides of SOD with NANB$_{5-1-1}$, with NANB with NANB$_{81}$, and with NANB$_{C33c}$ (these polypeptides are also called SOD-5-1-1,SOD-81, C100-3, and SOD-C33c, respectively), was accomplished by solid phase radioimmunoassay.

[0879] Microtiter plates were coated with any of the aforementioned antigens, which had been purified as described herein in Section IV.D.1. (SOD-NANB$_{5-1-1}$), Section IV.D.2. (SOD-NANB$_{81}$), Section IV.B.7. (C100-3), and Section IV.B.9. (SOD-33c). The assays were conducted as follows.

[0880] One hundred microliter aliquots containing 0.1 to 0.5 micrograms of the designated SOD-NANB fusion polypeptide in 0.125 M Na borate buffer, pH 8.3, 0.075 M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell Strips). The plate was incubated at 4° C. overnight in a humid chamber, after which, the protein solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent nonspecific binding, the wells were coated with bovine serum albumin (BSA) by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour; after this incubation the BSA solution was removed. The polypeptides in the coated wells were reacted with serum by adding 100 microliters of serum samples diluted 1:100 in 0.01M Na phosphate buffer, pH 7.2, 0.15 M NaCl (PBS) containing 10 mg/ml BSA, and incubating the serum containing wells for

1 hr at 37° C. After incubation, the serum samples were removed by aspiration, and the wells were washed 5 times with BBST. Anti-NANB$_{5-1-1}$ and Anti-NANB$_{81}$ bound to the fusion polypeptides was determined by the binding of ^{125}I-labeled F'(ab)$_2$ sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated at 37° C. for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

[0881] IV.D.1. Purification of Fusion Polypeptide SOD-NANB$_{5-1-1}$.

[0882] The fusion polypeptide SOD-NANB$_{5-1-1}$, expressed in-recombinant bacteria as described in Section IV.B.1., was purified from the recombinant E. coli by differential extraction of the cell extracts with urea, followed by chromatography on anion and cation exchange columns as follows.

[0883] Thawed cells from 1 liter of culture were resuspended in 10 ml of 20% (w/v) sucrose containing 0.01M Tris HCl, pH 8.0, and 0.4 ml of 0.5M EDTA, pH 8.0 was added. After 5 minutes at 0° C., the mixture was centrifuged at 4,000×g for 10 minutes. The resulting pellet was suspended in 10 ml of 25% (w/v) sucrose containing 0.05 M Tris HCl, pH 8.0, 1 mM phenylmethylsulfonylfluoride (PMSF) and 1 microgram/ml pepstatin A, followed by addition of 0.5 ml lysozyme (10 mg/ml) and incubation at 0° C. for 10 minutes. After the addition of 10 ml 1% (v/v) Triton X-100 in 0.05 M Tris HCl, pH 8.0, 1 ml EDTA, the mixture was incubated an additional 10 min at 0° C. with occasional shaking. The resulting viscous solution was homogenized by passage 6 times through a sterile 20-gauge hypodermic needle, and centrifuged at 13,000×g for 25 minutes. The pelleted material was suspended in 5 ml of 0.01 M Tris HCl pH 8.0, and the suspension centrifuged at 4,000×g for 10 minutes. The pellet, which contained SOD-NANB$_{5-1-1}$ fusion protein, was dissolved in 5 ml of 6 M urea in 0.02 M Tris HCl, pH 8.0, 1 mM dithiothreitol (Buffer A), and was applied to a column of Q-Sepharose Fast Flow equilibrated with Buffer A. Polypeptides were eluted with a linear gradient of 0.0 to 0.3 M NaCl in Buffer A. After elution, fractions were analyzed by polyacrylamide gel electrophoresis in the presence of SDS to determine their content of SOD-NANB$_{5-1-1}$. Fractions containing this polypeptide were pooled, and dialyzed against 6 M urea in 0.02 M sodium phosphate buffer, pH 6.0, 1 mM dithiothreitol (Buffer B). The dialyzed sample was applied on a column of S-Sepharose Fast Flow equilibrated with Buffer B, and polypeptides eluted with a linear gradient of 0.0 to 0.3 M NaCl in Buffer B. The fractions were analyzed by polyacrylamide gel electrophoresis for the presence of SOD-NANB$_{5-1-1}$, and the appropriate fractions were pooled.

[0884] The final preparation of SOD-NANB$_{5-1-1}$ polypeptide was examined by electrophoresis on polyacrylamide gels in the presence of SDS. Based upon this analysis, the preparation was more than 80% pure.

[0885] IV.D.2. Purification of Fusion Polypeptide SOD-NANB$_{81}$.

[0886] The fusion polypeptide SOD-NANB$_{81}$, expressed in recombinant bacteria as described in Section IV.B.2., was

purified from recombinant *E. coli* by differential extraction of the cell extracts with urea, followed by chromatography on anion and cation exchange columns utilizing the procedure described for the isolation of fusion polypeptide SOD-NANB$_{5-1-1}$ (see Section IV.D.1.).

[0887] The final preparation of SOD-NANB$_{81}$ polypeptide was examined by electrophoresis on polyacrylamide gels in the presence of SDS. Based upon this analysis, the preparation was more than 50% pure.

[0888] IV.D.3. Detection of Antibodies to HCV Epitopes by Solid Phase Radioimmunoassay.

[0889] Serum samples from 32 patients who were diagnosed as having NANBH were analyzed by radioimmunoassay (RIA) to determine whether antibodies to HCV epitopes present in fusion polypeptides SOD-NANB$_{5-1-1}$ and SOD-NANB$_{81}$ were detected.

[0890] Microtiter plates were coated with SOD-NANB$_{5-1-1}$ or SOD-NANB$_{81}$, which had been partially purified according to Sections IV.D.1. and IV.D.2., respectively. The assays were conducted as described supra.

[0891] The results of the detection of anti-NANB$_{5-1-1}$ and anti-NANB$_{81}$ in individuals with NANBH is presented in Table 1.

TABLE 1

Detection of Anti-5-1-1 and Anti-81 in Sera of
NANB, HAV and HBV Hepatitis Patients

Patient Reference		S/N	
Number	Diagnosis	Anti-5-1-1	Anti-81
1. 28[1]	Chronic NANB, IVD[2]	0.77	4.20
	Chronic NANB, IVD	1.14	5.14
	Chronic NANB, IVD	2.11	4.05
2. 29[1]	AVH[3], NANB, Sporadic	1.09	1.05
	Chronic, NANB	33.89	11.39
	Chronic, NANB	36.22	13.67
3. 30[1]	AVH, NANB, IVD	1.90	1.54
	Chronic NANB, IVD	34.17	30.28
	Chronic NANB, IVD	32.45	30.84
4. 31	Chronic NANB, PT[4]	16.09	8.05
5. 32[1]	Late AVH NANB, IVD	0.69	0.94
	Late AVH NANB, IVD	0.73	0.68
6. 33[1]	AVH, NANB, IVD	1.66	1.96
	AVH, NANB, IVD	1.53	0.56
7. 34[1]	Chronic NANB, PT	34.40	7.55
	Chronic NANB, PT	45.55	13.11
	Chronic NANB, PT	41.58	13.45
	Chronic NANB, PT	44.20	15.48
8. 35[1]	AVH NANE, IVD	31.92	31.95
	"Healed" recent NANB, AVH	6.87	4.45
9. 36	Late AVH NANB PT	11.84	5.79
10. 37	AVH NANB, IVD	6.52	1.33
11. 38	Late AVH NANB, PT	39.44	39.18
12. 39	Chronic NANB, PT	42.22	37.54
13. 40	AVH, NANB, PT	1.35	1.17
14. 41	Chronic NANB? PT	0.35	0.28
15. 42	AVH, NANB, IVD	6.25	2.34
16. 43	Chronic NANB, PT	0.74	0.61
17. 44	AVH, NANB, PT	5.40	1.83
18. 45	Chronic, NANB, PT	0.52	0.32
19. 46	AVH, NANB	23.25	4.45
20. 47	AVH, Type A	1.60	1.35
21. 48	AVH, Type A	1.30	0.66

TABLE 1-continued

Detection of Anti-5-1-1 and Anti-81 in Sera of
NANB, HAV and HBV Hepatitis Patients

Patient Reference		S/N	
Number	Diagnosis	Anti-5-1-1	Anti-81
22. 49	AVH, Type A	1.44	0.74
23. 50	Resolved Recent AVH, Type A	0.48	0.56
24. 51	AVH, Type A	0.68	0.64
	Resolved AVH, Type A	0.80	0.65
25. 52	Resolved Recent AVH, Type A	1.38	1.04
	Resolved Recent AVH, Type A	0.80	0.65
26. 53	AVH, Type A	1.85	1.16
	Resolved Recent AVH Type A	1.02	0.88
27. 54	AVH, Type A	1.35	0.74
28. 55	Late AVH, HBV	0.58	0.55
29. 56	Chronic HBV	0.84	1.06
30. 57	Late AVH, HBV	3.20	1.60
31. 58	Chronic HBV	0.47	0.46
32. 59[1]	AVH, HBV	0.73	0.60
	Healed AVH, HBV	0.43	0.44
33. 60[1]	AVH, HBV	1.06	0.92
	Healed AVH, HBV	0.75	0.68
34. 61[1]	AVH, HBV	1.66	0.61
	Healed AVH, HBV	0.63	0.36
35. 62[1]	AVH, HBV	1.02	0.73
	Healed AVH, HBV	0.41	0.42
36. 63[1]	AVH, HBV	1.24	1.31
	Healed AVH, HBV	1.55	0.45
37. 64[1]	AVH, HBV	0.82	0.79
	Healed AVH, HBV	0.53	0.37
38. 65[1]	AVH, HBV	0.95	0.92
	Healed AVH, HBV	0.70	0.50
39. 66[1]	AVH, HBV	1.03	0.68
	Healed AVH, HBV	1.71	1.39

[1]Sequential serum samples availablve from these patients
[2]IVD = Intravenus Drug User
[3]AVH = Acute viral hepatitis
PT = Post transfusion

[0892] As seen in Table 1, 19 of 32 sera from patients diagnosed as having NANBH were positive with respect to antibodies directed against HCV epitopes present in SOD-NANB$_{5-1-1}$ and SOD-NANB$_{81}$.

[0893] However, the serum samples which were positive were not equally immunologically reactive with SOD-NANB$_{5-1-1}$ and SOD-NANB$_{81}$. Serum samples from patient No. 1 were positive to SOD-NANB$_{81}$ but not to SOD-NANB$_{5-1-1}$ Serum samples from patients number 10, 15, and 17 were positive to SOD-NANB$_{5-1-1}$ but not to SOD-NANB$_{81}$ Serum samples from patients No. 3, 8, 11, and 12 reacted equally with both fusion polypeptides, whereas serum samples from patients No. 2, 4, 7, and 9 were 2-3 fold higher in the reaction to SOD-NANB$_{5-1-1}$ than to SOD-NANB$_{81}$. These results suggest that NANB$_{5-1-1}$ and NANB$_{81}$ may contain at least 3 different epitopes; i.e., it is possible that each polypeptide contains at least 1 unique epitope, and that the two polypeptides share at least 1 epitope.

[0894] IV.D.4. Specificity of the Solid Phase RIA for NANBH The specificity of the solid phase RIAs for

[0895] NANBH was tested by using the assay on serum from patients infected with HAV or with HBV and on sera

from control individuals. The assays utilizing partially purified SOD-NANB$_{5-1-1}$ and SOD-NANB$_{81}$ were conducted essentially as described in Section IV.D.3, except that the sera was from patients previously diagnosed as having HAV or HBV, or from individuals who were blood bank donors. The results for sera from HAV and HBV infected patients are presented in table 1. The RIA was tested using 11 serum specimens from HAV infected patients, and 20 serum specimens from HBV infected patients. As shown in table 1, none of these sera yielded a positive immunological reaction with the fusion polypeptides containing BB-NANBV epitopes.

[0896] The RIA using the NANB$_{5-1-1}$ antigen was used to determine immunological reactivity of serum from control individuals. Out of 230 serum samples obtained from the normal blood donor population, only 2 yielded positive reactions in the RIA (data not shown). It is possible that the two blood donors from whom these serum samples originated had previously been exposed to HCV.

[0897] IV.D.5. Reactivity of NANB$_{5-1-1}$ During the Course of NANBH Infection.

[0898] The presence of anti-NANB$_{5-1-1}$ antibodies during the course of NANBH infection of 2 patients and 4 chimpanzees was followed using RIA as described in Section IV.D.3. In addition the RIA was used to determine the presence or absence of anti-NANB$_{5-1-1}$ antibodies during the course of infection of HAV and HBV in infected chimpanzees.

[0899] The results, which are presented in Table 2, show that with chimpanzees and with humans, anti-NANB$_{5-1-1}$ antibodies were detected following the onset of the acute phase of NANBH infection. Anti-NANB$_{5-1-1}$ antibodies were not detected in serum samples from chimpanzees infected with either HAV or HBV. Thus anti-NANB$_{5-1-1}$ antibodies serve as a marker for an individual's exposure to HCV.

TABLE 2

Seroconversion In Sequential Serum Samples from
Hepatitis patients and Chimpanzees Using 5-5-5 Antigen

Patient/ Chimp	Sample Date (Days) (o = inoculation day)	Hepatitis Viruses	Anti-5-1-1 (S/N)	ALT (mu/ml)
Patient 29	T^a	NANB	1.09	1180
	T + 180		33.89	425
	T + 208		36.22	—
Patient 30	T	NANB	1.90	1830
	T + 307		34.17	290
	T + 799		32.45	276
Chimp 1	0	NANB	0.87	9
	76		0.93	71
	118		23.67	19
	154		32.41	—
Chimp 2	0	NANB	1.00	5
	21		1.08	52
	73		4.64	13
	138		23.01	—
Chimp 3	0	NANB	1.08	8
	43		1.44	205
	53		1.82	14
	159		11.87	6
Chimp 4	−3	NANB	1.12	11
	55		1.25	132
	83		6.60	—
	140		17.51	—

TABLE 2-continued

Seroconversion In Sequential Serum Samples from
Hepatitis patients and Chimpanzees Using 5-5-5 Antigen

Patient/ Chimp	Sample Date (Days) (o = inoculation day)	Hepatitis Viruses	Anti-5-1-1 (S/N)	ALT (mu/ml)
Chimp 5	0	HAV	1.50	4
	25		2.39	147
	40		1.92	18
	268		1.53	5
Chimp 6	−8	HAV	0.85	—
	15		—	106
	41		0.81	10
	129	1.33		—
Chimp 7	0	HAV	1.17	7
	22		1.60	83
	115		1.55	5
	139		1.60	—
Chimp 8	0	HAV	0.77	15
	26		1.98	130
	74		1.77	8
	205		1.27	5
Chimp 9	−290	HBV	1.74	—
	379		3.29	9
	435		2.77	6
Chimp 10	0	HBV	2.35	8
	111–118 (pool)		2.74	96–155 (pool)
	205		2.05	9
	240		1.78	13
Chimp 11	0	HBV	1.82	11
	28–56 (pool)		1.26	8–100 (pool)
	169		—	9
	223		0.52	10

^{a}T = day of initial sampling

[0900] IV.E. Purification of Polyclonal Serum Antibodies to NANB$_{5-1-1}$

[0901] On the basis of the specific immunological reactivity of the SOD-NANB$_{5-1-1}$ polypeptide with the antibodies in serum samples from patients with NANBH, a method was developed to purify serum antibodies which react immunologically with the epitope(s) in NANB$_{5-1-1}$. This method utilizes affinity chromatography. Purified SOD-NANB$_{5-1-1}$ polypeptide (see Section IV.D.1) was attached to an insoluble support; the attachment is such that the immobilized polypeptide retains its affinity for antibody to NANB$_{5-1-1}$. Antibody in serum samples is absorbed to the matrix-bound polypeptide. After washing to remove non-specifically bound materials and unbound materials, the bound antibody is released from the bound SOD-HCV polypeptide by change in pH, and/or by chaotropic reagents, for example, urea.

[0902] Nitrocellulose membranes containing bound SOD-NANB$_{5-1-1}$ were prepared as follows. A nitrocellulose membrane, 2.1 cm Sartorius of 0.2 micron pore size, was washed for 3 minutes three times with BBS. SOD-NANB$_{5-1-1}$ was bound to the membrane by incubation of the purified preparation in BBS at room temperature for 2 hours; alternatively it was incubated at 4° C. overnight. The solution containing unbound antigen was removed, and the filter was washed three times with BBS for three minutes per wash. The remaining active sites on the membrane were blocked with BSA by incubation with a 5 mg/ml BSA solution for 30 minutes. Excess BSA was removed by washing the membrane with 5 times with BBS and 3 times with distilled

water. The membrane containing the viral antigen and BSA was then treated with 0.05 M glycine hydrochloride, pH 2:5, 0.10 M NaCl (GlyHCl) for 15 minutes, followed by 3 three minute washes with PBS.

[0903] Polyclonal anti-NANB$_{5-1-1}$ antibodies were isolated by incubating the membranes containing the fusion polypeptide with serum from an individual with NANBH for 2 hours. After the incubation, the filters were washed 5 times with BBS, and twice with distilled water. Bound antibodies were then eluted from each filter with 5 elutions of GlyHCl, at 3 minutes per elution. The pH of the eluates was adjusted to pH 8.0 by collecting each eluate in a test tube containing 2.0 M Tris HCl, pH 8.0. Recovery of the anti-NANB$_{5-1-1}$ antibody after affinity chromatography is approximately 50%.

[0904] The nitrocellulose membranes containing the bound viral antigen can be used several times without appreciable decrease in binding capacity. To reuse the membranes, after the antibodies have been eluted the membranes are washed with BBS three times for 3 minutes. They are then stored in BBS at 4° C.

[0905] IV.F. The Capture of HCV Particles from Infected Plasma Using Purified Human Polyclonal Anti-HCV Antibodies; Hybridization of the Nucleic Acid in the Captured Particles to HCV cDNA

[0906] IV.F.1. The Capture of HCV Particles from Infected Plasma Using Human Polyclonal Anti-HCV Antibodies

[0907] Protein-nucleic acid complexes present in infectious plasma of a chimpanzee with NANBH were isolated using purified human polyclonal anti-HCV antibodies which were bound to polystyrene beads.

[0908] Polyclonal anti-NANB$_{5-1-1}$ antibodies were purified from serum from a human with NANBH using the SOD-HCV polypeptide encoded in clone 5-1-1. The method for purification was that described in Section IV.E.

[0909] The purified anti-NANB$_{5-1-1}$ antibodies were bound to polystyrene beads (¼" diameter, specular finish, Precision Plastic Ball Co., Chicago, Ill.) by incubating each at room temperature overnight with 1 ml of antibodies (1 microgram/ml in borate buffered saline, pH 8.5). Following the overnight incubation, the beads were washed once with TBST [50 mM Tris HCl, pH 8.0, 150 mM NaCl, 0.05% (v/v) Tween 20], and then with phosphate buffered saline (PBS) containing 10 mg/ml BSA.

[0910] Control beads were prepared in an identical fashion, except that the purified anti-NANB$_{5-1-1}$ antibodies were replaced with total human immunoglobulin.

[0911] Capture of HCV from NANBH infected chimpanzee plasma using the anti-NANB$_{5-1-1}$ antibodies bound to beads was accomplished as follows. The plasma from a chimpanzee with NANBH used is described in Section IV.A.1. An aliquot (1 ml) of the NANBV infected chimpanzee plasma was incubated for 3 hours at 37° C. with each of 5 beads coated with either anti-NANB$_{5-1-1}$ antibodies, or with control immunoglobulins. The beads were washed 3 times with TBST.

[0912] IV.F.2. Hybridization of the Nucleic Acid in the Captured. Particles to NANBV-cDNA

[0913] The nucleic acid component released from the particles captured with anti-NANB antibodies was analyzed for hybridization to HCV cDNA derived from clone 81.

[0914] HCV particles were captured from NANBH infected chimpanzee plasma, as described in IV.F.1. To release the nucleic acids from the particles, the washed beads were incubated for 60 min. at 37° C. with 0.2 ml per bead of a solution containing proteinase k (1 mg/ml), 10 mM Tris HCl, pH 7.5, 10 mM EDTA, 0.25% (w/v) SDS, 10 micrograms/ml soluble yeast RNA, and the supernatant solution was removed. The supernatant was extracted with phenol and chloroform, and the nucleic acids precipitated with ethanol overnight at −20° C. The nucleic acid precipitate was collected by centrifugation, dried, and dissolved in 50 mM Hepes, pH 7.5. Duplicate aliquots of the soluble nucleic acids from the samples obtained from beads coated with anti-NANB$_{5-1-1}$ antibodies and with control beads containing total human immunoglobulin were filtered onto to nitrocellulose filters. The filters were hybridized with a ^{32}P-labeled, nick-translated probe made from the purified HCV cDNA fragment in clone 81. The methods for preparing the probe and for the hybridization are described in Section IV.C.1.

[0915] Autoradiographs of a probed filter containing the nucleic acids from particles captured by beads containing anti-NANB$_{5-1-1}$ antibodies are shown in FIG. 40. The extract obtained using the anti-NANB$_{5-1-1}$ antibody (A$_1$,A$_2$) gave clear hybridization signals relative to the control antibody extract (A$_3$,A$_4$) and to control yeast RNA (B$_1$,B$_2$). Standards consisting of 1 pg, 5 pg, and 10 pg of the purified, clone 81 cDNA fragment are shown in C1-3, respectively.

[0916] These results demonstrate that the particles captured from NANBH plasma by anti-NANB$_{5-1-1}$-antibodies contain nucleic acids which hybridize with HCV cDNA in clone 81, and thus provide further evidence that the cDNAs in these clones are derived from the etiologic agent for NANBH.

[0917] IV.G. Immunological Reactivity of C100-3 with Purified Anti-NANB$_{5-1-1}$ Antibodies

[0918] The immunological reactivity of C100-3 fusion polypeptide with anti-NANB$_{5-1-1}$ antibodies was determined by a radioimmunoassay, in which the antigens which were bound to a solid phase were challenged with purified anti-NANB$_{5-1-1}$ antibodies, and the antigen-antibody complex detected with ^{125}I-labeled sheep anti-human antibodies. The immunological reactivity of C100-3 polypeptide was compared with that of SOD-NANB$_{5-1-1}$ antigen.

[0919] The fusion polypeptide C100-3 was synthesized and purified as described in Section IV.B.5. and in Section IV.B.6., respectively. The fusion polypeptide SOD-NANB$_{5-1-1}$ was synthesized and purified as described in Section IV.B.1. and in Section IV.D.1., respectively. Purified anti-NANB$_{5-1-1}$ antibodies were obtained as described in Section IV.E.

[0920] One hundred microliter aliquots containing varying amounts of purified C100-3 antigen in 0.125M Na borate buffer, pH 8.3, 0.075M NaCl (BBS) was added to each well of a microtiter plate (Dynatech Immulon 2 Removawell

Strips). The plate was incubated at 4° C. overnight in a humid chamber, after which, the protein solution was removed and the wells washed 3 times with BBS containing 0.02% Triton X-100 (BBST). To prevent non-specific binding, the wells were coated with BSA by addition of 100 microliters of a 5 mg/ml solution of BSA in BBS followed by incubation at room temperature for 1 hour, after which the excess BSA solution was removed. The polypeptides in the coated wells were reacted with purified anti-NANB$_{5-1-1}$ antibodies by adding 1 microgram antibody/well, and incubating the samples for 1 hr at 37° C. After incubation, the excess solution was removed by aspiration, and the wells were washed 5 times with BBST. Anti-NANB$_{5-1-1}$ bound to the fusion polypeptides was determined by the binding of ^{125}I-labeled F'(ab)$_2$ sheep anti-human IgG to the coated wells. Aliquots of 100 microliters of the labeled probe (specific activity 5-20 microcuries/microgram) were added to each well, and the plates were incubated at 37° C. for 1 hour, followed by removal of excess probe by aspiration, and 5 washes with BBST. The amount of radioactivity bound in each well was determined by counting in a counter which detects gamma radiation.

[0921] The results of the immunological reactivity of C100 with purified anti-NANB$_{5-1-1}$ as compared to that of NANB$_{5-1-1}$ with the purified antibodies are shown in Table 3.

TABLE 3

Immunological Reactivity of C100-3 compared to NANB$_{5-1-1}$ by Radioimmunoassay

	RIA (cpm/assay)					
AG(ng)	400	320	240	160	60	0
NANB$_{5-1-1}$	7332	6732	4954	4050	3051	57
C100-3	7450	6985	5920	5593	4096	67

[0922] The results in Table 3 show that anti-NANB$_{5-1-1}$ recognizes an epitope(s) in the C100 moiety of the C100-3 polypeptide. Thus NANB$_{5-1-1}$ and C100 share a common epitope(s). The results suggest that the cDNA sequence encoding this NANBV epitope(s) is one which is present in both clone 5-1-1 and in clone 81.

[0923] IV.H. Characterization of HCV

[0924] IV.H.1. Characterization of the Strandedness of the HCV Genome.

[0925] The HCV genome was characterized with respect to its strandedness by isolating the nucleic acid fraction from particles captured on anti-NANB$_{5-1-1}$ antibody coated polystyrene beads, and determining whether the isolated nucleic acid hybridized with plus and/or minus strands of HCV cDNA.

[0926] Particles were captured from HCV infected chimpanzee plasma using polystyrene beads coated with immunopurified anti-NANB$_{5-1-1}$ antibody as described in Section IV.F.1. The nucleic acid component of the particles was released using the method described in Section IV.F.2. Aliquots of the isolated genomic nucleic acid equivalent to 3 mls of high titer plasma were blotted onto nitrocellulose filters. As controls, aliquots of denatured HCV cDNA from clone 81 (2 picograms) was also blotted onto the same filters. The filters were probed with ^{32}P-labeled mixture of

plus or mixture of minus strands of single stranded DNA cloned from HCV cDNAs; the cDNAs were excised from clones 40b, 81, and 25c.

[0927] The single stranded probes were obtained by excising the HCV cDNAs from clones 81, 40b, and 25c with EcoRI, and cloning the cDNA fragments in M13 vectors, mp18 and mp19 [Messing (1983)]. The M13 clones were sequenced to determine whether they contained the plus or minus strands of DNA derived from the HCV cDNAs. Sequencing was by the dideoxychain termination method of Sanger et al. (1977).

[0928] Each of a set of duplicate filters containing aliquots of the HCV genome isolated from the captured particles was hybridized with either plus or minus strand probes derived from the HCV cDNAs. FIG. 41 shows the autoradiographs obtained from probing the NANBV genome with the mixture of probes derived from clones 81, 40b, and 25c. This mixture was used to increase the sensitivity of the hybridization assay. The samples in panel I were hybridized with the plus strand probe mixture. The samples in panel II were probed by hybridization with the minus strand probe mixture. The composition of the samples in the panels of the immunoblot are presented in Table 4.

TABLE 4

lane	A	B
1	HCV genome	*
2	—	*
3	*	cDNA 81
4	—	cDNA 81

*is an undescribed sample.

[0929] As seen from the results in FIG. 41, only the minus strand DNA probe hybridizes with the isolated HCV genome. This result, in combination with the result showing that the genome is sensitive to RNase and not DNase (see Section IV.C.2.), suggests that the genome of NANBV is positive stranded RNA.

[0930] These data, and data from other laboratories concerning the physicochemical properties of a putative NANBV(s), are consistent with the possibility that HCV is Flavi-like.

[0931] IV.H.2. Detection of Sequences in Captured Particles Which When Amplified by PCR Hybridize to HCV cDNA Derived from Clone 81

[0932] The RNA in captured particles was obtained as described in Section IV.H.1. The analysis for sequences which hybridize to the HCV cDNA derived from clone 81 was carried out utilizing the PCR amplification procedure, as described in Section IV.C.3, except that the hybridization probe was a kinased oligonucleotide derived from the clone 81 cDNA sequence. The results showed that the amplified sequences hybridized with the clone 81 derived HCV cDNA probe.

[0933] IV.H.3. Homology Between the Non-Structural Protein of Dengue Flavivirus (MNWWVD1) and the HCV Polypeptides

[0934] Encoded by the Combined ORF of Clones 14i Through 39c The combined HCV cDNAs of clones 14i

through 39c contain one continuous ORF, as shown in **FIG. 26**. The polypeptide encoded therein was analyzed for sequence homology with the region of the non-structural polypeptide(s) in Dengue flavivirus (MNWVD1). The analysis used the Dayhoff protein data base, and was performed on a computer. The results are shown in **FIG. 42**, where the symbol (:) indicates an exact homology, and the symbol (.) indicates a conservative replacement in the sequence; the dashes indicate spaces inserted into the sequence to achieve the greatest homologies. As seen from the figure, there is significant homology between the sequence encoded in the HCV cDNA, and the non-structural polypeptide(s) of Dengue virus. In addition to the homology shown in **FIG. 42**, analysis of the polypeptide segment encoded in a region towards the 3'-end of the cDNA also contained sequences which are homologous to sequences in the Dengue polymerase. Of consequence is the finding that the canonical Gly-Asp-Asp (GDD) sequence thought to be essential for RNA-dependent RNA polymerases is contained in the polypeptide encoded in HCV cDNA, in a location which is consistent with that in Dengue 2 virus. (Data not shown.)

[0935] IV.H.4. HCV-DNA is Not Detectable in NANBH Infected Tissue

[0936] Two types of studies provide results suggesting that HCV-DNA is not detectable in tissue from an individual with NANBH. These results, in conjunction with those described in IV.C. and IV.H.1. and IV.H.2. provide evidence that HCV is not a DNA containing virus, and that its replication does not involve cDNA.

[0937] IV.H.4.a. Southern Blotting Procedure

[0938] In order to determine whether NANBH infected chimpanzee liver contains detectable HCV-DNA (or HCV-cDNA), restriction enzyme fragments of DNA isolated from this source was Southern blotted, and the blots probed with [32]P-labeled HCV cDNA. The results showed that the labeled HCV cDNA did not hybridize to the blotted DNA from the infected chimpanzee liver. It also did not hybridize to control blotted DNA from normal chimpanzee liver. In contrast, in a positive control, a labeled probe of the beta-interferon gene hybridized strongly to Southern blots of restriction enzyme digested human placental DNA. These systems were designed to detect a single copy of the gene which was to be detected with the labeled probe.

[0939] DNAs were isolated from the livers of two chimpanzees with NANBH. Control DNAs were isolated from uninfected chimpanzee liver, and from human placentas. The procedure for extracting DNA was essentially according to Maniatis et al. (1982), and the DNA samples were treated with RNAse during the isolation procedure.

[0940] Each DNA sample was treated with either EcoRI, MboI, or HincII (12 micrograms), according to the manufacturer's directions. The digested DNAs were electrophoresed on 1% neutral agarose gels, Southern blotted onto nitrocellulose, and the blotted material hybridized with the appropriate nick-translated probe cDNA (3×10^6 cpm/ml of hybridization mix). The DNA from infected chimpanzee liver and normal liver were hybridized with [32]P-labeled HCV cDNA from clones 36 plus 81; the DNA from human placenta was hybridized with [32]P-labeled DNA from the beta-interferon gene. After hybridization, the blots were washed under stringent conditions, i.e., with a solution containing 0.1×SSC, 0.1% SDS, at 65° C.

[0941] The beta-interferon gene DNA was prepared as described by Houghton et al (1981).

[0942] IV.H.4.b. Amplification by the PCR Technique

[0943] In order to determine whether HCV-DNA could be detected in liver from chimpanzees with NANBH, DNA was isolated from the tissue, and subjected to the PCR amplification-detection technique using primers and probe polynucleotides derived from HCV cDNA from clone 81. Negative controls were DNA samples isolated from uninfected HepG2 tissue culture cells, and from presumably uninfected human placenta. Positive controls were samples of the negative control DNAs to which a known relatively small amount (250 molecules) of the HCV cDNA insert from clone 81 was added.

[0944] In addition, to confirm that RNA fractions isolated from the same livers of chimpanzees with NANBH contained sequences complementary to the HCV-cDNA probe, the PCR amplification-detection system was also used on the isolated RNA samples.

[0945] In the studies, the DNAs were isolated by the procedure described in Section IV.H.4.a, and RNAs were extracted essentially as described by Chirgwin et al. (1981).

[0946] Samples of DNA were isolated from 2 infected chimpanzee livers, from uninfected HepG2 cells, and from human placenta. One microgram of each DNA was digested with HindIII according to the manufacturer's directions. The digested samples were subjected to PCR amplification and detection for amplified HCV cDNA essentially as described in Section IV.C.3., except that the reverse transcriptase step was omitted. The PCR primers and probe were from HCV cDNA clone 81, and are described in Section IV.C.3.. Prior to the amplification, for positive controls, a one microgram sample of each DNA was "spiked" by the addition of 250 molecules of HCV cDNA insert isolated from clone 81.

[0947] In order to determine whether HCV sequences were present in RNA isolated from the livers of chimpanzees with NANBH, samples containing 0.4 micrograms of total RNA were subjected to the amplification procedure essentially as described in Section IV.C.3., except that the reverse transcriptase was omitted from some of the samples as a negative control. The PCR primers and probe were from HCV cDNA clone 81, as described supra.

[0948] The results showed that amplified sequences complementary to the HCV cDNA probe were not detectable in the DNAs from infected chimpanzee liver, nor were they detectable in the negative controls. In contrast, when the samples, including the DNA from infected chimpanzee liver, was spiked with the HCV cDNA prior to amplification, the clone 81 sequences were detected in all positive control samples. In addition, in the RNA studies, amplified HCV cDNA clone 81 sequences were detected only when reverse transcriptase was used, suggesting strongly that the results were not due to a DNA contamination.

[0949] These results show that hepatocytes from chimpanzees with NANBH contain no, or undetectable levels, of HCV DNA. Based upon the spiking study, if HCV DNA is present, it is at a level far below 0.06 copies per hepatocyte.

In contrast, the HCV sequences in total RNA from the same liver samples was readily detected with the PCR technique.

[0950] IV.H.5 Comparison of the Hydrophobic Profiles of HCV Polyproteins with West Nile Virus Polyprotein and with Dengue Virus NS1

[0951] The hydrophobicity profile of an HCV polyprotein segment was compared with that of a typical flavivirus, West Nile virus. The polypeptide sequence of the West Nile virus polyprotein was deduced from the known polynucleotide sequences encoding the non-structural proteins of that virus. The HCV polyprotein sequence was deduced from the sequence of overlapping cDNA clones. The profiles were determined using an antigen program which uses a window of 7 amino acid width (the amino acid in question, and 3 residues on each side) to report the average hydrophobicity about a given amino acid residue. The parameters giving the reactive hydrophobicity for each amino acid residue are from Kyte and Doolittle (1982). **FIG. 55** shows the hydrophobic profiles of the two polyproteins; the areas corresponding to the non-structural proteins of West Nile virus, ns1 through ns5, are indicated in the figure. As seen in the figure, there is a general similarity in the profiles of the HCV polyprotein and the West Nile virus polyprotein.

[0952] The sequence of the amino acids encoded in the 5'-region of HCV cDNA shown in **FIG. 47** has been compared with the corresponding region of one of the strains of Dengue virus, described supra., with respect to the profile of regions of hydrophobicity and hydrophilicity (data not shown). This comparison indicated that the polypeptides from HCV and Dengue encoded in this region, which corresponds to the region encoding NS1 (or a portion thereof), have a similar hydrophobic/hydrophilic profile.

[0953] The similarity in hydrophobicity profiles, in combination with the previously identified homologies in the amino acid sequences of HCV and Dengue Flavivirus in Section IV.H.3., suggests that HCV is related to these members of the Flavivirus family.

[0954] IV.H.6. Characterization of the Putative Polypeptides Encoded Within the HCV ORF

[0955] The sequence of the HCV cDNA sense strand, shown in **FIG. 62**, was deduced from the overlapping HCV cDNAs in the various clones described in Section IV.A. It may be deduced from the sequence that the HCV genome contains primarily one long continuous ORF, which encodes a polyprotein. The amino acid sequence of the putative HCV polyprotein deduced from the HCV cDNA sense strand sequence is shown in **FIG. 66**, where position 1 begins with the putative initiator methionine, and the amino acids are indicated by the one-letter code. In the figure, the numbers of the amino acids are at the right of the sequence. The letters above the sequence indicate heterogeneities which have been detected by sequencing a number of clones which overlap the same region; the letters in parentheses indicates that the heterogeneity is possibly due to 5' or 3' terminal cloning artifacts.

[0956] IV.H.6.a. The Hydrophilic and Antigenic Profile of the Polypeptide

[0957] Profiles of the hydrophilicity/hydrophobicity and the antigenic index of the putative polyprotein encoded in the HCV cDNA sequence shown in **FIG. 89** were deter-

mined by computer analysis. The program for hydrophilicity/hydrophobicity was as described in Section IV.H.5. The antigenic index results from a computer program which relies on the following criteria: 1)surface probability, 2) prediction of alpha-helicity by two different methods; 3) prediction of beta-sheet regions by two different methods; 4) prediction of U-turns by two different methods; 5) hydrophilicity/hydrophobicity; and flexibility. The traces of the profiles generated by the computer analyses are shown in **FIG. 67**. In the hydrophilicity profile, deflection above the abscissa indicates hydrophilicity, and below the abscissa indicates hydrophobicity. The probability that a polypeptide region is antigenic is usually considered to increase when there is a deflection upward from the abscissa in the hydrophilic and/or antigenic profile. It should be noted, however, that these profiles are not necessarily indicators of the strength of the immunogenicity of a polypeptide.

[0958] IV.H.6.c. Identification of Co-linear Peptides in HCV and Flaviviruses

[0959] The amino acid sequence of the putative polyprotein encoded in the HCV cDNA sense strand was compared with the known amino acid sequences of several members of Flaviviruses. The comparison shows that homology is slight, but due to the regions in which it is found, it is probably significant. The conserved colinear regions are shown in **FIG. 68**. The amino acid numbers listed below the sequences represent the number in the putative HCV polyprotein (see **FIG. 66**.)

[0960] The spacing of these conserved motifs is similar between the Flaviviruses and HCV, and implies that there is some similarity between HCV and these flaviviral agents.

[0961] IV.H.7. Sequence Variations in HCV Isolates from Different Individuals

[0962] Isolates of HCV which contain sequences which deviate from CDC/HCV1 were identified in human individuals, some of whom were serologically positive for anti-C100-3 antibodies (EC10 was antibody negative). Identification of these new isolates was accomplished by cloning and sequencing segments of the HCV genome which had been amplified by the PCR technique using CDC/HCl sequences. Amplification was accomplished essentially based on an HCV/cPCR method described in U.S. Ser. No. 398,667 filed Aug. 25, 1989, which is commonly owned by the herein assignee, and which is hereby incorporated herein by reference. The method utilizes primers and probes based upon the HCV cDNA sequences described herein. The first step in the method is the synthesis of a cDNA to either the HCV genome, or its replicative intermediate, using reverse transcriptase. After synthesis of the HCV cDNA, and prior to amplification, the RNA in the sample is degraded by techniques known in the art. A designated segment of the HCV cDNA is then amplified by the use of the appropriate primers. The amplified sequences are cloned, and clones containing the amplified sequences are detected by a probe which is complementary to a sequence lying between the primers, but which does not overlap the primers.

[0963] IV.H.7.a. HCV Isolates Isolated from Humans in the U.S.

[0964] Blood samples which were used as a source of HCV virions were obtained from the American Red Cross in Charlotte, N.C., and from the Community Blood Center of

Kansas, Kansas City, Mo. The samples were screened for antibodies to the HCV C100-3 antigen using an ELISA assay as described in Section IV.I.1, and subjected to supplemental Western blot analysis using a polyclonal goat anti-human HRP to measure anti-HCV antibodies. Two samples, #23 and #27, from the American Red Cross and from the Community Blood Center of Kansas, respectively, were determined to be HCV positive by these assays.

[0965] Viral particles present in the serum of these samples were isolated by ultracentrifugation under the conditions described by Bradley et al. (1985). RNA was extracted from the particles by digestion with proteinase K and SDS at final concentrations of 10 micrograms/ml proteinase K, and 0.1% SDS; digestion was for 1 hour at 37° C. Viral RNA was further purified by extraction with chloroform-phenol, as described in Section IV.A.1.

[0966] HCV RNA in the preparation of RNA was reverse transcribed into cDNA essentially as described in Section IV.A.1., except that the oligonucleotide JHC 7, which corresponds to the cDNA sequence 1958-1939, and which has the following sequence, was used as primer for the reverse transcriptase reaction. using reverse transcriptase. After synthesis of the HCV cDNA, and prior to amplification, the RNA in the sample is degraded by techniques known in the art. A designated segment of the HCV cDNA is then amplified by the use of the appropriate primers. The amplified sequences are cloned, and clones containing the amplified sequences are detected by a probe which is complementary to a sequence lying between the primers, but which does not overlap the primers.

[0967] IV.H.7.a. HCV Isolates Isolated from Humans in the U.S.

[0968] Blood samples which were used as a source of HCV virions were obtained from the American Red Cross in Charlotte, N.C., and from the Community Blood Center of Kansas, Kansas City, Mo. The samples were screened for antibodies to the HCV C100-3 antigen using an ELISA assay as described in Section IV.I.1, and subjected to supplemental western blot analysis using a polyclonal goat anti-human HRP to measure anti-HCV antibodies. Two samples, #23 and #27, from the American Red Cross and from the Community Blood Center of Kansas, respectively, were determined to be HCV positive by these assays.

[0969] Viral particles present in the serum of these samples were isolated by ultracentrifugation under the conditions described by Bradley et al. (1985). RNA was extracted from the particles by digestion with proteinase K and SDS at final concentrations of 10 micrograms/ml proteinase K, and 0.1% SDS; digestion was for 1 hour at 37° C. Viral RNA was further purified by extraction with chloroform-phenol, as described in Section IV.A.1.

[0970] HCV RNA in the preparation of RNA was reverse transcribed into cDNA essentially as described in Section IV.A.1., except that the oligonucleotide JHC 7, which corresponds to the cDNA sequence 1958-1939, and which has the following sequence, was used as primer for the reverse transcriptase reaction.

[0971] JHC 7: CCA GCG GTG GCC TGG TAT TG.

[0972] After both strands of the cDNA were synthesized, the resulting cDNA was then amplified by the PCR method,

essentially as described in Section IV.A.34, except that the oligonucleotide primers used, i.e., JHC 6 and ALX 80, were designed to amplify a 1080 nucleotide segment of the HCV genome from CDC/HCV1 nucleotides 673 to 1751. The primers, in addition, are designed to incorporate a NOT I restriction site at the 3'-end of the PCR product, and a blunt end at the 5'-terminus. The sequences of the primers is:

[0973] ALX 80: TTT GGG TAA GGT CAT CGA TAC CCT TAC GTG; and

[0974] JHC 6: ATA TGC GGC CGC CTT CCG TTG GCA TAA.

[0975] ALX 80 corresponds to nucleotides 673-702 of the CDC/HCV1 sequence; JHC 6 corresponds to nucleotides 1752-1738 of the HCV1 (in addition there are 12 extra nucleotides which encode a NotI site). The designation of nucleotides in JHC 6, i.e., a declining number, indicates the placement in the anti-sense strand.

[0976] After PCR amplification with the above described primers, the blunt end terminus was converted into a NOT I site as follows. A homopolymer tail of 15 dGs was attached to the PCR product using terminal deoxynucleotide transferase, and the products were again subjected to amplification by PCR using as primers JHC 6 and JHC 13. The latter primer, JHC 13, the sequence of which follows, is designed to contain a NOT I site in addition to an SP6 phage promoter. (The SP6 promoter is described in GENETIC ENGINEERING, J. Setlow Ed. (1988).

```
JHC 13:   AAT TCG CGG CCG CCA TAC GAT TTA GGT GAC
          ACT ATA GAA CCC CCC CCC CCC CCC.
```

[0977] In order to clone the amplified HCV cDNA, the PCR products were cleaved with NotI, precipitated with spermine to remove free oligonucleotides (Hoopes et al. (1981)), and cloned into the NotI site of pUC18S (see Section IV.A.34.). The HCV cDNAs in three clones derived from each HCV isolate, were subjected to sequence analysis. Analysis was essentially by the method described in Chen and Seeburg (1985).

[0978] Consensus sequences of the clones derived from HCV in samples 23 and 27 are shown in **FIG. 80** and **FIG. 81**, respectively. The variable sequences are also shown in these figures, as are the amino acids encoded in the consensus sequences.

[0979] **FIGS. 82 and 83** show comparisons of the aligned positive strand nucleotide sequences **(FIG. 82)** and putative amino acid sequences **(FIG. 83)** of samples 23, 27, and HCV1. The amino acid sequence of HCV1 in **FIG. 83** represents amino acid numbers 129-467 of the HCV polyprotein encoded by the large ORF in the HCV genomic RNA. An examination of **FIGS. 82 and 83** show that there are variations in the sequences of the three isolated clones. The sequence variations at the nucleotide level and the amino acid level are summarized in the table immediately below. In the table, the polypeptides designated S and NS1 represent amino acid numbers 130 to ¯380, and 380 to ¯470, respectively. The numbering is from the putative initiator methionine. The terminology S and NS1 is based upon the positioning of the sequences encoding the polypeptides using the Flavivirus model. As discussed above, however,

recent evidence suggests that there is not total correlation between HCV and the Flaviviruses with regard to viral polypeptide domains, particularly in the putative E/NS1 domains. Indeed, HCV polypeptides and their coding domains may exhibit substantial deviation from the Flavivirus model.

TABLE

	Sequence Homology					
	Nucleotide Encoding			Amino Acid Encoded		
	overall %	S %	NS1 %	overall %	S %	NS1 %
HCV1/HCV23	93	95	91	92	95	87
HCV1/HCV27	89	93	84	89	95	82
HCV23/HCV27	89	93	85	90	93	84

[0980] Although there are variations in the newly isolated HCV sequences, the cloned sequences from samples 23 and 27 (called HCV23 and HCV27) each contain 1019 nucleotides, indicating a lack of deletion and addition mutants in this region in the selected clones. The sequences in **FIGS. 82 and 83** also show that the isolated sequences are not rearranged in this region.

[0981] A comparison of the consensus sequences for HCV1 and for the other isolates of HCV is summarized in the Table, supra. The sequence variations between the chimpanzee isolate HCV1, and the HCVs isolated from humans are about the same as that seen between the HCVs of human origin.

[0982] It is of interest that the sequence variations in two of the putative domains is not uniform. The sequence in a putative S region appears to be relatively constant, and randomly scattered throughout the region. In contrast, a putative NS1 region has a higher degree of variability than the overall sequence, and the variation appears to be in a hypervariable pocket of about 28 amino acids which is located about 70 amino acids downstream from the putative N-terminus of the putative polyprotein.

[0983] Although it may be argued that the detected variations were introduced during the amplification process, it is unlikely that all of the variations are from this result. It has been estimated that Taq polymerase introduces errors into a sequence at approximately one base per 10 kilobases of DNA template per cycle (Saiki et al. (1988)). Based upon this estimate, up to 7 errors may have been introduced during the PCR amplification of the 1019 bp DNA fragment. However, the three subclones of HCV-23 and HCV-27 yielded 29 and 14 base variations, respectively. The following suggest that these variations are naturally occurring. About 60% of the base changes are silent mutations which do not change the amino acid sequence. Variations introduced by the Taq polymerase during PCR amplification would be expected to occur randomly; however, the results show that the variant sequences are clustered in at least one specific region. Moreover, a consensus sequence was derived by sequencing multiple different clones derived from the PCR amplified products.

[0984] IV.H.7.b. HCV Isolates from Humans in Italy and in the U.S.

[0985] Segments of HCV RNA present in different isolates were amplified by the HCV/cPCR method. These segments span a region of ˜0.6 Kb to ˜1.6 Kb downstream from the methionine encoding start codon of the putative HCV polyprotein. The isolates are from biological specimens obtained from HCV infected individuals. More specifically, isolate HCT #18 is from human plasma from an individual in the U.S.A., EC1 and EC10 are from a liver biopsy of an Italian patient, and Th is from a peripheral blood mononucleocyte fraction of an American patient. Comparable segments of HCV RNA have been isolated from a chimpanzee.

[0986] RNA was extracted from the human plasma specimens using phenol:CHCl3:isoamyl alcohol extraction. Either 0.1 ml or 0.01 ml of plasma was diluted to a final volume of 1.0 ml, with a TENB/proteinase K/SDS solution (0.05 M Tris-HCL, pH 8.0, 0.001 M EDTA, 0.1 M NaCl, 1 mg/ml Proteinase K, and 0.5% SDS) containing 10 to 40 micrograms/ml polyadenylic acid, and incubated at 37° C. for 60 minutes. After this proteinase K digestion, the resultant plasma fractions were deproteinized by extraction with TE (50 mM Tris-HCl, pH 8.0, 1 mM EDTA) saturated phenol, pH 6.5. The phenol phase was separated by centrifugation, and was reextracted with TENB containing 0.1% SDS. The resulting aqueous phases from each extraction were pooled, and extracted twice with an equal volume of phenol/chloroform/isoamyl alcohol [1:1(99:1)], and then twice with an equal volume of a 99:1 mixture of chloroform/isoamyl alcohol. Following phase separation by. centrifugation, the aqueous phase was brought to a final concentration of 0.2 M Na Acetate, and the nucleic acids were precipitated by the addition of two volumes of ethanol. The precipitated nucleic acids were recovered by ultracentrifugation in a SW 41 rotor at 38 K, for 60 minutes at 4° C., or in a microfuge for 10 minutes at 10K, 4° C.

[0987] RNA extracted from the liver biopsy was provided by Dr. F. Bonino, Ospedale Maggiore di S. Giovanni Battista, Torino, Italy.

[0988] The mononucleocyte fraction was obtained by sedimentation of the individual's aliquot of blood through Ficoll-Paque® (Pharmacia Corp), using the manufacturer's directions. Total RNA was extracted from the fraction using the guanidinium thiocyanate procedure described in Section IV.C.1 (see also Section IV.C.1; See also Choo et al (1989)).

[0989] Synthesis of HCV cDNA from the samples was accomplished using reverse transcriptase, and primers derived from clone 156e and from clone K91. These primers, which are anti-sense relative to the genomic RNA, have the following sequences.

```
156e16B:    5' CGA CAA GAA AGA CAG A 3',
            and
K91/1EB     5' CGT TGG ZAT AAC TGA T 3'.
```

[0990] Following ethanol precipitation, the precipitated RNA or nucleic acid fraction was dried, and resuspended in DEPC treated distilled water. Secondary structures in the nucleic acids were disrupted by heating at 65° C. for 10 minutes, and the samples were immediately cooled on ice.

cDNA was synthesized using-1 to 3 micrograms of total RNA from liver, or from nucleic acids (or RNA) extracted from 10 to 100 microliters of plasma. The synthesis utilized reverse transcriptase, and was in a 25 microliter reaction, using the protocol specified by the manufacturer, BRL. All reaction mixtures for cDNA synthesis contained 23 units of the RNAase inhibitor, RNASIN™ (Fisher/Promega). Following cDNA synthesis, the reaction mixtures were diluted with water, boiled for 10 minutes, and quickly chilled on ice.

[0991] Each set of samples was subjected to two rounds of PCR amplification. The primers for the reactions were selected to amplify regions designated "EnvL" and "EnvR". The "EnvL" region encompasses nucleotides 669-1243, and putative amino acids 117 to 308; the "EnvR" region encompasses nucleotides 1215-1629, and encodes putative amino acids 300-408 (the putative amino acids are numbered starting from the putative methionine initiation codon). The relationship of these regions relative to the putative polyprotein encoded in the HCV cDNA, and to the polypeptides encoded in the Flavirus model is shown in **FIG. 84**.

[0992] The primers for the first round of PCR reactions were derived from the HCV cDNA sequences in either clone ag30a, clone 156e, or clone k9-1. The primers used for the amplification of the EnvL region were 156e16B (shown supra), and ag30a16A for the sense strand; the amplification of the EnvR region utilized the primer K91/16B (shown supra), and 156e16a for the sense strand. The sequences of the sense strand primers are the following.

[0993] For EnvL, ag30a16A: 5'CTC TAT GGC AAT GAG G 3', and

[0994] For EnvR, 156e16A: 5'-AGC TTC GAC GTC ACA T 3'

[0995] The PCR reactions were performed essentially according to the manufacturer's directions (Cetus-Perkin-Elmer), except for the addition of 1 microgram of RNase A. The reactions were carried out in a final volume of 100 microliters. The PCR was performed for 30 cycles, utilizing a regimen of 94° C. (1 min), 37° C. (2 min), and 72° C. (3 min), with a 7 minute extension at 72° C. for the last cycle. The samples were then extracted with phenol:CHCl₃, 3 ethanol precipitated two times, resuspended in 10 mM Tris HCl, pH 8.0, and concentrated using Centricon-30 (Amicon) filtration. This procedure efficiently removes oligonucleotides less than 30 nucleotides in size; thus, the primers from the first round of PCR amplification are removed.

[0996] The Centricon-30 concentrated samples were then subjected to a second round of PCR amplification using probes designed from clones 202a and 156e for the EnvL region, and from 156e and 59a for the EnvR region. The primers for amplification of the EnvL region have the following sequences. 202aEnv41a:

```
5' CTT GAA TTC GCA ATT TGG GTA
AGG TCA TCG ATA CCC TTA CG 3'
```

[0997] and 156e38B':

```
5' CTT GAA TTC GAT AGA GCA ATT
GCA ACC TTG CGT CGT CC 3'.
```

[0998] The primers for amplification of the EnvR region in RNAs derived from humans have the following sequences.

156e38A':

```
5' CTT GAA TTC GGA CGA CGC AAG
GTT GCA ATT GCT CTA TC 3'
```

[0999] and 59aEnv39C:

```
5' CTT GAA TTC CAG CCG GTG TTG
AGG CTA TCA TTG CAG TTC 3'.
```

[1000] Amplification by PCR was for 35 cycles utilizing a regimen of 94° C. (1 min), 60° C. (1 min), and 72° C. (2 min), with a 7 minute extension at 72° C. for the last cycle. The samples were then extracted with phenol:CHCl₃, precipitated two times, and digested with EcoRI. The PCR reaction products were analyzed by separation of the products by electrophoresis on 6% polyacrylamide gels. DNA of approximately the estimated size of the expected PCR product was electroeluted from the gels, and subcloned into either a pGEM-4 plasmid vector or into lambda gt11. The expected product sizes for the EnvL and EnvR after the first round of amplification are 615 bp and 683 bp, respectively; after the second round of amplification the expected product sizes for EnvL and EnvR are 414 bp and 575 bp, respectively. The plasmids containing the amplified products were used to transform host cells; the pGEM-4 plasmid was used to transform DH5-alpha, and lambda gt11 was used to transform C600 delta-HFL. Clones of the transformed cells which either hybridized to the appropriate HCV probes (described below), or those which had inserts of the correct size were selected. The inserts were then cloned in M13 and sequenced.

[1001] The probes for all of the HCV/cPCR products consisted of ³²P labeled sections of HCV cDNA which had been prepared by PCR amplification of a region of clone 216 (using CA216a16A and 216a16B as primers), and of clone 84 (using CA84a16A and CA84a16B or CA84a16C as primers); ³²P was introduced into the PCR products by nick translation. The probes for the first and second round of EnvL amplification were from clone 216. Those for the first round of EnvR amplification were from 84 (i.e., CA84a16A and CA84a16B), for the second round of EnvL amplification were CA84a16A and CA84a16C. These probes did not overlap the primers used in the HCV/cPCR reactions. The sequence of the primers for the PCR amplification of the probes is in the following table.

TABLE

Primer	Clone	Sequence
CA216a16A	216	5' TGA ACT ATG CAA CAG G 3'
CA216a16B	216	5' GGA GTG TGC AGG ATG G 3'
CA84a16A	84	5' AAG GTT GCA ATT GCT C 3'
CA84a16B	84	5' ACT AAC AGG ACC TTC G 3'
CAB4a16C	84	5' TAA CGG GTC ACC GCA T 3'

[1002] Sequence information on variants in the EnvL region was obtained from 3 clones from HCT #18, 2 clones from TH, 3 clones from EC1, and from the HCV1 clones described in Section IV.A. A comparison of the composite nucleotide sequence of each isolate derived from these clones is shown in **FIG. 85**. In the figure, each sequence is shown 5' to 3' for the sense strand for the EnvL region, and the sequences have been aligned. The vertical lines and

capital letters indicate sequence homology, the absence of a line and an uncapitalized letter indicates a lack of homology. The sequences shown in the lines are as follows: line 1, Thorn; line 2, EC1; line 3, HCT #18; line 4, HCV1.

[1003] Sequence information on variants in the EnvR region was obtained from two clones of EC10, and from the HCV1 clones described in Section IV.A. The two EC10 clones differed by only one nucleotide. A comparison of the nucleotide sequences of EC10(clone 2) and a composite of the HCV1 sequences is shown in **FIG. 86**; each sequence is shown 5' to 3' for the sense strand of the EnvR region, and the sequences have been aligned. The double dots between the sequences indicate sequence homology.

[1004] A comparison of the amino acid sequences encoded in the EnvL (amino acids #117-308) and EnvR region (amino acids #300-438) for each of the isolates is shown in **FIG. 87** and **FIG. 88**, respectively. Included in the Figures are sequences for the isolates JH23 and JH27, described in Section IV.H.7.a. Also indicated are sequences from a Japanese isolate; these sequences were provided by Dr. T. Miyamura, Japan. In the figures, the amino acid sequence for the region is given in its entirety for HCV1, and the non-homologous amino acids in the various isolates are indicated.

[1005] As seen in **FIG. 87**, In the EnvL region there is overall about a 93% homology between HCV1 and the other isolates. HCT18, Th, and EC1 have about a 97% homology with HCV1; JH23 and JH27 have about 96% and about 95% homology, respectively, with HCV1. **FIG. 88** shows that the homologies in the EnvR region are significantly less than in the EnvL region; moreover, one subregion appears to be hypervariable (i.e., from amino acid 383-405). This data is summarized in the Table immediately below.

TABLE

Homology of EnvR Region

Isolate	Percent Homology with HCV1	
	AA330–AA438	AA383–AA405
JH23(U.S.)	83	57
JH27(U.S.)	80	39
Japanese	73	48
EC10 (Italy)	84	48

[1006] IV.H.8. Composite cDNA Sequence of HCV1

[1007] As described supra., in Section IV.A., overlapping clones of HCV cDNA from a lambda gt11 library have been isolated and sequenced. A composite cDNA sequence for HCV1, deduced from overlapping clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, p114a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1),26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, 16jh, 6k, and 131jh is shown in **FIG. 89**. Shown above the sequence are the position of the putative initiator methionine codon, and nucleotides which vary from the sequence, which produce changes in encoded amino acids. These variant nucleotides were detected by the sequencing of overlapping clones, isolated from the same lambda gt11 library, described in Section IV.A.1. Clonal heterogeneities which cause many "silent" mutations were detected also, but are not shown in the Figure.

[1008] The putative sequence of the major HCV polyprotein encoded in the composite of HCV1 cDNA is shown in

FIG. 90. The first amino acid in the sequence is the putative initiator methionine. The variant amino acids, due to the clonal heterogeneities, are indicated above the sequence. Since the lambda gt11 library was created from serum obtained from one individual (see Section IV.A.1.), the results suggest that variant viral sequences (both nucleotide and amino acid) are present in that individual.

[1009] An examination of the composite HCV cDNA sequence in **FIG. 89** shows that besides the large ORF, there are a number or ORFs upstream of that encoding the polyprotein, and within the sequence encoding the polyprotein there are a large number of smaller ORFs in the other two translational frames. The ORFs upstream of the HCV polyprotein are shown in the Table immediately below.

TABLE

ORFs Upstream of that Encoding the Large HCV Polyprotein

Nucl. #	Translation Frame	Amino Acid Sequence
10	1	MNHSPVRNYCLHAESV
63	3	MALV
74	2	MSVVQPPGPPLPGEP
193	1	MPGDLGVPPQDC

[1010] The reading frame, position, and size of the ORFs downstream of the sequence encoding the putative initiator MET of the polyprotein are shown in the Table below. The major polyprotein is that translated from reading frame 2.

TABLE

ORF's Downstream of the Putative Initiator MET Encoding Sequence

Reading Frame	Size(aa)	Position(bp)
1	168	1015
1	105	2662
1	119	5935
2	3025	278
3	160	324
3	111	1986
3	148	7212

[1011] In addition to the above, an examination of the sequence which is complementary to the genomic strand of HCV RNA also contains several small ORFs. One of these ORFs encodes a polypeptide of 385 amino acids.

[1012] IV.I. ELISA Assays Using HCV Polypeptides

[1013] IV.I.1. ELISA Determinations for HCV Infection Using HCV c100-3 as Test Antigen

[1014] All samples were assayed using the HCV c100-3 ELISA. This assay utilizes the HCV c100-3 antigen (which was synthesized and purified as described in Section IV.B.5), and a horseradish peroxidase (HRP) conjugate of mouse monoclonal anti-human IgG.

[1015] Plates coated with the HCV c100-3 antigen were prepared as follows. A solution containing Coating buffer (50 mM Na Borate, pH 9.0), 21 mil/plate, BSA (25 micrograms/ml), c100-3 (2.50 micrograms/ml) was prepared just prior to addition to the Removeawell Immulon I plates (Dynatech Corp.). After mixing for 5 minutes, 0.2 ml/well of the solution was added to the plates, they were covered and

incubated for 2 hours at 37° C., after which the solution was removed by aspiration. The wells were washed once with 400 microliters Wash Buffer (100 mM sodium phosphate, pH 7.4, 140 mM sodium chloride, 0.1% (W/V) casein, 1% (W/V) Triton x-100, 0.01% (W/V) Thimerosal). After removal of the wash solution, 200 microliters/well of Post-coat solution (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 0.1% (w/v) casein, 3% sucrose and 2 mM phenylmethylsulfonylfluoride (PMSF)) was added, the plates were loosely covered to prevent evaporation, and were allowed to stand at room temperature for 30 minutes. The wells were then aspirated to remove the solution, and lyophilized dry overnight, without shelf heating. The pre-pared plates may be stored at 2-8° C. in sealed aluminum pouches with dessicant (3 g Sorb-it packs).

[1016] In order to perform the ELISA determination, 20 microliters of serum sample or control sample was added to a well containing 200 microliters of sample diluent (100 mM sodium phosphate, pH 7.4, 500 mM sodium chloride, 1 mM EDTA, 0.1% (W/V) Casein, 0.01% (W/V) Thimerosal, 1% (W/V) Triton X-100, 100 micrograms/ml yeast extract). The plates were sealed, and incubated at 37° C. for two hours, after which the solution was removed by aspiration, and the wells were washed three times with 400 microliters of wash buffer (phosphate buffered saline (PBS) containing 0.05% Tween 20). The washed wells were treated with 200 micro-liters of mouse anti-human IgG-HRP conjugate contained in a solution of Ortho conjugate diluent (10 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, 50% (V/V) fetal bovine serum, 1% (V/V) heat treated horse serum, 1 mM $K_3Fe(CN)_6$, 0.05% (W/V) Tween 20, 0.02% (W/V) Thimerosal). Treatment was for 1 hour at 37° C., the solution was removed by aspiration, and the wells were washed three times with 400 ml wash buffer, which was also removed by aspiration. To determine the amount of bound enzyme conjugate, 200 microliters of substrate solution (10 mg O-phenylenediamine dihydrochloride per 5 ml of Developer solution) was added. Developer solution contains 50 mM sodium citrate adjusted to pH 5.1 with phosphoric acid, and 0.6 microliters/ml of 30% H_2O_2. The plates containing the substrate solution were incubated in the dark for 30 minutes at room temperature, the reactions were stopped by the addition of 50 microliters/ml 4N sulfuric acid, and the ODs determined.

[1017] The examples provided below show that the micro-titer plate screening ELISA which utilizes HCV c100-3 antigen has a high degree of specificity, as evidenced by an initial rate of reactivity of about 1%, with a repeat reactive rate of about 0.5% on random donors. The assay is capable of detecting an immunoresponse in both the post acute phase of the infection, and during the chronic phase of the disease. In addition, the assay is capable of detecting some samples which score negative in the surrogate tests for NANBH; these samples come from individuals with a history of NANBH, or from donors implicated in NANBH transmission.

[1018] In the examples described below, the following abbreviations are used:

ALT	Alanine amino transferase
Ani-HBc	Antibody against HBc
Anti-HBsAg	Antibody against HBsAg
HBc	Hepatitis B core antigen
ABsAg	Hepatitis B surface antigen

-continued

IgG	Immunoglobulin G
IgM	Immunoglobulin M
Iu/L	International units/Liter
NA	Not available
NT	Not tested
N	Sample size
Neg	Negative
OD	Optical density
Pos	Positive
S/CO	Signal/cutoff
SD	Standard deviation
x	Average or mean
WNL	Within normal limits

[1019] IV.I.1.a. HCV Infection in a Population of Random Blood Donors

[1020] A group of 1,056 samples (fresh sera) from random blood donors were obtained from Irwin Memorial Blood Bank, San Francisco, Calif. The test results obtained with these samples are summarized in a histogram showing the distribution of the OD values (FIG. 43). As seen in FIG. 43, 4 samples read >3, 1 sample reads between 1 and 3, 5 samples read between 0.4 and 1, and the remaining 1,046 samples read <0.4, with over 90% of these samples reading <0.1.

[1021] The results on the reactive random samples are presented in Table 5. Using a cut-off value equal to the mean plus 5 standard deviations, ten samples out of the 1,056 (0.95%) were initially reactive. of these, five samples (0.47%) repeated as reactive when they were assayed a second time using the ELISA. Table 5 also shows the ALT and Anti-HBd status for each of the repeatedly reactive samples. Of particular interest is the fact that all five repeat reactive samples were negative in both surrogate tests for NANBH, while scoring positive in the HCV ELISA.

TABLE 5

Result on Reactive Random Samples
N = 1051
x = 0.049*
SD = ±0.074
Cut-off: x + 5SD = 0.419 (0.400 + Negative Control)

Samples	Initial Reactives OD	Repeat Reactives OD	ALT**	Anti HBc***
4227	0.462	0.084	NA	(OD)
6292	0.569	0.294	NA	NA
6188	0.699	0.326	NA	NA
6157	0.735	0.187	NA	NA
6277	0.883	0.152	NA	NA
6397	1.567	1.392	30.14	1.433
6019	>3.000	>3.000	46.48	1.057
6651	>3.000	>3.000	48.53	1.343
6669	>3.000	>3.000	60.53	1.165
4003	>3.000	>3.000	WNL****	Negative

10/1056 = 0.95%
5/1065 = 0.47%
*Samples reading >1.5 were not included in calculating the Mean and SD
**ALT > 68 IU/L is above normal limits.
***Anti-HBc ≤ 0.535 (competition assay) is considered positive.
****WNL: Within normal limits

[1022] IV.I.1.b. Chimpanzee Serum Samples

[1023] Serum samples from eleven chimpanzees were tested with the HCV c100-3 ELISA. Four of these chimpanzees were infected with NANBH from a contaminated batch of Factor VIII (presumably Hutchinson strain), following an established procedure in a collaboration with Dr. Daniel Bradley at the Centers for Disease Control. As controls, four other chimpanzees were infected with HAV and three with HBV. Serum samples were obtained at different times after infection.

[1024] The results, which are summarized in Table 6, show documented antibody seroconversion in all chimpanzees infected with the Hutchinson strain of NANBH. Following the acute phase of infection (as evidenced by the significant rise and subsequent return to normal of ALT levels), antibodies to HCV c100-3 became detectable in the sera of the 4/4 NANBH infected chimpanzees. These samples had previously been shown, as discussed in Section IV.B.3., to be positive by a Western analysis, and an RIA. In contrast, none of the control chimpanzees which had been infected with HAV or HBV showed evidence of reactivity in the ELISA.

TABLE 6

			Chimpanzee Serum Samples			
	OD	S/CO	Inoculation Date	Bleed Date	ALT (IU/L)	Transfused
Negative Control	0.001					
Positive Control	1.504					
Cutoff	0.401					
Chimp 1	−0.007	0.00	May 24, 1984	May 24, 1984	9	NANB
	0.003	0.01		Aug. 07, 1984	71	
	>3.000	>7.48		Sep. 18, 1984	19	
	>3.000	>7.48		Oct. 24, 1984	—	
Chimp 2	—	—	Jun. 07 1984	—	—	NANB
	−0.003	0.00		May 31, 1984	5	
	−0.005	0.00		Jun. 28, 1984	52	
	0.945	2.36		Aug. 20, 1984	13	
	>3.000	>7.48		Oct. 24, 1984	—	
Chimp 3	0.005	0.01	Mar. 14, 1985	Mar. 14, 1985	8	NANB
	0.017	0.04		Apr. 26, 1985	205	
	0.006	0.01		May 06, 1985	14	
	1.010	2.52		Aug. 20, 1985	6	
Chimp 4	−0.006	0.00	Mar. 11, 1985	Mar. 11, 1985	11	HANB
	0.003	0.01		May 09, 1985	132	
	0.523	1.31		Jun. 06, 1985	—	
	1.574	3.93		Aug. 01. 1985	—	
Chimp 5	−0.006	0.00	Nov. 21, 1980	Nov. 21, 1980	4	HAV
	0.001	0.00		Dec. 16, 1980	147	
	0.003	0.01		Dec. 30, 1980	18	
	0.006	0.01		Jul. 29–Aug. 21, 1981	5	
Chimp 6	—	—	May 25, 1982	—	—	HAV
	−0.005	0.00		May 17, 1982	—	
	0.001	0.00		Jun. 10, 1982	106	
	−0.004	0.00		Jul. 06, 1982	10	
	0.290	0.72		Oct. 01, 1982	—	
Chimp 7	−0.008	0.00	May 25, 1982	May 25, 1982	7	HAV
	−0.004	0.00		Jun. 17, 1982	83	
	−0.006	0.00		Sep. 16, 1982	5	
	0.005	0.01		Oct. 09, 1982	—	
Chimp 8	−0.007	0.00	Nov. 21, 1980	Nov. 21, 1980	15	HAV
	0.000	0.00		Dec. 16, 1980	130	
	0.004	0.01		Feb. 03, 1981	8	
	0.000	0.00		Jun. 03–Jun. 10, 1981	4.5	
Chimp 9	—	—	Jul. 24, 1980	—	—	HBV
	0.019	0.050		Aug. 22–Oct. 10, 1979	—	
	—	—		Mar. 11, 1981	57	
	0.015	0.04		Jul. 01–Aug. 05, 1981	9	
	0.008	0.02		Oct. 01, 1981	6	
Chimp 10	—	—		Apr. 21–May 12, 1982	9	HBV
	0.011	0.03		Apr. 21–May 12, 1982	9	
	0.015	0.04		Sep. 01–Sep. 08, 1982	126	
	0.008	0.02		Dec. 02, 1982	9	
	0.010	0.02		Jan. 06, 1983	13	
Chimp 11	—	—	May 12, 1982	—	—	HBV
	0.000	0.00		Jan. 06–May 12, 1982	11	
	—	—		Jun. 23, 1982	100	
	−0.003	0.00		Jun. 09–Jul. 07, 1982	—	
	−0.003	0.00		Oct. 28, 1982	9	
	−0.003	0.00		Dec. 20, 1982	10	

[1025] IV.I.1.c. Panel 1: Proven Infectious Sera from Chronic Human NANBH Carriers

[1026] A coded panel consisted of 22 unique samples, each one in duplicate, for a total of 44 samples. The samples were from proven infectious sera from chronic NANBH carriers, infectious sera from implicated donors, and infectious sera from acute phase NANBH patients. In addition, the samples were from highly pedigreed negative controls, and other disease controls. This panel was provided by Dr. H. Alter of the Department of Health and Human Services, National Institutes of Health, Bethesda, Md. The panel was constructed by Dr. Alter several years ago, and has been used by Dr. Alter as a qualifying panel for putative NANBH assays.

[1027] The entire panel was assayed twice with the ELISA assay, and the results were sent to Dr. Alter to be scored. The results of the scoring are shown in Table 7. Although the Table reports the results of only one set of duplicates, the same values were obtained for each of the duplicate samples.

[1028] As shown in Table 7, 6 sera which were proven infectious in a chimpanzee model were strongly positive. The seventh infectious serum corresponded to a sample for an acute NANBH case, and was not reactive in this ELISA. A sample from an implicated donor with both normal ALT levels and equivocal results in the chimpanzee studies was non-reactive in the assay. Three other serial samples from one individual with acute NANBH were also non-reactive. All samples coming from the highly pedigreed negative controls, obtained from donors who had at least 10 blood donations without hepatitis implication, were non-reactive in the ELISA. Finally, four of the samples tested had previously scored as positive in putative NANBH assays developed by others, but these assays were not confirmable. These four samples scored negatively with the HCV ELISA.

TABLE 7

H. Alter's Panel 1:

Panel	1st Result	2nd Result
1) Proven Infectious by Chimpanzee Transmission		
A. Chronic NANB: Post-Tx		
JF	+	+
EB	+	+
PG	+	+
B. Implicated Donors with Elevated ALT		
BC	+	+
JJ	+	+
BB	+	+
C. Acute NANB; Post-Tx		
WH	–	–
2) Equivocally Infectious by Chimpanzee Transmission		
A. Implicated Donor with Normal ALT		
CC	–	–
3) Acute NANB; Post-Tx		
JL Week 1	–	–
JL Week 2	–	–
JL Week 3	–	–

TABLE 7-continued

H. Alter's Panel 1:

Panel	1st Result	2nd Result
4) Disease Controls		
A. Primary Biliary Cirrhosis		
EK	–	–
B. Alcoholic Hepatitis in Recovery		
HB	–	–
5) Pedigreed Negative Controls		
DH	–	–
DC	–	–
LV	–	–
ML	–	–
AH	–	–
6) Potential NANB "Antigens"		
JS-80-01T-0 (Ishida)	–	–
Asterix (Trepo)	–	–
Zurtz (Arnold)	–	–
Becassdine (Trepo)	–	–

[1029] IV.I.1.d. Panel 2: Donor/Recipient NANBH

[1030] The coded panel consisted of 10 unequivocal donor-recipient cases of transfusion associated NANBH, with a total of 188 samples. Each case consisted of samples of some or all the donors to the recipient, and of serial samples (drawn 3, 6, and 12 months after transfusion) from the recipient. Also included was a prebleed, drawn from the recipient before transfusion. The coded panel was provided by Dr. H. Alter, from the NIH, and the results were sent to him for scoring.

[1031] The results, which are summarized in Table 8, show that the ELISA detected antibody seroconversion in 9 of 10 cases of transfusion associated NANBH. Samples from case 4 (where no seroconversion was detected), consistently reacted poorly in the ELISA. Two of the 10 recipient samples were reactive at 3 months post transfusion. At six months, 8 recipient samples were reactive; and at twelve months, with the exception of case 4, all samples were reactive. In addition, at least one antibody positive donor was found in 7 out of the 10 cases, with case 10 having two positive donors. Also, in case 10, the recipient's pre-bleed was positive for HCV antibodies. The one month bleed from this recipient dropped to border-line reactive levels, while it was elevated to positive at 4 a 10 month bleeds. Generally, a S/CO of 0.4 is considered positive. Thus, this case may represent a prior infection of the individual with HCV.

[1032] The ALT and HBc status for all the reactive, i.e., positive, samples are summarized in Table 9. As seen in the table, ⅛ donor samples was negative for the surrogate markers and reactive in the HCV antibody ELISA. On the other hard, the recipient samples (followed up to 12 months after transfusion) had either elevated ALT, positive Anti-HBc, or both.

TABLE 8

H. Alter Donor/Recipient NANB Panel

Case	Donor OD	Donor S/CO	Recipient Prebleed OD	Recipient Prebleed S/CO	3 Months OD	3 Months S/CO	Post-TX 6 Months OD	Post-TX 6 Months S/CO	12 Months OD	12 Months S/CO
1.	—	—	.032	0.07	.112	0.26	>3.000	>6.96	>3.000	>6.96
2.	—	—	.059	0.14	.050	0.12	1.681	3.90	>3.000	>6.96
3.	.403	0.94	.049	0.11	.057	0.13	>3.000	>6.96	>3.000	>6.96
4.	—	—	.065	0.15	.073	0.17	.067	0.16	.217	0.50
5.	>3.000	>6.96	.034	0.08	.096	0.22	>3.000	>6.96	>3.000	>6.96
6.	>3.000	>6.96	.056	0.13	1.475	3.44	>3.000	>6.96	>3.000	>6.96
7.	>3.000	>6.96	.034	0.08	.056	0.13	>3.000	>6.96	>3.000	>6.96
8.	>3.000	>6.96	.061	0.14	.078	0.18	2.262	5.28	>3.000	>6.96
9.	>3.000	>6.96	.080	0.19	.127	0.30	.055	0.13	>3.000	>6.96
10.	>3.000 >3.000	>6.96 >6.96	>3.000	>6.96	.317*	0.74	>3.000**	>6.96	>3.000***	>6.96

*1 Month,
**4 Months,
***10 Months

[1033]

TABLE 9

ALT AND HBc STATUS FOR REACTIVE SAMPLES IN
H. ALTER PANEL 1

Samples		Anti-ALT*	HBc**
		Donors	
Case 3		Normal	Negative
Case 5		Elevated	Positive
Case 6		Elevated	Positive
Case 7		Not Available	Negative
Case 8		Normal	Positive
Case 9		Elevated	Not Available
Case 10		Normal	Positive
Case 10		Normal	Positive
		Recipients	
Case 1	6 mo	Elevated	Positive
	12 mo	Elevated	Not tested
Case 2	6 mo	Elevated	Negative
	12 mo	Elevated	Not tested
Case 3	6 mo	Normal	Not tested***
	12 mo	Elevated	Not tested***
Case 5	6 mo	Elevated	Not tested
	12 mo	Elevated	Not tested
Case 6	3 mo	Elevated	Negative
	6 mo	Elevated	Negative
	12 mo	Elevated	Not tested
Case 7	6 mo	Elevated	Negative
	12 mo	Elevated	Negative
Case 8	6 mo	Normal	Positive
	12 mo	Elevated	Not tested
Case 9	12 mo	Elevated	Not tested
Case 10	4 mo	Elevated	Not tested
	10 mo	Elevated	Not tested

*ALT ≧45 IU/L is above normal limits
**Anti-HBc ≦50% (competition assay) is considered positive.
***Prebleed and 3 mo samples were negative for HBc.

[1034] IV.I.1.e. Determination of HCV Infection in High Risk Group Samples

[1035] Samples from high risk groups were monitored using the ELISA to determine reactivity to HCV c100-3

antigen. These samples were obtained from Dr. Gary Tegtmeier, Community Blood Bank, Kansas City. The results are summarized in Table 10.

[1036] As shown in the table, the samples with the highest reactivity are obtained from hemophiliacs (76%). In addition, samples from individuals with elevated ALT and positive for Anti-HBc, scored 51% reactive, a value which is consistent with the value expected from clinical data and NANBH prevalence in this group. The incidence of antibody to HCV was also higher in blood donors with elevated ALT alone, blood donors positive for antibodies to Hepatitis B core alone, and in blood donors rejected for reasons other than high ALT or anti-core antibody when compared to random volunteer donors.

TABLE 10

NANBH HIGH RISK GROUP SAMPLES

Group		N	Distribution N	OD	% Reactive
Elevated ALT		35	3	>3.000	11.4%
	1	0.728			
Anti-HBc		24	5	>3.000	20.8%
Elevated ALT,		33	12	>3.000	51.5%
Anti-HBc	1	2.768			
	1	2.324			
	1	0.939			
	1	0.951			
	1	0.906			
Rejected Donors		25	5	>3.000	20.0%
Donors with History		150	19	>3.000	14.7%
of Hepatitis	1	0.837			
	1	0.714			
	1	0.469			
Haemophiliacs		50	31	>3.000	76.0%
	1	2.568			
	1	2.483			
	1	2.000			
	1	1.979			
	1	1.495			
	1	1.209			
	1	0.819			

[1037] IV.I.1.f.(1) Comparative Studies Using Anti-IgG or Anti-IgM Monoclonal Antibodies, or Polyclonal Antibodies as a Second Antibody in the HCV c100-3 ELISA

[1038] The sensitivity of the ELISA determination which uses the anti-IgG monoclonal conjugate was compared to that obtained by using either an anti-IgM monoclonal conjugate, or by replacing both with a polyclonal antiserum reported to be both heavy and light chain specific. The following studies were performed.

[1039] IV.I.6.a. Serial Samples from Seroconverters

[1040] Serial samples from three cases of NANB seroconverters were studied in the HCV c100-3 ELISA assay using in the enzyme conjugate either the anti-IgG monoclonal alone, or in combination with an anti-IgM monoclonal, or using a polyclonal antiserum. The samples were provided by Dr. Cladd Stevens, N.Y. Blood Center, N.Y.C., N.Y. The sample histories are shown in Table 11.

[1041] The results obtained using an anti-IgG monoclonal antibody-enzyme conjugate are shown in Table 12. The data shows that strong reactivity is initially detected in samples 1-4, 2-8, and 3-5, of cases 1, 2, and 3, respectively.

[1042] The results obtained using a combination of an anti-IgG monoclonal conjugate and an anti-IgM conjugate are shown in Table 13. Three different ratios of anti-IgG to anti-IgM were tested; the 1:10,000 dilution of anti-IgG was constant throughout. Dilutions tested for the anti-IgM monoclonal conjugate were 1:30,000, 1:60,000, and 1:120,000.

The data shows that, in agreement with the studies with anti-IgG alone, initial strong reactivity is detected in samples 1-4, 2-8, and 3-5.

[1043] The results obtained with the ELISA using anti-IgG monoclonal conjugate (1:10,000 dilution), or Tago polyclonal conjugate (1:80,000 dilution), or Jackson polyclonal conjugate (1:80,000 dilution) are shown in Table 14. The data indicates that initial strong reactivity is detected in samples 1-4, 2-8, and 3-5 using all three configurations; the Tago polyclonal antibodies yielded the lowest signals.

[1044] The results presented above show that all three configurations detect reactive samples at the same time after the acute phase of the disease (as evidenced by the ALT elevation). Moreover, the results indicate that the sensitivity of the HCV c100-3 ELISA using anti-IgG monoclonal-enzyme conjugate is equal to or better than that obtained using the other tested configurations for the enzyme conjugate.

TABLE 11

DESCRIPTION OF SAMPLES FROM CLADD STEVENS PANEL

	Date	HBsAg	Anti-HBs	Anti-HBc	ALT	Bilirubin
Case 1						
1-1	Aug. 05, 1981	1.0	91.7	12.9	40.0	−1.0
1-2	Sep. 02, 1981	1.0	121.0	15.1	274.0	1.4
1-3	Oct. 07, 1981	1.0	64.0	23.8	261.0	0.9
1-4	Nov. 19, 1981	1.0	67.3	33.8	75.0	0.9
1-5	Dec. 15, 1981	1.0	50.5	27.6	71.0	1.0
Case 2						
2-1	Oct. 19, 1981	1.0	1.0	116.2	17.0	−1.0
2-2	Nov. 17, 1981	1.0	0.8	89.5	46.0	1.1
2-3	Dec. 02, 1981	1.0	1.2	78.3	63.0	1.4
2-4	Dec. 14, 1981	1.0	0.9	90.6	152.0	1.4
2-5	Dec. 23, 1981	1.0	0.8	93.6	624.0	1.7
2-6	Jan. 20, 1982	1.0	0.8	92.9	66.0	1.5
2-7	Feb. 15, 1982	1.0	0.8	86.7	70.0	1.3
2-8	Mar. 17, 1982	1.0	0.9	69.8	24.0	−1.0
2-9	Apr. 21, 1982	1.0	0.9	67.1	53.0	1.5
2-10	May 19, 1982	1.0	0.5	74.8	95.0	1.6
2-11	Jun. 14, 1982	1.0	0.8	82.9	37.0	−1.0
Case 3						
3-1	Apr. 07, 1981	1.0	1.2	88.4	13.0	−1.0
3-2	May 12, 1981	1.0	1.1	126.2	236.0	0.4
3-3	May 30, 1981	1.0	0.7	99.9	471.0	0.2
3-4	Jun. 09, 1981	1.0	1.2	110.8	315.0	0.4
3-5	Jul. 06, 1981	1.0	1.1	89.9	273.0	0.4
3-6	Aug. 10, 1981	1.0	1.0	118.2	158.0	0.4
3-7	Sep. 08, 1981	1.0	1.0	112.3	84.0	0.3
3-8	Oct. 14, 1981	1.0	0.9	102.5	180.0	0.5
3-9	Nov. 01, 1981	1.0	1.0	84.6	154.0	0.3

[1045]

TABLE 12

ELISA RESULTS OBTAINED USING AN ANTI-IgG MONOCLONAL CONJUGATE

SAMPLE	DATE	ALT	OD	S/CO
Neg Control				.076
Cutoff				.476
PC (1:128)				1.390

TABLE 12-continued

ELISA RESULTS OBTAINED USING AN ANTI-IgG
MONOCLONAL CONJUGATE

SAMPLE	DATE	ALT	OD	S/CO
	Case #1			
1-1	Aug. 5, 1981	40.0	.178	.37
1-2	Sep. 2, 1981	274.0	.154	.32
1-3	Oct. 7, 1981	261.0	.129	.27
1-4	Nov. 19, 1981	75.0	.937	1.97
1-5	Dec. 15, 1981	71.0	>3.000	>6.30
	Case #2			
2-1	Oct. 19, 1981	17.0	.058	0.12
2-2	Nov. 17, 1981	46.0	.050	0.11
2-3	Dec. 2, 1981	63.0	.047	0.10
2-4	Dec. 14, 1981	152.0	.059	0.12
2-5	Dec. 23, 1981	624.0	.070	0.15
2-6	Jan. 20, 1982	66.0	.051	0.11
2-7	Feb. 15, 1982	70.0	.139	0.29
2-8	Mar. 17, 1982	24.0	1.867	3.92

TABLE 12-continued

ELISA RESULTS OBTAINED USING AN ANTI-IgG
MONOCLONAL CONJUGATE

SAMPLE	DATE	ALT	OD	S/CO
2-9	Apr. 21, 1982	53.0	>3.000	>6.30
2-10	May 19, 1982	95.0	>3.000	>6.30
2-11	Jun. 14, 1982	37.0	>3.000	>6.30
	Case #3			
3-1	Apr. 7, 1981	13.0	.090	.19
3-2	May 12, 1981	236.0	.064	.13
3-3	May 30, 1981	471.0	.079	.17
3-4	Jun. 9, 1981	315.0	.211	.44
3-5	Jul. 6, 1981	273.0	1.707	3.59
3-6	Aug. 10, 1981	158.0	>3.000	>6.30
3-7	Sep. 8, 1981	84.0	>3.000	>6.30
3-8	Oct. 14, 1981	180.0	>3.000	>6.30
3-9	Nov. 11, 1981	154.0	>3.000	>6.30

[1046]

TABLE 13

ELISA RESULTS OBTAINED USING ANTI-IgG and ANTI-IgM
MONOCLONAL CONJUGATE

			NANB ELISAs					
			Monoclonals IgG 1:10 K IgM 1:30 K		Monoclonals IgG 1:10 K IgM 1:60 K		Monoclonals IgG 1:10 K IgM 1:120 K	
SAMPLE	DATE	ALT	OD	S/CO	OD	S/CO	OD	S/CO
Neg Control Cutoff			.100		.080		.079	
PC (1:128)			1.083		1.328		1.197	
Case #1								
1-1	Aug. 05, 1981	40	.173		.162		.070	
1-2	Sep. 02, 1981	274	.194		.141		.079	
1-3	Oct. 07, 1981	261	.162		.129		.063	
1-4	Nov. 19, 1981	75	.812		.85		.709	
1-5	Dec. 15, 1981	71	>3.00		>3.00		>3.00	
Case #2								
2-1	Oct. 19, 1981	17	.442		.045		.085	
2-2	Nov. 17, 1981	46	.102		.029		.030	
2-3	Dec. 02, 1981	63	.059		.036		.027	
2-4	Dec. 14, 1981	152	.065		.041		.025	
2-5	Dec. 23, 1981	624	.082		.033		.032	
2-6	Jan. 20, 1982	66	.102		.042		.027	
2-7	Feb. 15, 1982	70	.188		.068		.096	
2-8	Mar. 17, 1982	24	1.728		1.668		1.541	
2-9	Apr. 21, 1982	53	>3.00		2.443		>3.00	
2-10	May 19, 1982	95	>3.00		>3.00		>3.00	
2-11	Jun. 14, 1982	37	>3.00		>3.00		>3.00	

TABLE 13-continued

ELISA RESULTS OBTAINED USING ANTI-IgG and ANTI-IgM
MONOCLONAL CONJUGATE

			NANB ELISAs					
			Monoclonals IgG 1:10 K IgM 1:30 K		Monoclonals IgG 1:10 K IgM 1:60 K		Monoclonals IgG 1:10 K IgM 1:120 K	
SAMPLE	DATE	ALT	OD	S/CO	OD	S/CO	OD	S/CO
Case #3								
3-1	Apr. 07, 1981	13	.193		.076		.049	
3-2	May 12, 1981	236	.201		.051		.038	
3-3	May 30, 1981	471	.132		.067		.052	
3-4	Jun. 09, 1981	315	.175		.155		.140	
3-5	Jul. 06, 1981	273	1.335		1.238		1.260	
3-6	Aug. 10, 1981	158	>3.00		>3.00		>3.00	
3-7	Sep. 08, 1981	84	>3.00		>3.00		>3.00	
3-8	Oct. 14, 1981	180	>3.00		>3.00		>3.00	
3-9	Nov. 11, 1981	154	>3.00		>3.00		>3.00	

[1047]

TABLE 14

ELISA RESULTS OBTAINED USING POLYCLONAL CONJUGATES

			NANB ELISAs					
			Monoclonal 1:10 K		Tago 1:80 K		Jackson 1:80 K	
SAMPLE	DATE	ALT	OD	S/CO	OD	S/CO	OD	S/CO
Neg Control			.076		.045		.154	
Cutoff			.476		.545		.654	
PC (1:128)			1.390		.727		2.154	
Case #1								
1-1	Aug. 05, 1981	40	.178	.37	.067	.12	.153	.23
1-2	Sep. 02, 1981	274	.154	.32	.097	.18	.225	.34
1-3	Oct. 07, 1981	261	.129	.27	.026	.05	.167	.26
1-4	Nov. 19, 1981	75	.937	1.97	.324	.60	.793	1.21
1-5	Dec. 15, 1981	71	>3.00	>6.30	1.778	3.27	>3.00	>4.29
Case #2								
2-1	Oct. 19, 1981	17	.058	.12	.023	.04	.052	.08
2-2	Nov. 17, 1981	46	.050	.11	.018	.03	.058	.09
2-3	Dec. 02, 1981	63	.047	.10	.020	.04	.060	.09
2-4	Dec. 14, 1981	152	.059	.12	.025	.05	.054	.08
2-5	Dec. 23, 1981	624	.070	.15	.026	.05	.074	.11
2-6	Jan. 20, 1982	66	.051	.11	.018	.03	.058	.09
2-7	Feb. 15, 1982	70	.139	.29	.037	.07	.146	.22
2-8	Mar. 17, 1982	24	1.867	3.92	.355	.65	1.429	2.19
2-9	Apr. 21, 1982	53	>3.00	>6.30	.748	1.37	>3.00	>4.59
2-10	Mau 19, 1982	95	>3.00	>6.30	1.025	1.88	>3.00	>4.59
2-11	Jun. 14, 1982	37	>3.00	>6.30	.917	1.68	>3.00	>4.59
Case #3								
3-1	Apr. 07, 1981	13	.090	.19	.049	.09	.138	.21
3-2	May 12, 1981	236	.064	.13	.040	.07	.094	.14
3-3	May 30, 1981	471	.079	.17	.045	.08	.144	.22
3-4	Jun. 09, 1981	315	.211	.44	.085	.16	.275	.42
3-5	Jul. 06, 1981	273	1.707	3.59	.272	.50	1.773	2.71
3-6	Aug. 10, 1981	158	>3.00	>6.30	1.347	2.47	>3.00	>4.59
3-7	Sep. 08, 1981	84	>3.00	>6.30	2.294	4.21	>3.00	>4.59
3-8	Oct. 14, 1981	180	>3.00	>6.30	>3.00	>5.50	>3.00	>4.59
3-9	Nov. 11, 1981	154	>3.00	>6.30	>3.00	>5.50	>3.00	>4.59

[1048] IV.I.1.f.(2). Samples from Random Blood Donors

[1049] Samples from random blood donors (see Section IV.I.1.) were screened for HCV infection using the HCV c100-3 ELISA, in which the antibody-enzyme conjugate was either an anti-IgG monoclonal conjugate, or a polyclonal conjugate. The total number of samples screened were 1077 and 1056, for the polyclonal conjugate and the monoclonal conjugate, respectively. A summary of the results of the screening is shown in Table 15, and the sample distributions are shown in the histogram in **FIG. 44**.

[1050] The calculation of the average and standard deviation was performed excluding samples that gave a signal over 1.5, i.e., 1073 OD values were-used for the calculations utilizing the polyclonal conjugate, and 1051 for the anti-IgG monoclonal conjugate. As seen in Table 15, when the polyclonal conjugate was used, the average was shifted from 0.0493 to 0.0931, and the standard deviation was increased from 0.074 to 0.0933. Moreover, the results also show that if the criteria of x+5SD is employed to define the assay cutoff, the polyclonal-enzyme conjugate configuration in the ELISA requires a higher cutoff value. This indicates a reduced assay specificity as compared to the monoclonal system. In addition, as depicted in the histogram in **FIG. 44,** a greater separation of results between negative and positive distributions occurs when random blood donors are screened in an ELISA using the anti-IgG monoclonal conjugate as compared to the assay using a commercial polyclonal label.

TABLE 15

COMPARISON OF TWO ELISA CONFIGURATIONS IN
TESTING SAMPLES FROM RANDOM BLOOD DONORS

CONJUGATE	POLYCLONAL (Jackson)	ANTI-IgG MONOCLONAL
Number of samples	1073	1051
Average (x)	0.0931	0.04926
Standard deviation (SD)	0.0933	0.07427
5 SD	0.4666	0.3714
CUT-OFF (5 SD + x)	0.5596	0.4206

[1051] IV.I.2. ELISA Assay Using Recombinant SOD-NANB$_{5-1-1}$

[1052] This assay utilizes the SOD-NANB$_{5-1-1}$ antigen, and is similar to the assay utilizing the c100-3 antigen (see Section IV.I.1.) except for the following.

[1053] The HCV polypeptide used in the assay is SOD-NANB$_{5-1-1}$ which is purified as described in Section IV.N.1.b., infra.

[1054] In the preparation of the plates, Immulon 2 plates replace Immulon 1 plates. In addition, BSA is omitted from the coating solution, and the coating solution contains 3.75 micrograms/ml of SOD-NANB$_{5-1-1}$ instead of c100-3.

[1055] The assay is also changed in that the sample diluent contains 1 mg/ml yeast extract, and also contains 500 micrograms/ml of the second E. coli extract (which is comprised of proteins in the soluble fraction of the lysozyme treated bacteria), and 100 micrograms/ml SOD. The extracts are prepared as described in Section IV N.1.a., infra.

[1056] IV.I.3. ELISA Assay Using Recombinant C33c

[1057] This assay utilizes the SOD-C33c antigen, and is similar to the assay utilizing the SOD-NANB$_{5-1-1}$ antigen (see Section IV.I.2.) except for the following.

[1058] The HCV polypeptide used in the assay is SOD-C33c, which is prepared as described in Section IV.B.9., supra. The plates coated are Immulon 1 plates instead of Immulon 2 plates, and the coating solution 1.25 micrograms/ml of SOD-C33c instead of c100-3. The assay is also changed in that the sample diluent contains 1 mg/ml yeast extract instead of 100 micrograms/ml of the second E. coli extract and 100 micrograms/ml of SOD.

[1059] IV.I.4. ELISA Assay Using a Synthetic Polypeptide Containing NANB$_{5-1-1}$ Sequences

[1060] This assay utilizes a synthetic peptide containing 42 amino acids encoded in HCV which are in the NANB$_{5-1-1}$ polypeptide, and which are also in the c100-3 polypeptide. The polypeptide, which was prepared by Peninsula Laboratory using chemical synthesis, has the following sequence.

[1061] NH 2-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-lu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro Tyr-Ile-Glu-Gln-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-OOH.

[1062] The assay is essentially as described in Section IV.I.1., except that the synthetic 5-1-1 polypeptide replaces the c100-3 polypeptide in the coating solution at a concentration of 2.5 ug/mL (micrograms). Also, Immulon 2 plates replace Immulon 1 plates, and the BSA is omitted from the coating solution.

[1063] IV.J. Detection of HCV Seroconversion in NANBH Patients from a Variety of Geographical Locations

[1064] Sera from patients who were suspected to have NANBH based upon elevated ALT levels, and who were negative in HAV and HBV tests were screened using the RIA essentially as described in Section IV.D., except that the HCV C100-3 antigen was used as the screening antigen in the microtiter plates. As seen from the results presented in Table 16, the RIA detected positive samples in a high percentage of the cases.

TABLE 16

Seroconversion Frequencies for Anti-c100-3
Among NANBH Patients in Different Countries

Country	The Netherlands	Italy	Japan
No. Examined	5	36	26
No. Positive	3	29	19
% Positive	60	80	73

[1065] IV.K. Detection of HCV Seroconversion in Patients with "Community Acquired" NANBH

[1066] Sera which was obtained from 100 patients with NANBH, for whom there was no obvious transmission route (i.e., no transfusions, i.v. drug use, promiscuity, etc. were identified as risk factors), was provided by Dr. M. Alter of the Center for Disease Control, and Dr. J. Dienstag of Harvard University. These samples were screened using an

RIA essentially as described in Section IV.D., except that the HCV c100-3 antigen was used as the screening antigen attached to the microtiter plates. The results showed that of the 100 serum samples, 55 contained antibodies that reacted immunologically with the HCV c100-3 antigen.

[1067] The results described above suggest that "Community Acquired" NANBH is also caused by HCV. Moreover, since it has been demonstrated herein that HCV is related to Flaviviruses, most of which are transmitted by arthropods, it is suggestive that HCV transmission in the "Community Acquired" cases also results from arthropod transmission.

[1068] IV.L. Comparison of Incidence of HCV Antibodies and Surrogate Markers in Donors Implicated in NANBH Transmission

[1069] A prospective study was carried out to determine whether recipients of blood from suspected NANBH positive donors, who developed NANBH, seroconverted to anti-HCV-antibody positive. The blood donors were tested for the surrogate marker abnormalities which are currently used as markers for NANBH infection, i.e., elevated ALT levels, and the presence of anti-core antibody. In addition, the donors were also tested for the presence of anti-HCV antibodies. The determination of the presence of anti-HCV antibodies was determined using a radioimmunoassay as described in Section IV.K. The results of the study are presented in Table 17, which shows: the patient number (column 1); the presence of anti-HCV antibodies in patient serum (column 2); the number of donations received by the patient, with each donation being from a different donor (column 3); the presence of anti-HCV antibodies in donor serum (column 4); and the surrogate abnormality of the donor (column 5) (NT or -- means not tested) (ALT is elevated transaminase, and ANTI-HBc is anti-core antibody).

[1070] The results in Table 17 demonstrate that the HCV antibody test is more accurate in detecting infected blood donors than are the surrogate marker tests. Nine out of ten patients who developed NANBH symptoms tested positive for anti-HCV antibody seroconversion. Of the 11 suspected donors, (patient 6 received donations from two different individuals suspected of being NANBH carriers), 9 were positive for anti-HCV antibodies, and 1 was borderline positive, and therefore equivocal (donor for patient 1). In contrast, using the elevated ALT test 6 of the ten donors tested negative, and using the anticore-antibody test 5 of the ten donors tested negative. Of greater consequence, though, in three cases (donors to patients 8, 9, and 10) the ALT test and the ANTI-HBc test yielded inconsistent results.

TABLE 17

DEVELOPMENT OF ANTI-HCV ANTIBODIES IN PATIENTS
RECEIVING BLOOD FROM DONORS SUSPECTED OF BEING
NANBH CARRIERS

Patient	Anti-HCV Seroconversion in Patient	No. of Donations/ Donors	Anti-HCV Positive Donor	Surrogate Abnormality*	
				Alt	Anti-HBc
1	yes	18	equiv	no	no
2	yes	18	yes	NT	yes
3	yes	13	yes	no	no
4	no	18	no	—	—

TABLE 17-continued

DEVELOPMENT OF ANTI-HCV ANTIBODIES IN PATIENTS
RECEIVING BLOOD FROM DONORS SUSPECTED OF BEING
NANBH CARRIERS

Patient	Anti-HCV Seroconversion in Patient	No. of Donations/ Donors	Anti-HCV Positive Donor	Surrogate Abnormality*	
				Alt	Anti-HBc
5	yes	16	yes	yes	yes
6	yes	11	yes (2)	no	no
				yes	yes
7	yes	15	yes	NT	no
8	yes	20	yes	no	yes
9	yes	5	yes	yes	no
10	yes	15	yes	no	yes

*Same donor as anti-NANBV Positive

[1071] IV.M. Amplification for Cloning of HCV cDNA Sequences Utilizing the PCR and Primers Derived from Conserved Regions of Flavivirus Genomic Sequences

[1072] The results presented supra., which suggest that HCV is a flavivirus or flavi-like virus, allows a strategy for cloning uncharacterized HCV cDNA sequences utilizing the PCR technique, and primers derived from the regions encoding conserved amino acid sequences in flaviviruses. Generally, one of the primers is derived from a defined HCV genomic sequence, and the other primer which flanks a region of unsequenced HCV polynucleotide is derived from a conserved region of the flavivirus genome. The flavivirus genomes are known to contain conserved sequences within the NS1, and E polypeptides, which are encoded in the 5'-region of the flavivirus genome. Corresponding sequences encoding these regions lie upstream of the HCV cDNA sequence shown in FIG. 26. Thus, to isolate cDNA sequences derived from this region of the HCV genome, upstream primers are designed which are derived from the conserved sequences within these flavivirus polypeptides. The downstream primers are derived from an upstream end of the known portion of the HCV cDNA.

[1073] Because of the degeneracy of the code, it is probable that there will be mismatches between the flavivirus probes and the corresponding HCV genomic sequence. Therefore a strategy which is similar to the one described by Lee (1988) is used. The Lee procedure utilizes mixed oligonucleotide primers complementary to the reverse translation products of an amino acid sequence; the sequences in the mixed primers takes into account every codon degeneracy for the conserved amino acid sequence.

[1074] Three sets of primer mixes are generated, based on the amino acid homologies found in several flaviviruses, including Dengue-2,4 (D-2,4), Japanese Encephalitis Virus (JEV), Yellow Fever (YF), and West Nile Virus (WN). The primer mixture derived from the most upstream conserved sequence (5'-1), is based upon the amino acid sequence gly-trp-gly, which is part of the conserved sequence asp-arg-gly-trp-gly-aspN found in the E protein of D-2, JEV, YF, and WN. The next primer mixture (5'-2) is based upon a downstream conserved sequence in E protein, phe-asp-gly-asp-ser-tyr-ileu-phe-gly-asp-ser-tyr-ileu, and is derived from phe-gly-asp; the conserved sequence is present in D-2, JEV, YF, and WN. The third primer mixture (5'-3), is based on the

amino acid sequence arg-ser-cys, which is part of the conserved sequence cys-cys-arg-ser-cys in the NS1 protein of D-2, D-4, JEV, YF, and WN. The individual primers which form the mixture in 5'-3 are shown in **FIG. 45**. In addition to the varied sequences derived from conserved region, each primer in each mixture also contains a constant region at the 5'-end which contains a sequence encoding sites for restriction enzymes, HindIII, MboI, and EcoRI.

[1075] The downstream primer, ssc5h20A, is derived from a nucleotide sequence in clone 5h, which contains HCV cDNA with sequences with overlap those in clones 14i and 11b. The sequence of ssc5 h20A is

[1076]   5' GTA ATA TGG TGA CAG AGT CA 3'.

[1077] An alternative primer, ssc5 h34A, may also be used. This primer is derived from a sequence in clone 5h, and in addition contains nucleotides at the 5'-end which create a restriction enzyme site, thus facilitating cloning. The sequence of ssc5h34A is

[1078]   5' GAT CTC TAG AGA AAT CAA TAT GGT GAC AGA GTC A 3'.

[1079] The PCR reaction, which was initially described by Saiki et al. (1986), is carried out essentially as described in Lee et al. (1988), except that the template for the cDNA is RNA isolated from HCV infected chimpanzee liver, as described in Section IV.C.2., or from viral particles isolated from HCV infected chimpanzee serum, as described in Section IV.A.1. In addition, the annealing conditions are less stringent in the first round of amplification (0.6M NaCl, and 25° C.), since the part of the primer which will anneal to the HCV sequence is only 9 nucleotides, and there could be mismatches. Moreover, if ssc5 h34A is used, the additional sequences not derived from the HCV genome tend to destabilize the primer-template hybrid. After the first round of amplification, the annealing conditions can be more stringent (0.066M NaCl, and 32° C.-37° C.), since the amplified sequences now contain regions which are complementary to, or duplicates of the primers. In addition, the first 10 cycles of amplification are run with Klenow enzyme I, under appropriate PCR conditions for that enzyme. After the completion of these cycles, the samples are extracted, and run with Taq polymerase, according to kit directions, as furnished by Cetus/Perkin-Elmer.

[1080] After the amplification, the amplified HCV cDNA sequences are detected by hybridization using a probe derived from clone 5h. This probe is derived from sequences upstream of those used to derive the primer, and does not overlap the sequences of the clone 5h derived primers. The sequence of the probe is

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5' CCC AGC GGC GTA CGC GCT GGA CAC GGA CGT GGC CGC
GTC GTG TGG CGG TGT TGT TCT CGT CGG GTT GAT GGC
GC 3'.
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[1081] IV.N.1. Immunoblot Assay for HCV Antibodies Using HCV Antigens

[1082] The immunoblot assay for HCV employs an immunoblot ELISA technique for the qualitative detection of antibodies to HCV in biological specimens The assay uses three purified recombinant antigens: the fusion polypeptide, SOD-NANB$_{5-1-1}$ (also called 5-1-1); the fusion polypeptide

SOD-C33c; the fusion polypeptide HCV C100-3; and human superoxide dismutase (hSOD). The latter antigen is included as a control to detect the presence of antibodies against SOD. The purification procedure for SOD-NANB$_{5-1-1}$ is described below in Section IV.N.1.b.; that for the fusion C100-3 polypeptide is described in Section IV.B.7.b; and that for the C33c polypeptide is described in Section IV.B.9.

[1083] IV.N.1.a. The Immunoblot Assay System for HCV

[1084] In the immunoblot assay system for HCV, the above described individual recombinant derived HCV antigens are immobilized in discreet bands-on nitro-cellulose strips (0.45 microns) by vacuum blotting solutions of individual antigens. During the incubation of the strips with the biological specimens, antibodies to HCV, if present, react with the corresponding antigens coated in bands on the nitrocellulose strips. After the removal of nonspecific antibodies by aspiration and washing, the strip is then reacted with goat anti-human IgG (heavy and light chain specific) labeled with horseradish peroxidase (HRP). Following incubation, decantation, and washing to remove excess conjugate, 4-chloro-1-naphthol solution containing hydrogen peroxide is added. The corresponding intensities of the blue-black colored bands which develop are proportional to the amount of specific antibody bound to each of the recombinant proteins on the strips. After the precipitation reaction is stopped by decantation and washing, the reactivity of specimens towards each antigen is determined by visually comparing the intensity of the individual antigen band with that of the low and high IgG internal controls included on each strip.

[1085] The reagents utilized in the test are the following. The conjugate is goat anti-human IgG (heavy and light chain specific) labeled with HRP in sodium phosphate buffer containing sodium chloridae and protein stabilizers. The stock substrate is 4-chloro-1-naphthol (3 g/L) in methanol. The developer buffer is sodium monobasic phosphate (1.3 g) containing sodium chloride (1.17 g/L) and hydrogen peroxide (1 ml/L). The developer buffer contains sodium monobasic phosphate (13.8 μL), NaCl (29.2 g/L), casein (1 μL), EDTA disodium salt (0.34 g/L), Triton X-100 (10 μL), 10% thimerosal (1 ml/L), yeast extract (1 g/L), BSA (10 g/L), the first *E. coli* extract (20 mg/L) and the second *E. coli* extract (0.5 g/L). The HCV antibody positive control is inactivated human serum containing antibodies to HCV; the serum is inactivated by heat and psoralen treatment. The HCV-antibody negative control is human serum which does not demonstrate antibodies to HCV. A working sample diluent for the assay is prepared by weighing out 0.5 gm nonfat dry milk powder into each 10 ml sample diluent, and mixing well before use. The working substrate for the assay is prepared by combining 1 part of the stock substrate with 5 parts of developer buffer.

[1086] The yeast extract used in the sample diluent is prepared as follows. Red Star baker's yeast (Universal Foods) is slurried with an approximately equal amount (weight to volume) of 64 mM Tris HCl, and the pH is adjusted to 7.0. The yeast is mechanically disrupted using a glass bead mill (Dynomill) using 0.5 mm nominal diameter beads, until 95% of the cells are broken. Concentrated sodium dodecylsulfate (SDS) is added to the lysate to yield a final concentration of SDS of ~2.14%. The lysate is then

agitated and heated to 70° C.±5° C. for 10 minutes. The heated lysate is diluted with 3 volumes of buffer containing 67M Tris HCl, pH 7.0, 2.25% SDS, and is cooled to 20-25° C. Gross debris is removed from the diluted lysate by centrifugation using a Westphalia SA1 continuous flow desludging centrifuge at a feed rate of about 1.5 liters per minute and back pressure of 20-23 psig. The desludge interval is about 3.5 minutes.

[1087] One of the *E. coli* extracts used in the diluent is comprised of bacterial proteins which are solubilized during urea treatment of an insoluble protein fraction. This extract is prepared from a lysate of strain D1210 cells transformed with the expression vector pSODcf1 (see Section IV.B.1.). The cells are cultured and pelleted as described in Section IV.N.7.b., except that the pSODcf1 transformants replace the transformants which harbor pSODcf1 containing the HCV sequence from clone 5-1-1. In order to prepare the extract, 40 g of pelleted cells are placed into a 500 ml Beckman bottle, and resuspended in 14.4 ml of TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA). Next, 18 ml of distilled water, 6.8 ml of lysozyme solution, 0.8 ml of 0.1M PMSF (in ethanol) are added to the suspension, and it is incubated on ice for 1 hour. After the incubation, a solution containing 120 ml sterile water, 240 microliters 0.5 M $MgCl_2$, 180 microliters DNAse I (20,000 u/ml in distilled water) and 1.2 ml of 0.1M PMSF is added for each 40 g of cells. The suspension is then sonicated in a Branson Sonifier 450 at setting 1 for 30 seconds and placed on ice for 1 minute; this cycle is repeated five more times, after which the sonicate is incubated at room temperature for 15 minutes. The solution is then sonicated at setting 5 for 30 seconds and placed on ice for 1 minute; this cycle is repeated 3 more times, after which the sonicate is incubated at room temperature for 15 minutes. This last cycle of sonication is repeated. After sonication, the sonicate is centrifuged at 10,000 rpm for 25 minutes. The supernatant is decanted, and the remaining pellet is extracted with urea as follows. Using an Omni mixer, the pellet is resuspended in 120 ml of 6 M urea, 50 mM Tris HCl, pH 8.0, 0.1% beta-mercaptoethanol (BME). The bottle is attached to a tilt shaker and rocked at 4° C. for four hours. The suspension is then centrifuged at 10,000 rpm for 25 minutes at 4° C. The supernatant containing urea is dialyzed against a 400-fold volume of buffer containing 50 mM sodium borate, 0.5 M NaCl, pH 8.4. Dialysis is for 1.5 hours at room temperature, using Spectrapor® dialysis tubing with a molecular weight cut-off of 12,000-14,000. Precipitate in the dialysate is removed by centrifugation at 10,000 rpm for 15 minutes, and the supernatant is subjected to a repeated round of dialysis and centrifugation, yielding a supernatant which is used as the bacterial extract.

[1088] The second *E. coli* extract used in the diluent is prepared from non-transformed *E. coli* cells, and is comprised of proteins in the soluble fraction of the lysozyme treated bacteria. The extract is prepared by adding to 15 ml of packed cells the following: 3 ml of lysozyme (5 mg/ml) in 0.25 M Tris HCl, pH 8.0, 150 microliters 0.1M PMSF in ethanol, 3.3 ml 0.25 M EDTA, pH 8.0, and 12.5 ml of a solution containing 1% triton and 0.4% deoxycholate. The cells are suspended using an Omnimixer, and are broken by mixing for 15-25 minutes at 2°-8° C.; if breakage is incomplete (i.e., the material is not viscous), an additional 150 microliters 0.1M PMSF is added, and the mixing is continued for an additional 15 to 25 minutes. After cell breakage, 20,000 units of DNAse I in 1 ml water and 15 ml 0.5 M

$MgCl_2$ are added, and the mixing is continued for 15 to 25 minutes at room temperature, until the DNA is digested (i.e., the mixture approaches the viscosity of water. The mixture is then centrifuged at 17,000×G for 20 minutes at 20 to 8° C., and the supernatant is decanted. The supernatant is then dialyzed against 1 liter of phosphate buffered saline (PBS) from 8 to 72 hours, using Spectropor tubing with a molecular weight cutoff of 6,000 to 8,000. The protein concentration of the dialysate is adjusted to 5 to 15 mg/ml.

[1089] The immunoblot assay is performed by setting up one tube per sample, each containing a nitrocellulose assay strip. Each strip is banded with the three aforementioned HCV antigens, with hSOD, and with two levels of human IgG (internal controls), one of which yields a weak positive reaction, and one of which yields a moderate positive reaction. Sufficient working sample diluent is added to each tube to cover the strip with liquid. An aliquot of the appropriate specimen or control sample is added to each tube. The tubes are covered, and inverted to mix, the tubes are placed in a rocker, and agitated for 4 hours at room temperature. (The motion of the sample solution over the strips, generated by the rocker, is important in achieving even band staining and maximum sensitivity). After the 4 hour incubation, the tubes are uncapped, the liquid aspirated, and the strips are washed with distilled water, and transferred to a wash vessel. Following another three washes with excess distilled water, and removal of the wash by decantation, the strips are reacted with conjugate (the aliquot added is in excess of that which is sufficient to cover all of the strips). During the reaction with conjugate, the vessel containing the strip and conjugate is agitated on a rotary shaker at approximately 110 rpm for 30 minutes at room temperature. Excess conjugate is removed by washing the strips three times with an excess of distilled water, and the final wash is decanted. An aliquot of working substrate is added to the wash vessel, and the vessel is agitated on a rotary shaker at approximately 110 rpm for 15 minutes at room temperature. After the reaction, the working substrate is decanted off, and the strips are washed twice with excess distilled water. The strips are transferred to absorbent paper to blot off the excess water, air dried for at least twenty minutes at room temperature (protected from the light), and read within three hours after completion of the assay.

[1090] IV-N.1.b. Purification of SOD-NANB$_{5\text{-}1\text{-}1}$

[1091] The HCV fusion polypeptide, SOD-NANB$_{5\text{-}1\text{-}1}$ (also called the 5-1-1 polypeptide), used in the immunoblot assay system for HCV is purified according to the following procedure.

[1092] The 5-1-1 polypeptide is expressed in recombinant bacteria D1210 transformed with the vector described in Section IV.B.1.. In order to prepare an overnight culture of the transformed bacteria, the cells (about 150 microliters of glycerol stock) were grown in 35 ml of L broth-containing 160±15 microliters of 2% ampicillin); growth is overnight at 37° C. with shaking at 300 rpm. For expression, each 1.5 liters of culture (L broth containing 6.5±0.25 ml 2% ampicillin) is inoculated with 15 ml of the overnight culture, and grown at 37° C. in a Fernbach flask with shaking for 2½ to 3 hours, until an O.D.$_{650}$ of 0.80±0.5 is attained; at this time expression is induced by the addition of 15 ml 200 mM IPTG. After induction, the cells are grown for 3 hours at 37°

C. with shaking. The cells are then harvested by pelleting in a J6-B centrifuge in a JS5.2 rotor at 3.5K rpm for 15 minutes.

[1093] A 6 M urea extract of the cell pellet is prepared. Five grams of cell pellet is resuspended in 1.8 ml of TE, and then 2.25 ml sterile water, 0.85 ml lysozyme solution (5 mg/ml in 0.25 N Tris HCl, pH 8.0), and 100 microliters of 0.1M PMSF in ethanol are added. The resuspended pellet is incubated on ice for one hour. After the incubation, 15 ml of distilled water, 30 microliters of 0.5 M MgCl$_2$, 22.5 microliters DNAse I (20,000 units/ml in water), and 150 microliters of PMSF are added. The mixture is sonicated and the insoluble material pelleted using the procedure for the preparation of the *E. coli* extract described supra, except that sonication at setting 1 is repeated for a total of four times, and sonication at setting 5 is repeated for a total of two times. The pellet, which has a volume of ˜1.5 ml is suspended in 15 ml of solution containing 6M urea, 50 mM Tris HCl, pH 8.0, and 0.1% BME, using a pipetter and with vortexing. The suspension is then rocked in a tilt shaker at 4° C. for 4 hours, and centrifuged at 12,000 rpm for 15 minutes at 4° C.

[1094] The 5-1-1 polypeptide contained in the supernatant from the above described centrifugation is purified from the urea extract by passage through a Q Sepharose Fast Flow ion exchange column (Pharmacia Corp.). A 30 ml column is equilibrated by pumping through approximately 80 ml of running buffer (6 M urea in 20 mM Tris HCl, pH 8.0, 1 mM Dithiothreitol (DTT)), or RB, at a speed of about 2 ml per minute. After the column is equilibrated, the entire 6 M urea extract is loaded onto the column, and is washed in with approximately 80 ml of running buffer; fractions of 2 ml are collected during the loading and the washing steps. After the load is washed into the column, the 5-1-1 polypeptide is eluted using a 0.0 to 0.5 M NaCl gradient in RB. The gradient solution is pumped over the column at 2 ml per minute; 1 ml fractions are collected; and the O.D.$_{280}$ is monitored throughout the load, wash, and elution. The 5-1-1 polypeptide is expected to elute from the column approximately two-thirds of the way through the gradient. The column fractions are analyzed by electrophoresis on 12.5% polyacrylamide gels containing SDS, and by Western blot using positive polyclonal antibodies to SOD and/or the 5-1-1 polypeptide and/or serum samples. The purity of the 5-1-1 polypeptide is estimated based upon the O.D.$_{280}$, the Western blot, and the polyacrylamide gel analysis. Based upon this, appropriate fractions are pooled, yielding a total volume of ˜30 ml. Each 10 ml of the pooled material is dialyzed at room temperature against 2 liters of buffer containing 50 mM sodium borate, pH 8.4, 0.5 M NaCl, 10 mM BME, and 2 mM EDTA for 1.5 hours, with 1 change of buffer after 1.5 hours; total dialysis time is approximately 3 hours. Protein concentration in the dialysate is determined by the Lowry method. The purified, dialyzed material is stored at –70° C.

[1095] The following listed materials are on deposit under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville, Md. 20852, and have been assigned the following Accession Numbers.

lambda-gt11	ATCC No.	Deposit Date
HCV cDNA library	40394	1 Dec. 1987
clone 81	40388	17 Nov. 1987
clone 91	40389	17 Nov. 1987
clone 1-2	40390	17 Nov. 1987
clone 5-1-1	40391	18 Nov. 1987
clone 12f	40514	10 Nov. 1988
clone 35f	40511	10 Nov. 1987
clone 15e	40513	10 Nov. 1988
clone K9-1	40512	10 Nov. 1988
JSC 308	20879	5 May 1988
pS356	67683	29 Apr. 1988

[1096] In addition, the following deposits were made on May 11, 1989.

Strain	Linkers	ATCC No.
D1210 (Cf1/5-1-1)	EF	67967
D1210 (Cf1/81)	EF	67968
D1210 (Cf1/CA74a)	EF	67969
D1210 (Cf1/35f)	AB	67970
D1210 (Cf1/279a)	EF	67971
D1210 (Cf1/C36)	CD	67972
D1210 (Cf1/13i)	AB	67973
D1210 (Cf1/C33b)	EF	67974
D1210 (Cf1/CA290a)	AB	67975
HB101 (AB24/C100 #3R)		67976

[1097] The following derivatives of strain D1210 were deposited on May 3, 1989.

Strain Derivative	ATCC No.
pCF1CS/C8f	67956
pCF1AB/C12f	67952
pCF1EF/14c	67949
pCF1EF/15e	67954
pCF1AB/C25c	67958
pCF1EF/C33c	67953
pCF1EF/C33f	67050
pCF1CD/33g	67951
pCF1CD/C39c	67955
pCF1EF/C40b	67957
pCF1EF/CA167b	67959

[1098] The following biological materials were deposited on May 12, 1989.

Material	ATCC No.
Lambda gt11(C35)	40603
Lambda gt10(beta-5a)	40602
D1210 (C40b)	67980
D1210 (M16)	67981

[1099] The following biological materials were deposited on Oct. 13, 1989.

Material	ATCC No.
AB122	20961
Lambda gt11	40678
pAB24/C200-C100	40679
pNS11d- 13–15	40680
pC100-d #3	68113
pC22	68114
pS2d #9	68115

[1100] The following biological materials were deposited on Oct. 17, 1989.

Material	ATCC No.
pCMV-NS1	68125
pMCMV-NS1	68126

[1101] Upon allowance and issuance of this application as a United States Patent, all restriction on availability of these deposits will be irrevocably removed; and access to the designated deposits will be available during pendency of the above-named application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 USC 1.22. Moreover, the designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit; or for the enforceable life of the U.S. patent, whichever is longer. The deposited materials mentioned herein are intended for convenience only, and are not required to practice the present invention in view of the descriptions herein, and in addition these materials are incorporated herein by reference.

INDUSTRIAL APPLICABILITY

[1102] The invention, in the various manifestations disclosed herein, has many industrial uses, some of which are the following. The HCV cDNAs may be used for the design of probes for the detection of HCV nucleic acids in samples. The probes derived from the cDNAs may be used to detect HCV nucleic acids in, for example, chemical synthetic reactions. They may also be used in screening programs for anti-viral agents, to determine the effect of the agents in inhibiting viral replication in cell culture systems, and animal model systems. The HCV polynucleotide probes are also useful in detecting viral nucleic acids in humans, and thus, may serve as a basis for diagnosis of HCV infections in humans.

[1103] In addition to the above, the cDNAs provided herein provide information and a means for synthesizing polypeptides containing epitopes of HCV. These polypeptides are useful in detecting antibodies to HCV antigens. A series of immunoassays for HCV infection, based on recombinant polypeptides containing HCV epitopes are described herein, and will find commercial use in diagnosing HCV induced NANBH, in screening blood bank donors for HCV-caused infectious hepatitis, and also for detecting contaminated blood from infectious blood donors. The viral antigens will also have utility in monitoring the efficacy of anti-viral

agents in animal model systems. In addition, the polypeptides derived from the HCV cDNAs disclosed herein will have utility as vaccines for treatment of HCV infections.

[1104] The polypeptides derived from the HCV cDNAs, besides the above stated uses, are also useful for raising anti-HCV antibodies. Thus, they may be used in anti-HCV vaccines. However, the antibodies produced as a result of immunization with the HCV polypeptides are also useful in detecting the presence of viral antigens in samples. Thus, they may be used to assay the production of HCV polypeptides in chemical systems. The anti-HCV antibodies may also be used to monitor the efficacy of anti-viral agents in screening programs where these agents are tested in tissue culture systems. They may also be used for passive immunotherapy, and to diagnose HCV caused NANBH by allowing the detection of viral antigen(s) in both blood donors and recipients. Another important use for anti-HCV antibodies is in affinity chromatography for the purification of virus and viral polypeptides. The purified virus and viral polypeptide preparations may be used in vaccines. However, the purified virus may also be useful for the development of cell culture systems in which HCV replicates.

[1105] Cell culture systems containing HCV infected cells will have many uses. They can be used for the relatively large scale production of HCV, which is normally a low titer virus. These systems will also be useful for an elucidation of the molecular biology of the virus, and lead to the development of anti-viral agents. The cell culture systems will also be useful in screening for the efficacy of antiviral agents. In addition, HCV permissive cell culture systems are useful for the production of attenuated strains of HCV.

[1106] For convenience, the anti-HCV antibodies and HCV polypeptides, whether natural or recombinant, may be packaged into kits.

[1107] The method used for isolating HCV cDNA, which is comprised of preparing a cDNA library derived from infected tissue of an individual, in an expression vector, and selecting clones which produce the expression products which react immunologically with antibodies in antibody-containing body components from other infected individuals and not from non-infected individuals, may also be applicable to the isolation of cDNAs derived from other heretofore uncharacterized disease-associated agents which are comprised of a genomic component. This, in turn, could lead to isolation and characterization of these agents, and to diagnostic reagents and vaccines for these other disease-associated agents.

1. A purified HCV polynucleotide.

2. A recombinant HCV polynucleotide.

3. A recombinant polynucleotide comprising a sequence derived from an HCV genome or from HCV cDNA.

4. A recombinant polynucleotide encoding an epitope of HCV.

5. A recombinant vector containing the polynucleotide of claim 2, or claim 3, or claim 4.

6. A host cell transformed with the vector of claim 5.

7. A recombinant expression system comprising an open reading frame (ORF) of DNA derived from an HCV genome or from HCV cDNA, wherein the ORF is operably linked to a control sequence compatible with a desired host.

8. A cell transformed with the recombinant expression system of claim 7.

9. A polypeptide produced by the cell of claim 8.

10. Purified HCV.

11. A preparation of polypeptides from the HCV of claim 10.

12. A purified HCV polypeptide.

13. A purified polypeptide comprising an epitope which is immunologically identifiable with an epitope contained in HCV.

14. A recombinant HCV polypeptide.

15. A recombinant polypeptide comprised of a sequence derived from an HCV genome or from HCV cDNA.

16. A recombinant polypeptide comprised of an HCV epitope.

17. A fusion polypeptide comprised of an HCV polypeptide.

18. A monoclonal antibody directed against an HCV epitope.

19. A purified preparation of polyclonal antibodies directed against HCV.

20. A particle which is immunogenic against HCV infection comprising a non-HCV polypeptide having an amino acid sequence capable of forming a particle when said sequence is produced in a eukaryotic host, and an HCV epitope.

21. A polynucleotide probe for HCV.

22. A kit for analyzing samples for the presence of polynucleotides derived from HCV comprising a polynucleotide probe containing a nucleotide sequence from HCV of about 8 or more nucleotides, in a suitable container.

23. A kit for analyzing samples for the presence of an HCV antigen comprising an antibody directed against the HCV antigen to be detected, in a suitable container.

24. A kit for analyzing samples for the presence of an antibodies directed against an HCV antigen comprising a polypeptide containing an HCV epitope present in the HCV antigen, in a suitable container.

25. A polypeptide comprised of an HCV epitope, attached to a solid substrate.

26. An antibody to an HCV epitope, attached to a solid substrate.

27. A method for producing a polypeptide containing an HCV epitope comprising incubating host cells transformed with an expression vector containing a sequence encoding a polypeptide containing an HCV epitope under conditions which allow expression of said polypeptide.

28. A polypeptide containing an HCV epitope produced by the method of claim 27.

29. A method for detecting HCV nucleic acids in a sample comprising:

(a) reacting nucleic acids of the sample with a probe for an HCV polynucleotide under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample; and

(b) detecting a polynucleotide duplex which contains the probe.

30. An immunoassay for detecting an HCV antigen comprising:

(a) incubating a sample suspected of containing an HCV antigen with a probe antibody directed against the HCV antigen to be detected under conditions which allow the formation of an antigen-antibody complex; and

(b) detecting an antigen-antibody complex containing the probe antibody.

31. An immunoassay for detecting antibodies directed against an HCV antigen comprising:

(a) incubating a sample suspected of containing anti-HCV antibodies with a probe polypeptide which contains an epitope of the HCV, under conditions which allow the formation of an antibody-antigen complex; and

(b) detecting the antibody-antigen complex containing the probe antigen.

32. A vaccine for treatment of HCV infection comprising an immunogenic polypeptide containing an HCV epitope wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

33. A vaccine for treatment of HCV infection comprising inactivated HCV in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

34. A vaccine for treatment of HCV infection comprising attenuated HCV in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

35. A tissue culture grown cell infected with HCV.

36. The HCV infected cell of claim 35, wherein the cell is of a human macrophage cell line, or is of a hepatocyte cell line, or is of a mosquito cell line, or is of a tick cell line, or is of a mouse macrophage cell line, or is an embryonic cell.

37. The HCV infected cell of claim 35, wherein the cell is of a cell line derived from liver of an HCV infected individual.

38. A method for producing antibodies to HCV comprising administering to an individual an isolated immunogenic polypeptide containing an HCV epitope in an amount sufficient to produce an immune response.

39. A method for producing antibodies to HCV comprising administering to an individual the polypeptide preparation of claim 11, wherein the preparation contains at least 1 immunogenic polypeptide, and the administering is of an amount sufficient to produce an immune response.

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