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(54) Title: GAMMARETROVIRUS ASSOCIATED WITH CANCER

(57) Abstract: The present invention provides for isolated nucleic acid sequences encoding viruses; isolated polypeptides comprising amino acid sequences of the virus; vectors comprising the viral nucleic acid sequences; cells comprising the vectors; antibodies and antigen binding fragments thereof which have binding specificity for the virus; methods of detecting or screening for the virus (e.g., in an individual); methods of identifying agents that inhibit the virus; methods of inducing an immune response to the virus; methods of treating disease associated with the presence of XMRV in an individual (e.g., cancer such as prostate cancer); methods of detecting asymptomatic cancer (e.g., prostate cancer); methods of identifying an individual at risk for developing cancer (e.g., prostate cancer); and kits for detecting the virus.

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GAMMARETROVIRUS ASSOCIATED WITH CANCER

RELATED APPLICATION(S)

This application claims the benefit of U.S. Provisional Application No. 60/669,473, filed on April 7, 2005 and U.S. Provisional Application No. 60/751,809, filed on December 19, 2005. The entire teachings of the above applications are incorporated herein by reference.

GOVERNMENT SUPPORT

The invention was supported, in whole or in part, by a grant NIH/NCI RO1 CA103943-01 from the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Sexually transmitted diseases have increased in the last 30 years. Such diseases have been linked to cancers (*e.g,* prostate cancer). Carcinoma of the prostate is the second leading cause of cancer deaths in American men and the most frequent visceral cancer (Kumar, V., *et al., S. L. (1997) in Basic Pathology,* 6th ed., pp. 584-588, W. B. Saunders Co., Philadelphia Kumar *et al,* 1997). Among populations in the U.S., African Americans have the highest risk. The American Cancer Society estimated that there were about 190,000 new cases and 30,000 deaths from prostate cancer in the US in 2003. Genetics, aging, hormonal, and environmental risk factors all play roles in the pathogenesis of prostate cancer (Nelson WG., *et al., N Engl J Med, 349*(4):366-81, 2003).

A need exists for improved detection and treatment methods for such cancers.

SUMMARY OF THE INVENTION

The present invention is directed to an isolated ***x***enotropic ***m***urine leukemia virus (MLV) ***r***elated ***v***irus (XMRV) that can cause cancer in the individual. That is,

the etiology of the cancer in the individual is a likely link to the presence of XMRV in the individual.

In one embodiment, the present invention is directed to an isolated XMRV present in a prostate tumor of an individual. In a particular embodiment, the XMRV is a human xenotropic virus (HXV). The individual can have a mutation in the hereditary prostate cancer-1 (HPC1) allele encoding an RNase L gene. In a particular, the mutation is a homozygous mutation (*e.g.*, homozygous for the RNase L mutation R462Q).

In one embodiment, the isolated virus comprises a nucleic acid sequence having at least 94% identity to SEQ ID NO: 1 and a complement thereof. In a particular embodiment, the isolated virus comprises SEQ ID NO: 1 or a complement thereof.

In another embodiment, the invention is directed to an isolated virus comprising an amino acid sequence encoded by a nucleic acid sequence having at least 94% identity to SEQ ID NO: 1. For example, the isolated virus can comprise an amino acid sequence encoded by SEQ ID NO: 1.

The present invention is also directed to isolated peptides. In one embodiment, the invention pertains to an isolated virus comprising SEQ ID NO: 2. In particular embodiments, the isolated polypeptide comprises an amino acid sequence having at least 97% similarity to SEQ ID NO: 3, an amino acid sequence having at least 97% similarity to SEQ ID NO: 4 and/or an amino acid sequence having at least 94% similarity to SEQ ID NO: 5.

The present invention is also directed to a vector comprising one or more of the nucleic acid sequences described herein. The invention further encompasses a cell comprising the vectors. In addition, the invention comprises methods of producing XMRV. In one embodiment, the method comprises maintaining a cell transfected or infected with XMRV. The method can further comprise isolating XMRV produced by the cell (*e.g.*, from a cell supernatant).

Also encompassed by the present invention is an antibody or antigen binding fragment thereof that specifically binds to a virus comprising an amino acid sequence encoded by a nucleic acid sequence having at least 94% identity to SEQ ID NO: 1. The antibody or antigen binding fragment can bind to a gag polypeptide

(*e.g.*, SEQ ID NO: 3), a pro-pol peptide (*e.g.*, SEQ ID NO:4) and/or an env polypeptide (*e.g.*, SEQ ID NO: 5) of the virus.

The present invention is also directed to methods of detecting XMRV. In one embodiment, detection of XRMV in an individual indicates that the individual has cancer or at risk of developing cancer (*e.g.*, prostate cancer). The method can comprise detecting the XMRV in the individual by detecting a nucleic acid sequence that encodes all or a portion of the XMRV (*e.g.*, a nucleic acid sequence having at least 94% identity to SEQ ID NO: 1 or a complement thereof). Alternatively, the method can comprise detecting the XMRV in the individual by detecting a polypeptide encoded by the XMRV (*e.g.*, a gag polypeptide, a pol polypeptide, an env polypeptide and combinations thereof).

In another embodiment, the invention is directed to a method of detecting XMRV in a sample comprising contacting the sample with a nucleic acid sequence that hybridizes to all of a portion of an XMRV nucleic acid sequence under conditions in which a hybridization complex can occur between the nucleic acid sequence and the XMRV nucleic acid sequence. Whether the hybridization complex is present in the sample is determined, wherein if the hybridization complex is detected, then XMRV is in the sample.

In another embodiment, the invention relates to a method of detecting XMRV in a sample comprising contacting the sample with an antibody or antigen binding fragment thereof that specifically binds to an XMRV polypeptide under conditions in which a complex can occur between the antibody and the XMRV polypeptide. Whether the complex is present in the sample is determined, wherein if the complex is detected, then XMRV is in the sample.

The present invention is also directed to a method of identifying an agent that inhibits an XMRV comprising contacting the XMRV with an agent to be assessed. Whether XMRV is inhibited in the presence of the agent is determined, wherein if XMRV is inhibited in the presence of the agent, then the agent inhibits XMRV. In a particular embodiment, the activity of the XMRV in the presence of the agent is determined by measuring the ability of the XMRV to produce retroviral particles with reverse transcriptase activity.

Methods of inducing an immune response to an XMRV in an individual in need thereof is also encompassed by the present invention. The method can comprise administering to the individual an effective amount of an agent which induces an immune response to the XMRV in the individual upon administration. The agent can be a subunit of XMRV, an attenuated XMRV and combinations thereof.

The present invention is also directed to a method of treating a cancer (*e.g.*, prostate cancer) in an individual wherein XMRV is present in the individual, such as in a tumor, comprising administering to the individual an effective amount of an agent that inhibits XMRV.

The present invention also pertains to a method of detecting asymptomatic (early stage) cancer (*e.g.*, early stage prostate cancer) in an individual wherein XMRV is present in the prostate of the individual, comprising detecting the presence of an XMRV in the individual, wherein the presence of XMRV in the individual is indicative of early stage prostate cancer in the individual.

Also encompassed by the present invention is a method of identifying an individual at risk for developing cancer (*e.g.*, prostate cancer), comprising detecting the presence of an XMRV in the individual, wherein the presence of XMRV in the individual is indicative of an individual at risk for developing prostate cancer.

The invention is also directed to kits for detecting the presence of XMRV in a sample. In one embodiment, the kit comprises a labeled moiety that detects XMRV in a sample (*e.g.*, a nucleic acid sequence that hybridizes to all of a portion of an XMRV nucleic acid sequence; an antibody or antigen binding fragment thereof that specifically binds to XMRV).

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 is a schemtaic illustrating the role of the 2-5 A/RNase L system in the antiviral activity of interferons; 2'-PDE, 2',5'-phosphodiesterase; P'tase, 5'-phosphatase.

Figure 2 is a schematic illustrating *RNASEL* mutations in different populations of prostate cancer cases aligned to the domain structure of RNase L; LOH, loss of heterozygosity (Carpten, J, *et al.*, *Nature Genetics* 2002; *30*:181-4; Rokman A., *et al.*, *Am J Hum Genet.* 2002 May; *70*(5):1299-304; Rennert, H., *et al.*, *Am J Hum Genet.* 2002 Oct; *71*(4):981-4; Wang D., *et al.*, *Proc Natl Acad Sci* U S A. 2002 Nov 26;99(24):15687-92; Casey G., *et al.*, *Nat Genet.* 2002 Dec; *32*(4):581-3; Xiang Y., *et al.*, *Cancer Res.* 2003; *63*(20):6795-801).

Figure 3 is a schematic illustrating the genomic structure of HXV aligned to viral transcripts.

Figure 4 is a dendrogram of the relationship between HXV_{35} (indicated in diagram as "PCRV") and other gammaretroviruses.

Figure 5 illustrates the predicted secondary structure of HXV_{35} genomic RNA performed using MFOLD (Zuker M, et al., RNA, 1998, 4(6):669-79, 1998).

Figure 6 is an agarose gel showing the presence of HXV RNA in human prostate cancer tissue as determined by RTPCR. Ethidium bromide stained 1 % agarose gel electrophoresis of nested RT-PCR products. An HXV RNA was detected as a GAG-nested RT-PCR product in VP 10 (but not in VP 107 and VP27) prostate RNA samples, whereas only non-specific amplification products were observed in HEMI-nested lanes. Method: One µg DNase-treated total RNA, 1 uL of reverse primer (100 pmol), and 7.5 uL of H_2O were used per reaction. Denaturation was at 65°C for 5 min and the primers were annealed at room temperature for 5 min. Ten µl reaction mix (l0X buffer (Stratascript) 2µL, 12.5mM dNTPs 0.8µL, H20 3.2µL, 0.1M DTT 2µL, and RT 2µL) were added and the sample were incubated at 42°C for 1 hr. Two µL of each RT reaction was then used to seed the PCRs. All primers were at 100 pmol/uL. The PCR recipe for a 50 uL reaction is as follows: 10X PCR buffer (Stratascript) 5 µL, 50mM $MgCl_2$ 2µL, outside forward primer 0.5µL, outside reverse primer 0.5µL, 25mM dNTPs 0.5µL (or 1µL of 12.5mM), Taq DNA polymerase 0.5µL (5U/µL Invitrogen Inc.), H_2O to 50µLs. PCR parameters: denaturation at 94°C for 2 min, [94°C for 45 sec, outside primer annealing for 45 sec, 72°C for 1.5 minutes] X 30 cycles, followed by an elongation step at 72°C for 7 min. One µl of the outside PCR was used to seed the inside PCR. The thermalcycling parameters for the inside

PCR were exactly the same as the outside, with the exception of primer annealing temperatures. Ld = DNA ladder, - = w/o cDNA.

Figure 7 is an agarose gel showing the presence of HXV-related RNA in LNCaP (clone R) cell line as determined by RT-PCR. Ethidium bromide stained 1 % agarose gel electrophoresis of RT-PCR products. One μg of reverse transcribed (DNase-treated) total RNA from prostate cancer cell lines $LNCaP_R$, PC3, and DU 145 and normal prostate epithelial cell (PrEC) RNA were amplified by RT-PCR using primers specific to the viral *env* region (top) and GAPDH (bottom). Ld = DNA ladder, - without cDNA, + with cDNA.

Figure 8 shows that LNCaP cells produce HXV-related virus as determined by reverse transcriptase assays. RT activity observed in virus infected LNCaP cell media at 2, 4 and 8 days of growth (twelve-hour exposure). Media from 2, 4 and 8 days was diluted 1:10 with fresh media before RT assay. Mock reactions containing no media were used as a negative control.

Figure 9 shows the presence of virus in LNCaP cell line as determined by fluorescence *in situ* hybridization. FISH analysis on cytoblocks prepared from $LNCaP_R$ (A), PC3 (B) and DU145 (C) cells. The virus FISH probe was generated using a 2.14kb segment of the viral *env* genome. (A) Positive fluorescent green signals were seen in both the cytoplasm and nucleus of the $LNCaP_R$ cells indicating labeling of both viral RNA and DNA. (B) Absence of fluorescent signal in PC3 cells and (C) DU145 cells. Method: The deparaffinized slides were rehydrated through a series of decreasing ethanol concentrations. The rehydrated tissue was subjected to target retrieval for 40 min at ~95°C, then allowed to cool to room temperature for 20 min. The tissue was rinsed in H_20, and then 300μl proteinase K was applied directly to slides for 10 min at room temperature. The tissue was rinsed again in H_2O and dehydrated through increasing EtOH concentrations, then allowed to air dry. Ten ul of probe mix was applied and the slides were coverslipped, debubbled and sealed with rubber cement. The probe and target DNA were codenatured at 73°C. Hybridization occurred at 37°C overnight. The slides were stringently washed for 3 sec, and incubated for 1 min in a 2X SSC wash at 57°C. The slides were then rinsed with 2X SSC. Vectashield Mounting Medium with DAPI (Vectashield Inc.) counterstain was applied and the slides were left to incubate in the dark at room temperature for at least

30 min to allow the DAPI to fully bind the nucleic acids for clearer nuclear visualization. BLUE: DAPI, GREEN: Virus

Figure 10 shows the presence of viral RNA in human prostate cancer tissue. Confocal fluorescent microscopy of FISH on human prostate cancer TMA from two different homozygous mutant RNase L patients (A) Patient VP29, (B, C, D,) Patient VP62. Method: The deparaffinized human prostate cancer TMA slides were rehydrated through a series of decreasing EtOH concentrations. The rehydrated tissue was subjected to target retrieval for 40 min at ~95°C, then allowed cool down to room temperature for 20 min. The tissue was rinsed in H_20, and then -300µl proteinase K was applied directly to slides for 10 min at room temperature. The tissue was rinsed again in H_20 and dehydrated through increasing ethanol concentrations, then allowed to air dry. Ten ul's of probe mix was applied and the slides were coverslipped, debubbled and sealed with rubber cement. The probe and target DNA were co-denatured at 73°C. Hybridization occurred at 37°C overnight. The slides were stringently washed for 3 sec, and incubated for 1 min in a 2X SSC wash at 57°C. The slides were then rinsed with 2X SSC. Vectashield Mounting Medium with DAPI (Vectashield Inc.) counterstain was applied and the slides were left to incubate in the dark at room temperature for at least 30 min to allow the DAPI to fully bind the nucleic acids for clearer nuclear visualization. BLUE: DAPI, GREEN: Virus

Figure 11 shows the amino acid sequences of the HXV_{35} gag peptide (SEQ ID NO: 3), the HXV gag peptides (SEQ ID NOS.: 7, 8, 9) used to raise antibody in rabbits and a hydropathy plot generated from HXV35 gag protein.

Figure 12 shows western blots using (A) anti-NC, and (B) anti-MA antibodies on separate proteins from (lane 1) $LNCaP_R$, (lane 2) PC3, and (lane 3) DU145 cells.

Figure 13 shows the amino acid sequence of HXV_{35} (SEQ ID NO: 2).

Figure 14 shows that amino acid sequence of HXV_{35} gag peptide (SEQ ID NO: 3).

Figure 15 shows the amino acid sequence of the HXV_{35} pro-pol peptide (SEQ ID NO: 4).

Figure 16 shows the amino acid sequence of the HXV_{35} env peptide (SEQ ID NO:5).

Figure 17 is a partial nucleotide sequence of an HXV-LNCaP viral sequence, obtained as an RNA RTPCR product (7084-7750 bp) (SEQ ID NO:6) and which has 97% identity with the HXV_{35} env nucleotide sequence and has 97.6% similarity with the HXV_{35} env amino acid sequence, indicating that the HXV-LNCaP and the HXV_{35} are variants of the same virus.

Figures 18A-18D show the immunohistochemical analysis of prostate from patient VP62 which indicates that homozygous R462Q RNase L prostates are XMRV positive by FISH. Figures 18A-18D, Left panel: Immunohistochemistry (IHC)(red) with a mouse anti-cytokeratin AE1/AE3 (20:1 mixture of AE1 to AE3) monoclonal Ab cocktail from Roche. The anti-keratin AE1 Ab recognizes the 56.5, 50, 50', 48 and 40 kDa keratins of the acidic subfamily. The anti-keratin AE3 Ab recognizes all 6 members of the basic subfamily. The IHC (red) labels prostate epithelial cells. The green label is FISH for HXV_{35} env probe as described in the legend to Fig. 9. Blue is DAPI staining of nuclei. Figures 18A-18D, Right panel. Hematoxylin and eosin staining.

Figure 19 immunohistochemical analysis of LNCaP, clone R, which shows that LNCaP clone R is XMRV positive by IHC with antibody to p30 capsid. Figure 19, Left panel: immunohistochemistry with specific antiserum prepared in goats to Rauscher mouse leukemia virus p30 protein (ATCC, catalog no. VR-1564AS-Gt) showing labeling of HXV gag protein plus DAPI (blue) staining of nuclei. Right panel: DAPI staining of nuclei.

Figure 20 shows doubling labeling by IHC and FISH of HXV in LNCaP infected cells which shows that LNCaP clone R is XMRV positive by FISH and IHC. Immunohistochemistry with specific antiserum prepared in goats to Rauscher mouse leukemia virus p30 protein (ATCC, catalog no. VR-1564AS-Gt) showing labeling of HXV gag protein plus DAPI (blue) staining of nuclei plus FISH labeling(green) of virus nucleic acid as described in Figures 18A-18D.

Figure 21 shows the results of a reverse transcriptase assay which shows that the virus (XMRV-LN) produced by LNCaP cells is infectious when used to infect DU145 human prostate cancer cells. DU145 cells were infected with 500 uls of LNCaP infected supernatant for 3 hours in the presence of 8ug/ml polybrene, -FBS. Virus was monitored by reverse transcriptase assay.

Figures 22A-22B shows XMRV detection by DNA microarrays and RT-PCR. Figure 22A shows Virochip hybridization patterns obtained for tumor samples from 19 patients. The samples (x axis) and the 502 retroviral oligonucleotides present on the microarray (y axis) were clustered using hierarchical clustering. The color bar indicates the range of observed hybridization intensities. The magnified view shows a selected cluster containing oligonucleotides with the strongest positive signal. Samples from patients with QQ *RNASEL* genotype are shown in red, and those from RQ and RR individuals as well as controls are in black. Figure 22B shows results of nested RT-PCR specific for XMRV *gag* gene. Amplified *gag* PCR fragments along with the corresponding human GAPDH amplification controls were separated by gel electrophoresis using the same lane order as in the microarray cluster.

Figures 23A-23C show the complete genome of XMRV. Figure 23A is chematic map of the 8185 nt XMRV genome. LTR regions (R, U5, U3) are indicated with boxes. Predicted open reading frames encoding Gag, Gag-Pro-Pol, and Env polyproteins are labeled in green. The corresponding start and stop codons (AUG, UAG, UGA, UAA) as well as the alternative Gag start codon (CUG) are shown with their nucleotide positions. Similarly, splice donor (SD) and acceptor (SA) sites are shown and correspond to the spliced 3.2 Kb Env subgenomic RNA (wiggled line). Figure 23B shows the cloning and sequencing of XMRV VP35 genome. Clones obtained by probe recovery from hybridizing microarray oligonucleotides (blue bars) or by PCR from tumor cDNA (black bars) were sequenced. Primers used to amplify individual clones (Table 10) were derived either from the genome of MTCR (black arrows) or from overlapping VP35 clones (blue arrows). Figure 23C shows the genome sequence similarity plots comparing XMRV VP35 with XMRV VP42, MuLV DG-75, and MTCR. The pair-wise alignments were made using AVID (Bray, N., *et al., Genome Res., 13*:97-102 (2003)), and plots were generated using mVISTA (Frazer, KA, *et al., Nucleic Acids Res., 32*:W273-279 (2004)) with the default window size of 100 nucleotides. Y axis scale represents percent nucleotide identities from 50 to 100%.

Figures 24 shows the phylogenetic analysis of XMRV based on complete genome sequences. Complete genomes of XMRV VP35 and VP42; MTCR;

MuLVs DG-75, MCF1233, Akv, Moloney, Friend, and Rauscher; Feline leukemia virus (FLV); Koala retrovirus (KoRV); and Gibbon ape leukemia virus (GALV) were aligned using ClustalX (see Materials and Methods). An unrooted neighbor joining tree was generated based on this alignment, excluding gaps and using Kimura's correction for multiple base substitutions. Bootstrap values (N=1000 trials) are indicated. MuLV genomes are labeled as xenotropic (X), polytropic (P), or ecotropic (E) based on published experimental evidence (Raisch KP, *et al.*, *Virology 308*: 83-91; O'Neill RR., *et al.*, *J Virol 53*: 100-106; Perryman S., *et al.*, *Nucleic Acids Res 19*: 6950; Shinnick TM., *et al.*, *Nature 293*: 543-548; Sijts EJ., *et al.*, *Virus Res 34*: 339-349; Khimani AH., *et al.*, *Virology 238*: 64-67; Lenz J., *et al.*, *J Virol 42*: 519-529).

Figure 25 shows multiple-sequence alignment of protein sequences from XMRV and related MuLVs spanning SU glycoprotein variable regions (VRA and VRB) known to determine receptor specificity (Battini JL., *et al.*, *J Virol 66*: 1468-1475; Tailor CS., *et al.*, *Immunol 281*: 29-106). Env protein sequences from XMRV VP35 (SEQ ID NO.: 10) and VP42 (SEQ ID NO.: 11); MTCR (SEQ ID NO.: 14); MuLVs DG-75 (SEQ ID NO.: 12), NZB-9-1 (SEQ ID NO.: 13), MCF1233 (SEQ ID NO.: 15), Akv (SEQ ID NO.: 19), Moloney (SEQ ID NO.: 18), Friend (SEQ ID NO.: 16), and Rauscher (SEQ ID NO.: 17) were aligned using ClustalX with the default settings. Sequences are labeled as xenotropic (X), polytropic (P), or ecotropic (E) based on published experimental evidence (Raisch KP., *et al.*, *Virology 308*: 83-91; , O'Neill RR., *et al.*, *J Virol 53*: 100-106; Perryman S., *et al.*, *Nucleic Acids Res 19*: 6950; Shinnick TM., *et al.*, *Nature 293*: 543-548; Sijts EJ., *et al.*, *Virus Res 34*: 339-349, Khimani AH., *et al.*, *Virology 238*: 64-67; Lenz J., *et al.*, *J Virol 42*: 519-529). Variable regions VRA and VRB are shown as boxes. Nucleotide positions to the right of the alignment are relative to the Env start codon.

Figure 26 shows multiple-sequence alignment of 5'gag leader nucleotide sequences from XMRV and related MuLVs. Sequences extending from the alternative CUG start codon to the AUG start codon of gag derived from XMRV VP35 (SEQ ID NO.: 20) and VP42 (SEQ ID NO.:21); MTCR (SEQ ID NO.:22), MuLVs DG-75, (SEQ ID NO.: 24), MCF1233, (SEQ ID NO.: 23) and Friend (SEQ ID NO.: 25) were aligned with ClustalX using the default settings. Predicted amino

acid translation corresponding to the VP35 sequence is shown above the alignment; "*" indicates a stop. Nucleotide positions to the right of the alignment are relative to the C of the alternative CUG start codon.

Figures 27A-27B shows a comparison of XMRV sequences derived from tumor samples of different patients. Figure 27A shows a phylogenetic tree based on the 380 nt XMRV gag RT-PCR fragment from the 9 positive tumor samples and the corresponding sequences from MTCR; and MuLVs DG-75, MCF1233, Akv, Moloney, Rauscher and Friend. The sequences were aligned using ClustalX, and the corresponding tree was generated using the neighbor joining method (see materials and methods). XMRV fragments from tumor samples are indicated in red. Figure 27B sows a phylogenetic tree based on a 2500 nt pol PCR fragment from the 9 XMRV-positive tumor samples. PCR fragments were obtained using amplified cDNA as the template. The tree was constructed as described in Figure 27A.

Figures 28A-28B shows the complete nucleotide sequence of XMRV VP35 (SEQ ID NO.: 26). Numbers to the left indicate nucleotide coordinates relative to the first nucleotide. Predicted open reading frames for Gag (SEQ ID NO.: 27); Gag-Pro-Pol (SEQ ID NO.: 28) and Env (SEQ ID NO.: 29) polyproteins are shown below the corresponding nucleotides. Characteristic 24-nt deletion in the 5' gag leader is indicated with a triangle. Other genome features as well as primers used in the nested *gag* RT-PCR are shown as arrows.

Figures 29A- 29B show phylogenetic analysis of XMRV based on predicted Gag-Pro-Pol (Figure 29A) and Env (Figure 29B) polyproteins. Predicted Gag-Pro-Pol and Env sequences of XMRV VP35 and VP42 as well as the corresponding sequences from MTCR; MuLVs DG-75, MCF1233, Akv, Moloney, Friend, and Rauscher; Feline leukemia virus (FLV); Koala retrovirus (KoRV); and Gibbon ape leukemia virus (GALV) were aligned using ClustalX. Resulting alignments were used to generate unrooted neighbor joining trees (see Example 2, Materials and Methods). Bootstrap values (N=1000 trials) are indicated.

Figure 30 shows the presence of XMRV nucleic acid in prostatic tissues determined by FISH. Prostatic tissue from prostate cancer cases VP62 (panels A to C) and VP88 (panels D to F) were visualized by H&E staining (left) after being probed with SpectrumGreenTM labeled XMRV-35 DNA probes (enlargements on

right). Nuclei were counterstained with DAPI in FISH panels. Arrows in H&E photographs indicate FISH positive cells. Bars shown in panels are 10 μm. Enlargements are images capture with a 63X 1.4 N.A. objective zoom 2.

Figure 31 shows the characterization of XMRV-infected prostatic stromal cells by FISH and concomitant FISH/immunofluorescence. Using a tissue microarray, prostatic tissue from prostate cancer case VP62 analyzed by FISH with XMRV-35 probes (green) (panels A&C) and corresponding H&E staining (panels B&D), respectively. Arrowheads indicate FISH positive cells. The enlargement images (on right) are FISH positive cells (arrows) captured as described in the legend to Figure 30. The FISH positive cell in panels A and B is a stromal fibroblast; in panels C and D a mitotic figure in a stromal cell, and in E and F a stromal hematopoietic element. (Panel E) Concomitant staining for XMRV by FISH (green) and cytokeratin AE1/AE3 by immunofluorescence (red). Bars shown in panels are 10 μm.

Figure 32 shows FISH on prostatic tissues of case VP88 with XMRV-35 probes (green) (panel A) and control probes (red and green) specific for two arms of chromosome 1 (panel B) (Example 3, Materials and Methods). Bars shown in panels are 10 μm. Enlargements were performed as described in the legend to Figure 31.

Figure 33 shows the presence of Gag protein in prostate tissues. IHC with monoclonal antibody to SFFV Gag p30 was performed on prostatic tissue of cases VP62 (panels A to D), VP88 (panels E to H), and VP51 (panels I, J). Visualization of bi-functional chromagen indicating Gag protein shown by immunofluorescence (panels A, B, E, F and enlargements [right]) and bright field (panels C, D, G, H and enlargements [right]) is detected by granular cytoplasmic staining (red) in stromal cells of the homozygous RNase L 462Q cases VP62 and VP88, but not in the homozygous RNase L 462R case VP51 (panels I&J). The positive cells in G and H are stromal lymphocytes. Bars in panels A, B and I were 5 μm and in panels E and F were 10 μm. Enlargements were performed as described in the legend to Figure 31.

Figures 34A-34D show the following. Figure 34A shows RT-PCR results indicating the presence of a gammaretrovirus in LNCaP-R cells. PCR amplicons for

a 700bp env-LTR region are separated on a 1% agarose gel stained with ethidium bromide. Prostate cell lines used are indicated at the top. RT-PCR for the eighth exon of human GAPDH mRNA is included for comparison. Figure 34B shows phylogenetic analysis of XMRV LNCaP-R based on complete genome sequences. Complete genomes of XMRV LNCaP-R; XMRV VP35 and VP42 (Urisman A., *et al.*, *PLOS Pathogens*; MTCR; MuLVs DG-75, MCF1233, Akv, Moloney, Friend, and Rauscher; Feline leukemia virus (FLV); Koala retrovirus (KoRV); and Gibbon ape leukemia virus (GALV) were aligned with ClustalX using default settings. An unrooted neighbor-joining tree was generated (see Materials and Methods) based on the alignment. Bootstrap values (N=1000 trials) are indicated. MuLV genomes are labeled as xenotropic (X), polytropic (P), or ecotropic (E). Figure 34C shows multiple-sequence alignment of 5'gag leader nucleotide sequences from XMRV-LNCaP RV and related MuLVs. Using default settings of ClustalX (Example 3, Materials and Methods) sequences extending from the alternative CUG start codon to the AUG start codon of gag derived from XMRV-LNCaP RV (SEQ ID NO.: 30), XMRV-VP35 (SEQ ID NO.: 20) and VP42 (SEQ ID NO.: 21), MTCR (SEQ ID NO.: 22), MuLVs DG-75 (SEQ ID NO.: 24), MCF1233 (SEQ ID NO.: 23), and Friend MuLV (SEQ ID NO.: 25) were aligned. Nucleotide positions to the right of the alignment are relative to the first position of the alternative CUG start codon. "*" above the aligned nucleotide sequences indicates a stop codon. Figure 34D shows genome sequence similarity plots comparing XMRV-VP35, MTCR, MCF1233 and MuLV DG-75 relative to XMRV-LNCaP RV. The alignments were created using AVID (Bray N., *et al.*, *Genome Res 13*: 97-102). Plots were visualized using VISTA (Frazer KA., *et al.*, *Nucleic Acids Res 32*: W273-279) with the default window size of 100 nucleotides. Y axis scale represents percent nucleotide identities from 50 to 100%. Similarity plots are shown relative to the predicted open reading frames for XMRV LNCaP-R, which are represented as black bars.

Figures 35A-35C show the following. Figure 35A is a schematic map of the 8185 nt XMRV-LNCaP RV genome. LTR regions (R, U5, U3) are indicated with open boxes. Predicted open reading frames encoding Gag, Gag-Pro-Pol, and Env polyproteins are labeled in green. The nucleotide positions of the corresponding

start and stop codons (AUG, UAG, UGA, UAA) as well as the alternative Gag start codon (CUG) are indicated. Similarly, splice donor (SD) and acceptor (SA) nucleotide positions corresponding to the spliced 3.2kb env subgenomic RNA sites are shown. Northern Blot probes used in (Figure 35B) are represented as black bars relative to the positon within the genome. Figure 35B shows the presence of viral transcripts in the LNCaP-R cell line by Northern Blot analysis. Total RNA isolated from the cell lines LNCaP R, Raji, NIH3T3 and DU145 was separated on a 1.2% formaldehyde agarose gel, blotted and probed with radiolabeled DNA corresponding to nucleotide positions 7780-7991 within the LTR region (left). Position of the full length and spliced message are represented with an arrow. The blot was stripped (Material and Methods) and reprobed using a radiolabeled XMRV VP35 Gag probe (positions 603-957) capable of detecting only the full length transcript (right). Size markers to the left indicate the sizes of the human 18S ribosomal RNA (1.9kb) and the 28S ribosomal RNA (5kb). Figure 35C shows the screening for the presence of LNCaP-RV in different cell lines with affinity purified polyclonal antibody against an XMRV VP35 Gag (NC) peptide (red) in LNCaP-R, LNCaP-FGC, DU145 and PC3 cells (as indicated). Nuclei were counterstained using DAPI. Immunofluorescence images were captured using a Texas Red filter. Bar shown in the panel is 30 μm.

Figures 36A-36C show the following. Figure 36A shows viral integration sites in the LNCaP-R cell line. Southern blot of genomic DNA isolated from the human cell lines LNCaP-R, LNCaP-FGC, DU145 and the mouse cell line NIH3T3 digested with PstI and probed with radiolabeled probe derived from XMRV-VP35 U3 LTR region (nucleotide positions 7780-7991). Positions of molecular weight markers separated in a 0.8% agarose gel are indicated on the right. Multiple integration sites within the DNA of LNCaP-R cells are pointed out with arrows on the left. Figure 36B shows ethidium bromide staining of the PstI digested genomic DNA in (Figure 36A) shows relative amounts of genomic DNA loaded. Figure 36C shows sequences of three different XMRV LNCaP-R integration sites (SEQ ID NOS.: 31, 32, 33). The sites were determined by a modified linker mediated PCR technique (see Example 3, Material and Methods). Viral sequences are boxed; above each sequence chromosome positions of the corresponding integration sites

are indicated.

Figures 37A-37C show XMRV LNCaP-R is an infectious virus. LNCaP-FGC cells were either mock infected (-Virus) or infected with LNCaP-RV (+Virus) (Example 3, Material and Methods). Figure 37A shows reverse transcriptase assays using cell supernatants were performed in duplicate (-Virus) or in quadruplicate (+Virus) at the indicated times after infection. Autoradiograph of the reverse transcriptase assays spotted to DEAE paper is shown on the left. Quantitation of reverse transcriptase activity by phosphorimage analysis is shown on the right. Figure 37B shows Northern Blot analysis of the mock (-) or LNCaP-RV (+) infected cell lines 24 hr after infection. Total RNA was extracted, separated on a 1.2% Formaldehyde gel, blotted and probed with a radiolabeled XMRV VP35 LTR probe (positions 7780- 7991). Size markers to the left indicate the sizes of the human 18S ribosomal RNA (1.9kb) and the 28S ribosomal RNA (5kb). Figure 37C shows Southern Blot analysis of the mock (-) or LNCaP-RV (+) infected cell lines 36 hr after infection. Genomic DNA of LNCaP-FGC and DU145 cells infected or mock infected was extracted (see Example 3, Material and Methods), digested with PstI, separated on a 0.8% Agarose-gel, blotted and probed with a radiolabeled XMRV VP35 U3 LTR probe (positions 7780-7991). Numerous de novo integration events are indicated by brackets to the left. NIH3T3 genomic DNA was used as a positive control. Molecular weight markers are shown on the right.

DETAILED DESCRIPTION OF THE INVENTION

As described herein, the role of the antiviral enzyme, RNase L, in prostate tumor biology was investigated. A clinical study was performed in which 150 prostate cancer patients scheduled for prostatectomies were genotyped for the most common germline mutation in RNase L (R462Q). RNA isolated from the prostate tissues was processed for analysis on Virochips, a comprehensive DNA microarray of viral sequences. A novel gammaretrovirus related to xenotropic strains of murine leukemia virus (referred to herein as xenotropic murine leukemia virus (MLV) related virus (XMRV) was identified and cloned from patients homozygous for the RNase L mutation. In a particular embodiment, the XMRV is human xenotropic virus (HXV).HXV infection of the prostate was common in patients homozygous for the

RNase L mutation R462Q (about 60% incidence), but relatively infrequent in heterozygous and in homozygous wild type patients (incidence of 10% or less). *In situ* methods confirmed the presence of HXV nucleic acid in prostates.

Accordingly, the present invention provides for isolated nucleic acid sequences encoding XMRV isolated polypeptides comprising amino acid sequences of XMRV; vectors comprising the viral nucleic acid sequences; cells comprising the vectors; antibodies and antigen binding fragments thereof which have binding specificity for XMRV; methods of producing XMRV; methods of detecting or screening for XMRV (*e.g.,* in an individual); methods of identifying agents that inhibit XMRV; methods of inducing an immune response to XMRV; methods of treating disease associated with the presence of XMRV in an individual (*e.g.,* cancer such as prostate cancer); methods of detecting asymptomatic cancer (*e.g.,* prostate cancer); methods of identifying an individual at risk for developing cancer (*e.g.,* prostate cancer); and kits for detecting the XMRV.

Carcinoma of the prostate is the second leading cause of cancer deaths in American men and the most frequent visceral cancer (Kumar, V., *et al., Basic Pathology*, 6th ed., pp. 584-588, W. B. Saunders Co., Philadelphia). Among populations in the U.S., African Americans have the highest risk. The American Cancer Society estimated that there were about 190,000 new cases and 30,000 deaths from prostate cancer in the US in 2003. Genetics, aging, hormonal, and environmental risk factors all play roles in the pathogenesis of prostate cancer (Nelson WG., *et al., N Engl J Med, 349*(4):366-81, 2003). Remarkably, men with three or more first-degree relatives with prostate cancer have an 11-fold increased risk compared with men that have no family history of the disease (Steinberg GD., *et al., Prostate, 17*(4):337-47, 1990). Segregation analysis supports the existence of rare autosomal dominant, highly penetrant gene(s) in hereditary prostate cancer (HPC) with early onset (Carter BS., *et al., Proc Natl Acad Sci, 89*(8):3367-71, 1992). Several different HPC genes are predicted to collectively account for about 43% of early onset (less than or equal to 55 years) disease and 9% of all cases of prostate cancer. These inherited prostate cancer susceptibility genes are believed to function at an early stage in the molecular pathogenesis of prostate cancer, during the progression of normal prostate epithelium to proliferative inflammatory atrophy (PIA) (Nelson WG., *et al.,*

N Engl J Med, 349(4):366-81, 2003). Chronic or recurrent microbial infections are suspected initiating events in PIA. PIA lesions, in turn, may be precursors of prostate intraepithelial neoplasia (PIN) and, after many years, lead to overt carcinoma and finally to metastatic cancer. The prostate has been suggested by others to be a resident organ for mutiple viral infections including the human BK polyomavirus (Das D., *et al., Oncogene, 23*(42):7031-46, 2004) and HPV (Zambrano A., *et al., Prostate, 53*(4):263-76, 2002). In addition, a large case-control study showed an association between prostate cancer frequency and a history of sexually transmitted diseases (Hayes RB., et al., *Br J Cancer, 82*(3):718-25, 2000). Interestingly, five candidate prostate cancer susceptibility alleles function in immunity and/or inflammation *(HPC1/RNASEL, TLR4, MIC-1, MSR-1* and *PON1)* (Carpten, J., *et al., Nature Genetics* 30:181-4, 2002; Zheng SL., *et al., Cancer Res. 64*(8):2918-22, 2004; Lindmark F., *et al., J Natl Cancer Inst. 96*(16):1248-54, 2004; Xu, J., *et al., Nat. Genet.* 32(2):321-5, 2002; Marchesani M., *et al., J Natl Cancer Inst. 95*(11):812-8, 2003).

HPC1, the prototype of the gene family, was linked to chromosome 1q24-25 in 1996 (Smith *et al.,* 1996) and to the *RNASEL* gene at 1q25 in 2002 (Carpten, J., *et al., Nature Genetics* 30:181-4, 2002*). HPCI/RNASEL* encodes a regulated nuclease, RNase L, that functions in the interferon (IFN) antiviral response (Clemens MJ, et al., *Cell 13*(3):565-72, 1979; Zhou A., et al., *Cell 72*(5):753-65, 1993; Hassel BA, *et al., EMBO J. 12*(8):3297-304, 1993). IFN treatment of cells induces a family of 2-5A synthetases that are stimulated by double stranded RNA to convert ATP to PPi and a series of short 2' to 5' linked oligoadenylates, collectively referred to as 2-5A (Kerr IM, *et al., Proc Natl Acad Sci U S A. 75*(1):25660, 1978). (FIG. 1). The only well established function of 2-5A is activation of RNase L leading to inhibition of the replication of certain viruses, including Coxsackievirus (Flodstrom-Tullberg, M., *et al., J. Immunol., 174,* 1171-1177, 2005). Upon binding 2-5A, RNase L converts from inactive monomers to active dimers (Dong B., *et al., J Biol Chem. 270*(8):4133-7, 1995). Sustained activation of RNase L by 2-5A binding leads to cleavage of 28S and 18S rRNA and to caspase-dependent apoptosis (Rusch L., et al., *J Interferon Cytokine Res. 20*(12):1091-100, 2000). RNase L-mediated apoptosis is accompanied by cytochrome C release from mitochondria and requires JNK and

caspase-3 activity (Iordanov MS, *et al.*, *Mol Cell Biol. 20*(2):617-27, 2000); Li G., *et al.*, *J Biol Chem. 279*(2):1123-31, 2004; Malathi K., et al., *Cancer Res. 64*(24):9144-51, 2004). Previously it has been demonstrated that activation of RNase L by 2-5A leads to apoptosis of late-stage human prostate cancer cell lines whereas naturally-occurring mutations in *RNASEL* allow cell survival (Xiang Y., *et al.*, *Cancer Res. 63*(20):6795-801, 2003). Involvement of *RNASEL/HPC1* in hereditary prostate cancer is supported by identification and association of different mutations (MII, E265X, 471ΔAAAG, & R462Q) with disease onset and/or frequency (Carpten, *et al.*, *Nature Genetics 30*:181-4, 2002; Rokman A., *et al.*, *Am J Hum Genet. 70*(5):1299-304, 2002; Rennert H., *et al.*, *Am J Hum Genet. 71*(4):981-4, 2002; Casey, G., *et al.*, *Nat Genet. 32*(4):581-3, 2002; Silverman, R.H, *Biochemistry* 72, 25;*42*(7):1805-12., 2003) (FIG. 2). Functional or epidemiological data for a role of *RNASEL* in HPC have been observed in most, but not all studies (Downing SR., *et al.*, *Clin Prostate Cancer 2*:177-80, 2003; Kotar K., *et al.*, *J Med Genet 40*: e22, 2003.

Recently the R462Q variant of *RNASEL* has been implicated in unselected (including both familial and non-familial) prostate cancer cases (Casey G., *et al.*, *Nat Genet. 32*(4):581-3, 2002). Interestingly, the R462Q variant of RNase L had about 3-fold reduced catalytic activity *in vitro*. The reduced ribonuclease activity of RNase L R462Q is due to a decreased capacity to dimerize into the active form of the enzyme (Xiang Y., *et al.*, *Cancer Res. 63*(20):6795-80, 2003). An expanded study was performed on DNA isolated from 423 unselected prostate cancer cases and 454 unaffected sibling controls (Casey G., *et al.*, *Nat Genet. 32*(4):581-3, 2002). *A* significant association of the R462Q variant with cases was observed (P=0.011). The odds ratios indicated that carrying one copy of the R462Q variant gene increased risk of prostate cancer by about 1.5-fold, while having two variant alleles doubled the risk. On the other hand, another variant of RNase L, D541E, was not associated with increased risk of prostate cancer and did not affect RNase L activity. Results implicated R462Q in up to 13% of cases, which would make it the most prevalent genetic marker for prostate cancer (and possibly for any of the common cancers). Therefore, R462Q could be an important risk marker for prostate cancer in the general male population.

As described herein, viruses in tumor-bearing prostates were identified and compared to virus frequency in men with different *RNASEL* genotypes. Because inactivating mutations in RNase L are relatively rare, the studies focused on the missense variant R462Q. Traditional viral detection methods have several disadvantages including failure of some viruses to grow in cell culture, limits to the number of DNA sequences that can be simultaneously amplified by multiplex PCR, antibody availability and evolving viral serotypes. Therefore, to determine an association of certain viruses with prostate cancer, a microarray-based detection method (Virochip) was used for genotyping viral pathogens developed at UCSF by Drs. DeRisi and Ganem (Wang D., *et al.*, *Proc Natl Acad Sci U S A. 99*(24):15687-92, 2002; Wang D., *et al.*, *Biol. 1*(2):E2. Epub, 2003). These microarrays contain long (70-mer) oligonucleotides that can detect and identify several hundred different types of viruses. Because the array contains highly conserved sequences within viral nucleic acids, it can detect viruses not explicitly represented. A novel gammaretrovirus related to xenotropic strains of murine leukemia virus (referred to herein as xenotropic murine leukemia virus (MLV) related virus (XMRV) was identified and cloned from patients homozygous for the RNase L mutation.

Accordingly, the present invention provides an isolated or recombinant ***x***enotropic ***m***urine leukemia virus (MLV) ***r***elated ***v***irus (XMRV). The present invention also relates to isolated or recombinant XMRV proviruses and retroviral particles (*e.g.*, produced by cells infected with XMRV). In one embodiment, the XMRV virus is an isolated or recombinant human xenotropic virus (HXV).

The invention embodies virus deposited with the A.T.C.C., 10801 University Boulevard, Manassass, VA, 02110-2209 on March 30, 2005, designated A.T.C.C. No. _____, or virus derived therefrom. The virus deposited with the A.T.C.C. is designated Human Xenotropic Virus (HXV) – LNCaP (HXV-LN) isolated from an LNCaP clone, animal (human). HXV is a retrovirus (gamma) related to murine leukemia virus (MLV), gag reacts with Rausher MLV P30 antibody (A.T.C.C. Accession No. VR-15645A-Gt) on Western blots. As indicated herein, the virus can be grown in cell lines LNCaP (A.T.C.C. Accession No. CRL-1740) and DU145 (A.T.C.C. Accession No. HTB-81). Cell line media and conditions for growth of the cell lines in which the virus can be grown include

RPMI 1640 with O-Glucose 2.0g/L, Glutamine (2.05mM) 300mg/L, Pyridoxine HCl 1.0mg/L, sodium bicarbonate 2g/L, fetal bovine serum (heat-inactivated) 10%, PEN/STREP 200 units, air, 95%: CO_2, 5%, 37°C. Infected cell (*e.g.,* LNCaP cells) supernatant fluid can be centrifuged (*e.g.,* at 12,000g for 15 minutes) followed by filtration (*e.g.,* through two successive 0.2 um filters).

Nucleic acid molecules

The present invention provides isolated XMRV nucleic acid molecules. By an "XMRV nucleic acid molecule" is meant a nucleic acid molecule that encodes an XMRV polypeptide. Such nucleic acid molecules include, for example, the XRMV nucleic acid molecule described in detail herein; an isolated nucleic acid comprising SEQ ID NO: 1; a complement of an isolated nucleic acid comprising SEQ ID NO: 1; an isolated nucleic acid encoding an XMRV polypeptide of SEQ ID NO: 2; a complement of an isolated nucleic acid encoding an XRMV polypeptide of SEQ ID NO: 2; a nucleic acid that is hybridizable under high stringency conditions to a nucleic acid molecule that encodes SEQ ID NO: 1 or a complement thereof; a nucleic acid molecule that is hybridizeable under high stringency conditions to a nucleic acid comprising SEQ ID NO: 1; and an isolated nucleic acid molecule that has at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 92, 94% 95%, 96%, 97%, 98% or 99% sequence identity with all or a portion of SEQ ID NO: 1, or a complement thereof. In one embodiment, the percent identity is determined over the full length of the XMRV nucleic acid molecule (e.g., the full length of SEQ ID NO:1). In another embodiment, the percent identity is determined over a portion of the XMRV nucleic acid molecule (*e.g.,* the portion encoding the gag, pro-pol and/or env polypeptide of XMRV). For example, the isolated nucleic acid molecule can have at least 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with a portion of SEQ ID NO: 1 that encodes the pol polypeptide of an XMRV (*e.g.,* SEQ ID NO:4).

The isolated nucleic acid molecules of the present invention can be RNA, for example, mRNA, or DNA, such as cDNA. DNA molecules can be double-stranded or single-stranded; single stranded RNA or DNA can be either the coding (sense) or non-coding (antisense) strand. The nucleic acid molecule can include all or a

portion of the coding sequence of the gene and can further comprise additional non-coding sequences such as introns and non-coding 3' and 5' sequences (including regulatory sequences, for example). Additionally, the nucleic acid molecule can be fused to a marker sequence, for example, a sequence that encodes a polypeptide to assist in isolation or purification of the polypeptide. Such sequences include, but are not limited to, FLAG tags, as well as sequences that encode a glutathione-S-transferase (GST) fusion protein and those that encode a hemagglutinin A (HA) polypeptide marker from influenza.

An "isolated," "substantially pure," or "substantially pure and isolated" nucleic acid molecule, as used herein, is one that is separated from nucleic acids that normally flank the gene or nucleotide sequence (as in genomic sequences) and/or has been completely or partially purified from other transcribed sequences (e.g., as in an RNA or cDNA library). For example, an isolated nucleic acid of the invention may be substantially isolated with respect to the complex cellular milieu in which it naturally occurs, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. In some instances, the isolated material will form part of a composition (for example, a crude extract containing other substances), buffer system, or reagent mix. In other circumstances, the material may be purified to essential homogeneity, for example, as determined by agarose gel electrophoresis or column chromatography such as HPLC. Preferably, an isolated nucleic acid molecule comprises at least about 50%, 80%, 90%, 95%, 98% or 99% (on a molar basis) of all macromolecular species present.

The XMRV nucleic acid molecule can be fused to other coding or regulatory sequences and still be considered isolated. Thus, recombinant DNA contained in a vector is included in the definition of "isolated" as used herein. Also, isolated nucleic acid molecules include recombinant DNA molecules in heterologous host cells, as well as partially or substantially purified DNA molecules in solution. "Isolated" nucleic acid molecules also encompass *in vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention. An isolated nucleic acid molecule or nucleotide sequence can include a nucleic acid molecule or nucleotide sequence that is synthesized chemically or by recombinant means. Therefore,

recombinant DNA contained in a vector are included in the definition of "isolated" as used herein.

Isolated nucleotide molecules also include recombinant DNA molecules in heterologous organisms, as well as partially or substantially purified DNA molecules in solution. *In vivo* and *in vitro* RNA transcripts of the DNA molecules of the present invention are also encompassed by "isolated" nucleotide sequences. Such isolated nucleotide sequences are useful in the manufacture of the encoded polypeptide, as probes for isolating homologous sequences (e.g., from other mammalian species) or for detecting expression of the gene in tissue (e.g., human tissue), such as by Northern blot analysis.

The present invention also pertains to variant XMRV nucleic acid molecules that are not necessarily found in nature but that encode an XMRV polypeptide. Thus, for example, DNA molecules that comprise a sequence that is different from a naturally-occurring XRMV nucleotide sequence but which, due to the degeneracy of the genetic code, encode an XMRV polypeptide of the present invention are also the subject of this invention.

The invention also encompasses XMRV nucleotide sequences encoding portions (fragments), or encoding variant polypeptides such as analogues or derivatives of an XMRV polypeptide. In one embodiment, a fragment of an XMRV nucleotide sequence comprises SEQ ID NO: 6. Such variants can be naturally-occurring, such as in the case of allelic variation or single nucleotide polymorphisms, or non-naturally-occurring, such as those induced by various mutagens and mutagenic processes. Intended variations include, but are not limited to, addition, deletion, and substitution of one or more nucleotides that can result in conservative or non-conservative amino acid changes, including additions and deletions. Preferably, the XMRV nucleotide (and/or resultant amino acid) changes are silent or conserved; that is, they do not alter the characteristics or activity of the XMRV polypeptide.

Other alterations of the XMRV nucleic acid molecules of the invention can include, for example, labeling, methylation, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, and carbamates), charged linkages (e.g., phosphorothioates or phosphorodithioates),

pendent moieties (e.g., polypeptides), intercalators (e.g., acridine or psoralen), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids).

The invention also pertains to XMRV nucleic acid molecules that hybridize under high stringency hybridization conditions, such as for selective hybridization, to a nucleotide sequence described herein (e.g., nucleic acid molecules that specifically hybridize to a nucleotide sequence encoding XMRV polypeptides described herein, and, optionally, have an activity of the XMRV polypeptide). In one embodiment, the invention includes variants described herein that hybridize under high stringency hybridization conditions (e.g., for selective hybridization) to a nucleotide sequence comprising the nucleotide sequence of SEQ ID NO: 1, and the complement of SEQ ID NO: 1. In another embodiment, the invention includes variants described herein that hybridize under high stringency hybridization conditions (e.g., for selective hybridization) to a nucleotide sequence encoding an amino acid sequence of SEQ ID NO: 2. In a preferred embodiment, the variant that hybridizes under high stringency hybridizations encodes a polypeptide that has a biological activity of an XMRV polypeptide (e.g., ability to infect prostate tissue).

Activities of XMRV include the ability to infect a cell (*e.g.,* a prostate cell), produce a provirus and produce retroviral particles.

Such nucleic acid molecules can be detected and/or isolated by specific hybridization (e.g., under high stringency conditions). "Stringency conditions" for hybridization is a term of art that refers to the incubation and wash conditions, e.g., conditions of temperature and buffer concentration, that permit hybridization of a particular nucleic acid to a second nucleic acid; the first nucleic acid may be perfectly (i.e., 100%) complementary to the second, or the first and second may share some degree of complementarity that is less than perfect (e.g., about or at least 70%, 75%, 85%, 94% 95%, 96%, 97%, 98%, 99%). For example, certain high stringency conditions can be used that distinguish perfectly complementary nucleic acids from those of less complementarity. "High stringency conditions," "moderate stringency conditions," and "low stringency conditions" for nucleic acid hybridizations are explained in Current Protocols in Molecular Biology (See Ausubel et al., *supra*, the entire teachings of which are incorporated by reference herein). The exact conditions that determine the stringency of hybridization depend

not only on ionic strength (e.g., 0.2XSSC or 0.1XSSC), temperature (e.g., room temperature, 42°C or 68°C), and the concentration of destabilizing agents such as formamide or denaturing agents such as SDS, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences, and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, equivalent conditions can be determined by varying one or more of these parameters while maintaining a similar degree of identity or similarity between the two nucleic acid molecules. Typically, conditions are used such that sequences at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90% or at least about 95% or more identical to each other remain hybridized to one another. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions that will allow a given sequence to hybridize (e.g., selectively) with the most similar sequences in the sample can be determined.

Exemplary hybridization conditions are described in Krause and Aaronson, Methods in Enzymology, 200:546-556 (1991), and also in Ausubel, et al., *supra*, which describes the determination of washing conditions for moderate or low stringency conditions. Washing is the step in which conditions are usually set so as to determine a minimum level of complementarity of the hybrids. Generally, starting from the lowest temperature at which only homologous hybridization occurs, each °C by which the final wash temperature is reduced (holding SSC concentration constant) allows an increase by 1% in the maximum extent of mismatching among the sequences that hybridize. Generally, doubling the concentration of SSC results in an increase in Tm of 17°C. Using these guidelines, the washing temperature can be determined empirically for high, moderate, or low stringency, depending on the level of mismatch sought.

For example, a low stringency wash can comprise washing in a solution containing 0.2XSSC/0.1% SDS for 10 minutes at room temperature; a moderate stringency wash can comprise washing in a prewarmed solution (42°C) solution containing 0.2XSSC/0.1% SDS for 15 minutes at 42°C; and a high stringency wash can comprise washing in prewarmed (68°C) solution containing 0.1XSSC/0.1%SDS

for 15 minutes at 68°C. Furthermore, washes can be performed repeatedly or sequentially to obtain a desired result as known in the art. Equivalent conditions can be determined by varying one or more of the parameters given as an example, as known in the art, while maintaining a similar degree of identity or similarity between the target nucleic acid molecule and the primer or probe used.

The present invention also provides isolated XMRV nucleic acid molecules that contain a fragment or portion that hybridizes under highly stringent conditions to a nucleotide sequence comprising a nucleotide sequence selected from SEQ ID NO: 1, and the complement of SEQ ID NO: 1, and also provides isolated nucleic acid molecules that contain a fragment or portion that hybridizes under highly stringent conditions to a nucleotide sequence encoding an amino acid sequence selected from SEQ ID NO: 2. The nucleic acid fragments of the invention are at least about 15, preferably, at least about 18, 20, 23, or 25 nucleotides, and can be 30, 40, 50, 100, 200 or more nucleotides in length. Fragments that are, for example, 30 or more nucleotides in length, that encode antigenic polypeptides described herein are particularly useful, such as for the generation of antibodies as described herein.

In a related aspect, the XMRV nucleic acid fragments of the invention are used as probes or primers in assays such as those described herein. "Probes" or "primers" are oligonucleotides that hybridize in a base-specific manner to a complementary strand of nucleic acid molecules. Such probes and primers include polypeptide nucleic acids, as described in Nielsen et al., Science, 254, 1497-1500 (1991). As also used herein, the term "primer" in particular refers to a single-stranded oligonucleotide that acts as a point of initiation of template-directed DNA synthesis using well-known methods (e.g., PCR, LCR) including, but not limited to those described herein.

Typically, a probe or primer comprises a region of nucleotide sequence that hybridizes to at least about 15, typically about 20-25, and more typically about 40, 50 or 75, consecutive nucleotides of a nucleic acid molecule comprising a contiguous nucleotide sequence selected from: SEQ ID NO: 1, the complement of SEQ ID NO: 1, and a sequence encoding an amino acid sequence of SEQ ID NO: 2.

In preferred embodiments, a probe or primer comprises 100 or fewer nucleotides, preferably, from 6 to 50 nucleotides, and more preferably, from 12 to 30

nucleotides. In other embodiments, the probe or primer is at least 70% identical to the contiguous nucleotide sequence or to the complement of the contiguous nucleotide sequence, preferably, at least 80% identical, more preferably, at least 90% identical, even more preferably, at least 95% identical, or even capable of selectively hybridizing to the contiguous nucleotide sequence or to the complement of the contiguous nucleotide sequence. Often, the probe or primer further comprises a label, e.g., radioisotope, fluorescent compound, enzyme, or enzyme co-factor.

The nucleic acid molecules of the invention such as those described above can be identified and isolated using standard molecular biology techniques and the sequence information provided in SEQ ID NO: 1, and /or SEQ ID NO: 2. For example, nucleic acid molecules can be amplified and isolated by the polymerase chain reaction using synthetic oligonucleotide primers designed based on one or more of the nucleic acid sequences provided above and/or the complement of those sequences. Or such nucleic acid molecules may be designed based on nucleotide sequences encoding the amino acid sequences provided in SEQ ID NO: 2. See generally PCR Technology: Principles and Applications for DNA Amplification (ed. H.A. Erlich, Freeman Press, NY, NY, (1992); PCR Protocols: A Guide to Methods and Applications (Eds. Innis et al., Academic Press, San Diego, CA, (1990); Mattila et al., Nucleic Acids Res., 19: 4967 (1991); Eckert et al., PCR Methods and Applications, 1: 17 (1991); PCR (eds. McPherson et al., IRL Press, Oxford)); and U.S. Patent No. 4,683,202. The nucleic acid molecules can be amplified using cDNA, mRNA, or genomic DNA as a template, cloned into an appropriate vector and characterized by DNA sequence analysis.

Suitable amplification methods include the ligase chain reaction (LCR) (See Wu and Wallace, Genomics, 4:560 (1989); and Landegren et al., Science, 241:1077 (1988)), transcription amplification (Kwoh et al., Proc. Natl. Acad. Sci. USA, 86:1173 (1989)), and self-sustained sequence replication (See Guatelli et al., Proc. Nat. Acad. Sci. USA, 87:1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, that produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

The amplified DNA can be radiolabeled and used as a probe for screening a cDNA library, for example, one derived from human cells or any other desired cell type. Corresponding clones can be isolated, DNA can be obtained following *in vivo* excision, and the cloned insert can be sequenced in either or both orientations by art-recognized methods to identify the correct reading frame encoding a polypeptide of the appropriate molecular weight. For example, the direct analysis of the nucleotide sequence of nucleic acid molecules of the present invention can be accomplished using well-known methods that are commercially available. See, for example, Sambrook et al., Molecular Cloning, A Laboratory Manual (2nd Ed., CSHP, New York (1989)); and Zyskind et al., Recombinant DNA Laboratory Manual, (Acad. Press, (1988)). Using these or similar methods, the polypeptide and the DNA encoding the polypeptide can be isolated, sequenced, and further characterized.

Antisense and interfering RNA (*e.g.,* siRNA; shRNA) nucleic acid molecules of the invention can be designed using the nucleotide sequence of SEQ ID NO: 1, and/or the complement of SEQ ID NO: 1, and/or a portion of those sequences, and/or the complement of those portions or sequences, and/or a sequence encoding the amino acid sequence of SEQ ID NO: 2, or encoding a portion of SEQ ID NO: 2. The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

The antisense or interfering RNA of the invention comprise a sequence complementary to at least a portion of an RNA transcript of an informative gene. Absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex. The ability to hybridize will depend on both the degree of complementarity and the length of the RNA sequence. Generally, the larger the hybridizing nucleic acid, the more base mismatches with the RNA it may contain and still form a stable duplex. One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

In general, the isolated XMRV nucleic acid sequences of the invention can be used as to identify homologous viral sequencec and to detect an XMRV in an

individual or in a sample. The nucleic acid molecules of the present invention can also be used as therapeutic agents.

The XMRV nucleic acid molecules of the present invention can further be used to derive primers, to raise anti-polypeptide antibodies using DNA immunization techniques, and as an antigen to raise anti-DNA antibodies or elicit immune responses. Portions or fragments of the nucleotide sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. The XMRV nucleotide sequences of the invention can be used to identify and express recombinant polypeptides for analysis, characterization, or therapeutic use, or as markers for tissues in which the corresponding polypeptide is expressed, either constitutively, during tissue differentiation, or in diseased states. The nucleic acid sequences can additionally be used as reagents in the screening and/or diagnostic assays described herein, and can also be included as components of kits (e.g., reagent kits) for use in the screening and/or diagnostic assays described herein.

Standard techniques, such as the polymerase chain reaction (PCR) and DNA hybridization, may be used to clone XMRV homologs in other species, for example, mammalian homologs (*e.g.*, primate, feline, canine, rodent, ovine, bovine homologs). XMRV homologs may be readily identified using low-stringency DNA hybridization or low-stringency PCR with XMRV probes or primers. Degenerate primers encoding XMRV polypeptides may be used to clone XMRV homologs by RT-PCR.

Alternatively, additional XMRV homologs can be identified by utilizing consensus sequence information for XMRV polypeptides to search for similar polypeptides in other species. For example, polypeptide databases for other species can be searched for proteins with the XMRV domains characteristic of an XMRV nucleic acid molecule described herein. Candidate polypeptides containing such a motif can then be tested for their XMRV biological activities, using methods described herein.

Expression of the nucleic acid molecules

Another aspect of the invention pertains to nucleic acid constructs containing an XMRV nucleic acid molecule, for example, one selected from the group consisting of SEQ ID NOs: 1 or 6, and the complement of any of SEQ ID NOs: 1 or 6 (or portions thereof). Yet another aspect of the invention pertains to XMRV nucleic acid constructs containing a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 2 or portions of SEQ ID NO: 2 (*e.g.,* SEQ ID NOs: 3, 4, 5). The constructs comprise a vector (e.g., an expression vector) into which a sequence of the invention has been inserted in a sense or antisense orientation.

One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors, expression vectors, are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses) that serve equivalent functions.

Preferred recombinant expression vectors of the invention comprise a nucleic acid molecule of the invention in a form suitable for expression of the nucleic acid molecule in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into

the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences).

It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed and the level of expression of polypeptide desired. The expression vectors of the invention can be introduced into host cells to thereby produce polypeptides, including fusion polypeptides, encoded by nucleic acid molecules as described herein.

The recombinant expression vectors of the invention can be designed for expression of a polypeptide of the invention in prokaryotic or eukaryotic cells, e.g., bacterial cells, such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example, using T7 promoter regulatory sequences and T7 polymerase.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a nucleic acid molecule of the invention can be expressed in bacterial cells (e.g., *E. coli*), insect cells, yeast, or mammalian cells (such as Chinese hamster ovary cells (CHO)

or COS cells, human 293T cells, HeLa cells, NIH 3T3 cells, mouse erythroleukemia (MEL) cells, LNCaP cells, and DU145 cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "infection", "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing a foreign nucleic acid molecule (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*supra*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin, or methotrexate. Nucleic acid molecules encoding a selectable marker can be introduced into a host cell on the same vector as the nucleic acid molecule of the invention or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid molecule can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) an XMRV polypeptide of the invention. Accordingly, the invention further provides methods for producing an XMRV polypeptide using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the XMRV polypeptide is produced. In another embodiment, the method further comprises isolating the polypeptide from the medium or the host cell.

Polypeptides

The present invention features isolated or recombinant XMRV polypeptides, and fragments, derivatives, and variants thereof, as well as polypeptides encoded by nucleotide sequences described herein (e.g., other variants). As used herein, the term "polypeptide" refers to a polymer of amino acids, and not to a specific length; thus, peptides, oligopeptides, and proteins are included within the definition of a polypeptide.

As used herein, a polypeptide is said to be "isolated," "substantially pure," or "substantially pure and isolated" when it is substantially free of cellular material, when it is isolated from recombinant or non-recombinant cells, or free of chemical precursors or other chemicals when it is chemically synthesized. In addition, a polypeptide can be joined to another polypeptide with which it is not normally associated in a cell (e.g., in a "fusion protein") and still be "isolated," "substantially pure," or "substantially pure and isolated." An isolated, substantially pure, or substantially pure and isolated polypeptide may be obtained, for example, using affinity purification techniques described herein, as well as other techniques described herein and known to those skilled in the art.

By an "XMRV polypeptide" is meant a polypeptide having XMRV biological activity, for example, the ability to infect prostate cells. An XMRV polypeptide is also a polypeptide whose activity can be inhibited by molecules having XMRV inhibitory activity. Examples of XMRV polypeptides include a substantially pure polypeptide comprising or consisting of SEQ ID NO: 2; and a polypeptide having preferably at least 75%, 80%, 82%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 2, as determined using the BLAST program and parameters described herein. In another embodiment, examples of XMRV polypeptides include a substantially pure polypeptide comprising or consisting of SEQ ID NO: 2; and a polypeptide having preferably at least 75%, 80%, 82%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% sequence similarity to SEQ ID NO: 2, as determined using the BLAST program and parameters described herein.

A polypeptide of the invention can be purified to homogeneity. It is understood, however, that preparations in which the polypeptide is not purified to

homogeneity are useful. The critical feature is that the preparation allows for the desired function of the polypeptide, even in the presence of considerable amounts of other components. Thus, the invention encompasses various degrees of purity. In one embodiment, the language "substantially free of cellular material" includes preparations of the polypeptide having less than about 30% (by dry weight) other proteins (i.e., contaminating protein), less than about 20% other proteins, less than about 10% other proteins, less than about 5%, or less than about 1% other proteins.

When a polypeptide is recombinantly produced, it can also be substantially free of culture medium, i.e., culture medium represents less than about 20%, less than about 10%, or less than about 5% of the volume of the polypeptide preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of the polypeptide in which it is separated from chemical precursors or other chemicals that are involved in its synthesis. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of the polypeptide having less than about 30% (by dry weight) chemical precursors or other chemicals, less than about 20% chemical precursors or other chemicals, less than about 10% chemical precursors or other chemicals, or less than about 5% chemical precursors or other chemicals.

In one embodiment, a polypeptide of the invention comprises an amino acid sequence encoded by a nucleic acid molecule of SEQ ID NO: 1, and complements and portions thereof. The polypeptides of the invention also encompasses fragments and sequence variants (*e.g.,* allelelic variants). Variants also encompass polypeptides derived from other organisms, but having substantial homology to a polypeptide encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, and complements and portions thereof, or having substantial homology to a polypeptide encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1. Variants also include polypeptides substantially homologous or identical to these polypeptides but derived from another organism, i.e., an ortholog. Variants also include polypeptides that are substantially homologous or identical to these polypeptides that are produced by chemical synthesis. Variants also include polypeptides that are substantially homologous or identical to these polypeptides that are produced by recombinant methods.

As used herein, two polypeptides (or a region of the polypeptides) are substantially homologous or identical when the amino acid sequences are at least about 82%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98% or 99% homologous or identical. A substantially identical or homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid molecule hybridizing to SEQ ID NO: 1, or a portion thereof, under stringent conditions as more particularly described herein.

The percent identity of two nucleotide or amino acid sequences can be determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first sequence). The nucleotides or amino acids at corresponding positions are then compared, and the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions x 100). In certain embodiments, the length of the XMRV amino acid or nucleotide sequence aligned for comparison purposes is at least 30%, 40%, 50%, 60%,70%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the length of the reference sequence, for example, those sequences provided in FIGS. 1 and 2. The actual comparison of the two sequences can be accomplished by well-known methods, for example, using a mathematical algorithm. A preferred, non-limiting example of such a mathematical algorithm is described in Karlin et al., Proc. Natl. Acad. Sci. USA, 90:5873-5877 (1993). Such an algorithm is incorporated into the BLASTN and BLASTX programs (version 2.2) as described in Schaffer et al., Nucleic Acids Res., 29:2994-3005 (2001). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., BLASTN) can be used. In one embodiment, the database searched is a non-redundant (NR) database, and parameters for sequence comparison can be set at: no filters; Expect value of 10; Word Size of 3; the Matrix is BLOSUM62; and Gap Costs have an Existence of 11 and an Extension of 1. In another embodiment, the percent identity between two polypeptides or two polynucleotides is determined over the full-length of the polypeptide or polynuecleotide of interest.

Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller,

CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software package (Accelrys, San Diego, California). When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12 , and a gap penalty of 4 can be used. Additional algorithms for sequence analysis are known in the art and include ADVANCE and ADAM as described in Torellis and Robotti, Comput. Appl. Biosci., 10: 3-5 (1994); and FASTA described in Pearson and Lipman, Proc. Natl. Acad. Sci USA, 85: 2444-8 (1988).

In another embodiment, the percent identity between two amino acid sequences can be accomplished using the GAP program in the GCG software package using either a Blossom 63 matrix or a PAM250 matrix, and a gap weight of 12, 10, 8, 6, or 4 and a length weight of 2, 3, or 4. In yet another embodiment, the percent identity between two nucleic acid sequences can be accomplished using the GAP program in the GCG software package, using a gap weight of 50 and a length weight of 3.

The invention also encompasses XMRV polypeptides having a lower degree of identity but having sufficient similarity so as to perform one or more of the same functions performed by an XMRV polypeptide encoded by a nucleic acid molecule of the invention. Similarity is determined by conserved amino acid substitution. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Conservative substitutions are likely to be phenotypically silent. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Glu; substitution between the amide residues Asn and Gln; exchange of the basic residues Lys and Arg; and replacements among the aromatic residues Phe and Tyr. Guidance concerning which amino acid changes are likely to be phenotypically silent are found in Bowie et al., Science 247: 1306-1310 (1990).

A variant polypeptide can differ in amino acid sequence by one or more substitutions, deletions, insertions, inversions, fusions, and truncations or a combination of any of these. Further, variant polypeptides can be fully functional

(*e.g.*, ability to infect cells and produce progeny virus) or can lack function in one or more activities (*e.g.*, ability to produce progeny virus). Fully functional variants typically contain only conservative variation or variation in non-critical residues or in non-critical regions. Functional variants can also contain substitution of similar amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree. Non-functional variants typically contain one or more non-conservative amino acid substitutions, deletions, insertions, inversions, or truncations or a substitution, insertion, inversion, or deletion in a critical residue or critical region.

Amino acids that are essential for function (*e.g.*, infection) can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham et al., Science, 244: 1081-1085 (1989)). The latter procedure introduces a single alanine mutation at each of the residues in the molecule (one mutation per molecule). The resulting mutant molecules are then tested for biological activity *in vitro*. Sites that are critical for polypeptide activity can also be determined by structural analysis, such as crystallization, nuclear magnetic resonance, or photoaffinity labeling (See Smith et al., J. Mol. Biol., 224: 899-904 (1992); and de Vos et al. Science, 255: 306-312 (1992)).

The invention also includes XMRV polypeptide fragments of the polypeptides of the invention. Fragments can be derived from a polypeptide comprising SEQ ID NO: 2, or from a polypeptide encoded by a nucleic acid molecule comprising SEQ ID NO: 1, or a portion thereof, complements thereof, or other variant thereof. The present invention also encompasses fragments of the variants of the polypeptides described herein. Useful fragments include those that retain one or more of the biological activities of the polypeptide, as well as fragments that can be used as an immunogen to generate polypeptide-specific antibodies. In particular embodiments, XMRV polypeptide fragments of the polypeptides of the invention comprise a gag polypeptide (*e.g.*, SEQ ID NO:3), a pro-pol polypeptide (*e.g.*, SEQ ID NO: 4), an env polypeptide (*e.g.*, SEQ ID NO: 4) and combinations thereof.

Biologically active fragments include peptides that are, for example, 6, 9, 12, 15, 18, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more amino acids in length.

Fragments can be discrete (not fused to other amino acids or polypeptides) or can be fused to one or more components of a polypeptide. Further, several fragments can be comprised within a single larger polypeptide. In one embodiment, a fragment designed for expression in a host can have heterologous pre- and pro-polypeptide regions fused to the amino terminus of the polypeptide fragment and an additional region fused to the carboxyl terminus of the fragment.

Standard molecular biology methods for generating polypeptide fragments are known in the art. Once the fragments are generated, they can be tested for biological activity, using, for example, any of the methods described herein.

The invention thus provides chimeric or fusion polypeptides. These comprise an XMRV polypeptide of the invention operatively linked to a heterologous protein or polypeptide having an amino acid sequence not substantially homologous to the polypeptide. "Operatively linked" indicates that the polypeptide and the heterologous protein are fused in-frame. The heterologous protein can be fused to the N-terminus or C-terminus of the polypeptide. In one embodiment, the fusion polypeptide does not affect the function of the polypeptide per se. For example, the fusion polypeptide can be a GST-fusion polypeptide in which the polypeptide sequences are fused to the C-terminus of the GST sequences. Other types of fusion polypeptides include, but are not limited to, enzymatic fusion polypeptides, for example, β-galactosidase fusions, yeast two-hybrid GAL fusions, poly-His fusions, FLAG-tagged fusions and Ig fusions. Such fusion polypeptides can facilitate the purification of recombinant polypeptide. In certain host cells (e.g., mammalian host cells), expression and/or secretion of a polypeptide can be increased by using a heterologous signal sequence. Therefore, in another embodiment, the fusion polypeptide contains a heterologous signal sequence at its N-terminus.

EP-A 0464 533 discloses fusion proteins comprising various portions of immunoglobulin constant regions. The Fc is useful in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). In drug discovery, for example, human proteins have been fused with Fc portions for

the purpose of high-throughput screening assays to identify antagonists. (See Bennett et al., Journal of Molecular Recognition, 8: 52-58 (1995) and Johanson et al., The Journal of Biological Chemistry, 270,16: 9459-9471 (1995)). Thus, this invention also encompasses soluble fusion polypeptides containing a polypeptide of the invention and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclass (IgG, IgM, IgA, IgE).

A chimeric or fusion polypeptide can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of nucleic acid fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive nucleic acid fragments that can subsequently be annealed and re-amplified to generate a chimeric nucleic acid sequence (see Ausubel et al., "Current Protocols in Molecular Biology," John Wiley & Sons, (1998), the entire teachings of which are incorporated by reference herein). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST protein). A nucleic acid molecule encoding a polypeptide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the polypeptide.

The substantially pure, isolated, or substantially pure and isolated XMRV polypeptide can be purified from cells that naturally express it, purified from cells that have been altered to express it (recombinant), or synthesized using known protein synthesis methods. In one embodiment, the polypeptide is produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the polypeptide is cloned into an expression vector, the expression vector is introduced into a host cell, and the polypeptide is expressed in the host cell. Alternatively, the cell can be infected with XMRV virus. The XMRV polypeptide can then be isolated from the cells or the supernatant of cells by an appropriate purification scheme using standard protein purification techniques.

In general, XMRV polypeptides of the present invention can be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration

columns using art-recognized methods. The polypeptides of the present invention can be used to raise antibodies or to elicit an immune response. The polypeptides can also be used as a reagent, e.g., a labeled reagent, in assays to quantitatively determine levels of the polypeptide or a molecule to which it binds (e.g., a receptor or a ligand) in biological fluids. The polypeptides can also be used as markers for cells or tissues in which the corresponding polypeptide is preferentially expressed, either constitutively, during tissue differentiation, or in a diseased state. The polypeptides can also be used to isolate a corresponding binding agent, and to screen for peptide or small molecule antagonists or agonists of the binding interaction. The polypeptides of the present invention can also be used as therapeutic agents.

Antibodies

Polyclonal and/or monoclonal antibodies that selectively bind all or a portion of an XMRV polypeptide, homologs and varaiants thereof are also provided. The invention provides antibodies to an XMRV polypeptide or polypeptide fragment of the invention, e.g., having an amino acid sequence encoded by SEQ ID NO: 2, or a portion thereof, or having an amino acid sequence encoded by a nucleic acid molecule comprising all or a portion of SEQ ID NO: 1, or another variant, or portion thereof.

The term "purified antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that selectively binds an antigen. A molecule that selectively binds to a polypeptide of the invention is a molecule that binds to that polypeptide or a fragment thereof, but does not substantially bind other molecules in a sample, e.g., a biological sample that naturally contains the polypeptide. Preferably the antibody is at least 60%, by weight, free from proteins and naturally occurring organic molecules with which it naturally associated. More preferably, the antibody preparation is at least 75% or 90%, and most preferably, 99%, by weight, antibody. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')2 fragments that can be generated by treating the antibody with an enzyme such as pepsin.

The invention provides polyclonal and monoclonal antibodies that selectively bind to an XMRV polypeptide of the invention. The term "monoclonal antibody" or "monoclonal antibody composition," as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a polypeptide of the invention. A monoclonal antibody composition thus typically displays a single binding affinity for a particular polypeptide of the invention with which it immunoreacts.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with a desired immunogen, e.g., an XMRV polypeptide of the invention or fragment thereof. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules directed against the polypeptide can be isolated from the mammal (e.g., from tissue, blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction.

At an appropriate time after immunization, e.g., when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein, Nature 256:495-497 (1975), the human B cell hybridoma technique (Kozbor et al., Immunol. Today 4:72 (1983)), the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96 (1985)) or trioma techniques. The technology for producing hybridomas is well known (see generally Current Protocols in Immunology, Coligan et al., (eds.) John Wiley & Sons, Inc., New York, NY (1994)). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds a polypeptide of the invention.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal

antibody to a polypeptide of the invention (see, e.g., Current Protocols in Immunology, *supra*; Galfre et al., Nature, 266:55052 (1977); R.H. Kenneth, in Monoclonal Antibodies: A New Dimension In Biological Analyses, Plenum Publishing Corp., New York, New York (1980); and Lerner, Yale J. Biol. Med. 54:387-402 (1981)). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods that also would be useful.

In one alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody to an XMRV polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the polypeptide to thereby isolate immunoglobulin library members that bind the polypeptide. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al., Bio/Technology 9:1370-1372 (1991); Hay et al., Hum. Antibod. Hybridomas 3:81-85 (1992); Huse et al., Science 246:1275-1281 (1989); and Griffiths et al., EMBO J. 12:725-734 (1993).

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art.

In general, antibodies of the invention (e.g., a monoclonal antibody) can be used to isolate an XMRV polypeptide of the invention by standard techniques, such as affinity chromatography or immunoprecipitation. A polypeptide-specific antibody can facilitate the purification of natural polypeptide from cells and of

recombinantly produced polypeptide expressed in host cells. Moreover, an antibody specific for an XMRV polypeptide of the invention can be used to detect the polypeptide (e.g., in a cellular lysate, cell supernatant, blood sample, or tissue sample).

The antibodies of the present invention can also be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, and acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, green fluorescent protein, and aequorin, and examples of suitable radioactive material include, for example, ^{125}I, ^{131}I, ^{35}S, ^{32}P and ^{3}H.

Retroviral vectors

The XMRV of the present invention can also be used as an expression vector and/or targeting vector wherein a moiety is inserted into or attached to an XMRV using known methods, thereby producing a recombinant XMRV.

In one embodiment the moiety of interest is a nucleic acid which is introduced into the genome of an XMRV (*e.g.*, using homologous recombination), thereby producing a recombinant XMRV, and the recombinant XMRV can express the moiety of interest. In addition, the recombinant XMRV can be used to deliver the moiety of interest to cells that are targeted by (can be infected by) XMRV (*e.g.*, a prostate tumor cell) under conditions in which the nucleic acid is expressed in the targeted cell. The nucleic acid of interest can be, for example, a nucleic acid which encodes a marker (*e.g.*, neomycin, β-galactosidase, green fluorescent protein) and/or

a therapeutic agent (*e.g.*, interferon, interleukin, antineoplastins, synthetic peptides) and can include regulatory sequences (*e.g.*, promoters (constitutive, inducible), enhancers).

In particular embodiments, the XMRV is attenuated (*e.g.*, avirulent). Attenuated XMRV can be obtained using known methods (*e.g.*, serial passage). The genome of the XMRV can be modified wherein the gene coding for one or more or all of the viral proteins (*e.g.*, gag, pol, env) have been replaced by a nucleic acid of interest, thereby producing an XMRV vector plasmid. In particular embodiments, one or more XMRV genes have been replaced, thereby producing a modified XMRV vector plasmid which cannot replicate. A packaging cell line that produces viral proteins but lacks the ability to produce replication competent virus can be used to package the modified XMRV into retroviral particles. The XMRV vector plasmid which includes the nucleic acid of interest can be transfected into the packaging cell line wherein the XMRV vector plasmid is transcribed and packaged into modified retroviral particles (recombinant retroviral particles). The modified retroviral particles can be used to infect cells targeted by XMRV and the nucleic acid of interest present in the XMRV vector plasmid can then be expressed in the infected cells. A cell infected with such a modified retroviral particle cannot produce new virus since one or more of the viral proteins are not present in the infected cells. However, the nucleic acid of interest is integrated into the infected cell's DNA and can now be expressed in the infected cell.

Diagnostic and Screening Assays

The present invention also pertains to diagnostic assays for assessing the presence of XMRV expression, or for assessing activity of XMRV polypeptides of the invention. In one embodiment, the assays are used in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether XMRV is present in an individual or a sample from an individual, or whether an individual is at risk for (has a predisposition for or a susceptibility to) developing cancer (*e.g.*, a cancer that can develop due to transmission of XMRV (*e.g.*, sexual transmission), such as prostate, cervical or ovarian cancer). The invention also provides for prognostic (or predictive) assays for determining whether an individual is

susceptible to developing cancer. For example, the presence of XMRV in an individual could indicate that the individual has an increased risk of developing cancer. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of symptoms associated with cancer.

Another aspect of the invention pertains to assays for monitoring the influence of agents, or candidate compounds (e.g., drugs or other agents) on the nucleic acid molecule expression or biological activity of polypeptides of the invention, as well as to assays for identifying candidate compounds that bind to an XMRV polypeptide. These and other assays and agents are described in further detail in the following sections.

Diagnostic assays

XMRV nucleic acid molecules, probes, primers, polypeptides, and antibodies to an XMRV polypeptide can be used in methods of diagnosis of a susceptibility to, or likelihood of an individual having cancer, as well as in kits useful for diagnosis of a susceptibility to cancer.

In one embodiment, the invention is directed to a method of diagnosing or detecting cancer (*e.g.,* prostate cancer) using hybridization methods, such as Southern analysis, Northern analysis, or *in situ* hybridizations (see Ausubel, et al., *supra*). For example, a biological sample from a test subject (a "test sample") of DNA (*e.g.,* cDNA) or RNA is obtained from an individual suspected of having, being susceptible to or predisposed for, cancer (the "test individual"). The test sample can be from any source that contains XMRV nucleic acid molecules such as a blood sample or a tissue sample. The DNA, RNA, or cDNA sample is then examined to determine whether an XMRV nucleic acid molecule is present. The presence of the XMRV nucleic acid can be indicated by hybridization of RNA or cDNA to a nucleic acid probe. A "nucleic acid probe," as used herein, can be a DNA probe or an RNA probe. The probe can be any of the nucleic acid molecules described above (e.g., the entire nucleic acid molecule, a fragment, a vector comprising the gene, a probe, or primer, etc.).

To detect XMRV nucleic acid a hybridization sample is formed by contacting the test sample which is suspected of containing XMRV nucleic acid, with at least one nucleic acid probe. A preferred probe is a labeled nucleic acid probe capable of hybridizing to XMRV nucleic acids described herein. The nucleic acid probe can be, for example, a full-length nucleic acid molecule, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250, or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to appropriate mRNA or genomic DNA. For example, the nucleic acid probe can be all or a portion of SEQ ID NO: 1, or the complement of SEQ ID NO: 1; or can be a nucleic acid molecule encoding all or a portion of SEQ ID NO: 2. Other suitable probes for use in the diagnostic assays of the invention are described above.

The hybridization sample is maintained under conditions that are sufficient to allow hybridization of the nucleic acid probe to XMRV nucleic acid. More than one nucleic acid probe can also be used concurrently in this method. Hybridization of any one of the nucleic acid probes is indicative of a susceptibility to, or likelihood of an individual having cancer (*e.g.,* prostate cancer).

Hybridization can be detected using Southern blot analysis, Northern blot analysis, arrays of oligonucleotide probes that are complementary to target nucleic acid sequence segments from an individual. For example, in one embodiment, an oligonucleotide array can be used. Oligonucleotide arrays typically comprise a plurality of different oligonucleotide probes that are coupled to a surface of a substrate in different known locations. Oligonucleotide arrays include "Virochip" and "GENECHIPS™" (U.S. Patent No. 5,143,854 and PCT patent publication Nos. WO 90/15070 and 92/10092). These arrays can generally be produced using mechanical synthesis methods or light directed synthesis methods that incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis methods. See Fodor et al., Science 251:767-777 (1991), U.S. Patent No. 5,143,854; PCT Publication No. WO 90/15070; PCT Publication No. WO 92/10092, and U.S. Patent No. 5,424,186, the entire teachings of each of which are incorporated by reference herein. Techniques for the synthesis of these arrays using mechanical

synthesis methods are described in, e.g., U.S. Patent No. 5,384,261, the entire teachings of which are incorporated by reference herein.

Once an oligonucleotide array is prepared, a nucleic acid of interest is hybridized to the array and scanned for the target nucleic acid molecule. Hybridization and scanning are generally carried out by methods described herein and also in, e.g., PCT Publication Nos. WO 92/10092 and WO 95/11995, and U.S. Patent No. 5,424,186, the entire teachings of which are incorporated by reference herein.

In addition, the level of XMRV nucleic acid can be detected using, for example, *in situ* hybridization techniques known to one skilled in the art, or by examining the level of expression, activity, and/or composition of an XMRV polypeptide, by a variety of methods, including enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, immunohistochemistry, and immunofluorescence. A test sample from an individual can also be assessed for the presence of an alteration in the level of an XMRV nucleic acid or in the expression and/or an alteration in composition of the polypeptide encoded by an XMRV nucleic acid.

Detection of XMRV in a sample can be compared with the expression or composition of an XMRV in a control sample. A control sample is a sample that corresponds to the test sample (e.g., is from the same type of cells), and is from an individual in which XMRV is not present.

Various means of examining expression or composition of an XMRV polypeptide can be used, including spectroscopy, colorimetry, electrophoresis, isoelectric focusing, and immunoassays such as immunoblotting (see also Ausubel et al., supra; particularly chapter 10). For example, in one embodiment, an antibody capable of binding to the polypeptide (e.g., as described above), preferably an antibody with a detectable label, can be used. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')2) can be used. The term "labeled," with regard to the antibody, is intended to encompass direct labeling of the antibody by coupling (i.e., physically linking) a detectable substance to the antibody, as well as indirect labeling of the antibody by reacting it with another reagent that is directly labeled. An example of indirect

labeling is detection of a primary antibody using a fluorescently labeled secondary antibody.

Western blotting analysis, using an antibody as described above that specifically binds to an XMRV polypeptide can be used to identify the presence in a test sample of an XMRV polypeptide.

In one embodiment of this method, the level or amount of an XMRV polypeptide in a test sample is compared with the level or amount of an XMRV polypeptide in a control sample. A level or amount of the polypeptide in the test sample that is higher or lower than the level or amount of the polypeptide in the control sample, such that the difference is statistically significant, is indicative of an alteration in the expression of the XMRV polypeptide, and can be indicative of a susceptibility to cancer.

Kits (e.g., reagent kits) useful in the methods of diagnosis comprise components useful in any of the methods described herein, including, for example, hybridization probes or primers as described herein (e.g., labeled probes or primers), reagents for detection of labeled molecules, antibodies that bind to an XMRV polypeptide, means for amplification of nucleic acids comprising XMRV, or means for analyzing the nucleic acid sequence of XMRV, or for analyzing the amino acid sequence of an XMRV polypeptide, etc.

Screening assays and agents identified

The invention provides methods (also referred to herein as "screening assays") for identifying the presence of a nucleic acid of the invention, as well as for identifying the presence of a polypeptide encoded by a nucleic acid of the invention. For example, the present invention provides for a method of screening and monitoring for the presence of XMRV in, for example, tissue, units of blood, plasma and/or platelets in a depository for such samples (*e.g.,* a blood bank; an organ bank).

In one embodiment, the presence (or absence) of a nucleic acid molecule of interest (e.g., a nucleic acid that has significant homology with a nucleic acid of XMRV) in a sample can be assessed by contacting the sample with a nucleic acid comprising a nucleic acid of the invention (e.g., a nucleic acid having the sequence of SEQ ID NO: 1, or the complement thereof, or a nucleic acid encoding an amino

acid having the sequence of SEQ ID NO: 2, or a fragment or variant of such nucleic acids), under stringent conditions as described above, and then assessing the sample for the presence (or absence) of hybridization. In a preferred embodiment, high stringency conditions are conditions appropriate for selective hybridization. In another embodiment, a sample containing the nucleic acid molecule of interest is contacted with a nucleic acid containing a contiguous nucleotide sequence (e.g., a primer or a probe as described above) that is at least partially complementary to a part of the nucleic acid molecule of interest (e.g., an XMRV nucleic acid), and the contacted sample is assessed for the presence or absence of hybridization. In a preferred embodiment, the nucleic acid containing a contiguous nucleotide sequence is completely complementary to a part of the nucleic acid molecule of XMRV.

In any of the above embodiments, all or a portion of the nucleic acid of interest can be subjected to amplification prior to performing the hybridization.

In another embodiment, the presence (or absence) of an XMRV polypeptide, such as a polypeptide of the invention or a fragment or variant thereof, in a sample can be assessed by contacting the sample with an antibody that specifically binds to the polypeptide of XMRV (e.g., an antibody such as those described above), and then assessing the sample for the presence (or absence) of binding of the antibody to the XMRV polypeptide.

In another embodiment, the invention provides methods for identifying agents or compounds (e.g., fusion proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes) that alter or modulate (e.g., increase or decrease; enhance or inhibit) the activity of the polypeptides described herein, or that otherwise interact with the polypeptides herein. For example, such compounds can be compounds or agents that bind to polypeptides described herein; that have a stimulatory or inhibitory effect on, for example, the activity of the polypeptides of the invention; or that change (e.g., enhance or inhibit) the ability of the polypeptides of the invention to interact with molecules with which XMRV polypeptides normally interact (XMRV binding agents).

The candidate compound can cause an increase in the activity of the polypeptide. For example, the activity of the polypeptide can be increased by at

least 1.5-fold to 2-fold, at least 3-fold, or, at least 5-fold, relative to the control. Alternatively, the polypeptide activity can be a decrease, for example, by at least 10%, at least 20%, 40%, 50%, or 75%, or by at least 90%, relative to the control.

In one embodiment, the invention provides assays for screening candidate compounds or test agents to identify compounds that bind to or modulate the activity of polypeptides described herein (or biologically active portion(s) thereof), as well as agents identifiable by the assays. As used herein, a "candidate compound" or "test agent" is a chemical molecule, be it naturally-occurring or artificially-derived, and includes, for example, peptides, proteins, synthesized molecules, for example, synthetic organic molecules, naturally-occurring molecule, for example, naturally occurring organic molecules, nucleic acid molecules, and components thereof.

In general, candidate compounds for uses in the present invention may be identified from large libraries of natural products or synthetic (or semi-synthetic) extracts or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available, e.g., from Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI). Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are generated, if desired, according to methods known in the art, e.g., by standard extraction and fractionation methods. For example, candidate

compounds can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer or small molecule libraries of compounds (Lam, Anticancer Drug Des. 12: 145 (1997)). Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods.

If desired, compounds shown to be useful agents for treatment are chemically modified according to methods known in the art. Compounds identified as being of therapeutic value may be subsequently analyzed using animal models for diseases in which it is desirable to alter the activity or expression of the nucleic acids or polypeptides of the present invention.

In one embodiment to identify candidate compounds that alter the biological activity of an XMRV polypeptide, a cell, tissue, cell lysate, tissue lysate, or solution containing or expressing an XMRV polypeptide (e.g., SEQ ID NO: 2), can be contacted with a candidate compound to be tested under conditions suitable for XMRV infection of a cell. Methods for assessing viral infectivity are known in the art (*e.g.,* assess the ability of the XMRV to produce retroviral particles with reverse transcriptase activity).

Alternatively, the XMRV nucleic acid molecule or polypeptide can be contacted directly with the candidate compound to be tested. The level (amount) of XMRV biological activity is assessed (e.g., either directly or indirectly), and is compared with the level of biological activity in a control. If the level of the biological activity in the presence of the candidate compound differs, by an amount that is statistically significant, from the level of the biological activity in the absence of the candidate compound, or in the presence of the candidate compound vehicle only, then the candidate compound is a compound that alters the biological activity of an XMRV polypeptide. For example, an increase in the level of an XMRV biological activity relative to a control, indicates that the candidate compound is a

compound that enhances (is an agonist of) XMRV activity. Similarly, a decrease in the level of XMRV biological activity relative to a control, indicates that the candidate compound is a compound that inhibits (is an antagonist of) XMRV activity. In another embodiment, the level of biological activity of an XMRV polypeptide or derivative or fragment thereof in the presence of the candidate compound to be tested, is compared with a control level that has previously been established. A level of the biological activity in the presence of the candidate compound that differs from the control level by an amount that is statistically significant indicates that the compound alters XMRV biological activity.

The present invention also relates to an assay for identifying compounds that alter the expression of an XMRV nucleic acid molecule (e.g., antisense nucleic acids, interfering RNA (*e.g.,* siRNA, shRNA), fusion proteins, polypeptides, peptidomimetics, prodrugs, receptors, binding agents, antibodies, small molecules or other drugs, or ribozymes) that alter (e.g., increase or decrease) expression (e.g., transcription or translation) of the nucleic acid molecule or that otherwise interact with the nucleic acids described herein, as well as compounds identifiable by the assays. For example, a solution containing a nucleic acid encoding an XMRV polypeptide can be contacted with a candidate compound to be tested. The level and/or pattern of XMRV expression is assessed, and is compared with the level and/or pattern of expression in a control. If the level and/or pattern in the presence of the candidate compound differs, by an amount or in a manner that is statistically significant, from the level and/or pattern in the absence of the candidate compound, or in the presence of the candidate compound vehicle only, then the candidate compound is a compound that alters the expression of XMRV.

This invention further pertains to novel compounds identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use a compound identified as described herein in an appropriate animal model. For example, a compound identified as described herein (e.g., an antibody) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such a compound. Alternatively, a compound identified as described herein can be used in an animal model to determine the mechanism of action of such a compound. Furthermore, this invention pertains to uses of novel compounds

identified by the above-described screening assays for treatments as described herein. In addition, a compound identified as described herein can be used to alter activity of an XMRV polypeptide, or to alter expression of XMRV, by contacting the polypeptide or the nucleic acid molecule (or contacting a cell comprising the polypeptide or the nucleic acid molecule) with the compound identified as described herein.

Pharmaceutical composition

The present invention also pertains to pharmaceutical compositions comprising nucleic acids described herein, particularly nucleotides encoding the polypeptides described herein; comprising polypeptides described herein (e.g., SEQ ID NO: 2, and/or variants thereof); and/or comprising a compound that alters (e.g., increases or decreases) XMRV expression or XMRV polypeptide activity as described herein. For instance, the compositions can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. The formulation should suit the mode of administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (e.g., NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrolidone, etc., as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like that do not deleteriously react with the active compounds.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard

carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

Methods of introduction of these compositions include, but are not limited to, intradermal, intramuscular, intraperitoneal, intraocular, intravenous, subcutaneous, topical, oral and intranasal. Other suitable methods of introduction can also include gene therapy (as described below), rechargeable or biodegradable devices, particle acceleration devises ("gene guns") and slow release polymeric devices. The pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other compounds.

The composition can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, compositions for intravenous administration typically are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active compound. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

For topical application, nonsprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water, can be employed. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, enemas, lotions, sols, liniments, salves, aerosols, etc., that are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. The compound may be incorporated into a cosmetic formulation. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient,

preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., pressurized air.

Compounds described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The compounds are administered in a therapeutically effective amount. The amount of compounds that will be therapeutically effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the symptoms of an angiogenic disease, a vascular disease, a heart disease, or a circulatory disease, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, that notice reflects approval by the agency of manufacture, use of sale for human administration. The pack or kit can be labeled with information regarding mode of administration, sequence of drug administration (e.g., separately, sequentially or concurrently), or the like. The pack or kit may also include means for reminding the patient to take the therapy. The pack or kit can be a single unit dosage of the combination therapy or it can be a plurality of unit dosages. In particular, the compounds can be separated, mixed together in any combination, present in a single vial or tablet. Compounds

assembled in a blister pack or other dispensing means is preferred. For the purpose of this invention, unit dosage is intended to mean a dosage that is dependent on the individual pharmacodynamics of each compound and administered in FDA approved dosages in standard time courses.

Methods of Therapy

The present invention also pertains to methods of treatment (prophylactic, diagnostic, and/or therapeutic) for a disease associated with XMRV, using an XMRV therapeutic compound. An "XMRV therapeutic compound" is a compound that inhibits XMRV polypeptide activity and/or XMRV nucleic acid molecule expression, as described herein (e.g., an agonist or antagonist). XMRV therapeutic compounds can inhibit XMRV polypeptide activity or nucleic acid molecule expression by a variety of means, such as, for example, by inducing an immune response to an XMRV, interfering with XMRV polypeptide activity (e.g., by binding to an XMRV polypeptide), or by downregulating expression of the XMRV nucleic acid molecule. In one embodiment, the XMRV therapeutic compound is a vaccine. Representative XMRV therapeutic compounds include the following: nucleic acids or fragments or derivatives thereof described herein, polypeptides described herein; peptidomimetics; fusion proteins or prodrugs thereof; antibodies; other small molecules; and other compounds that inhibit XMRV nucleic acid expression or polypeptide activity, for example, those compounds identified in the screening methods described herein. In particular embodiments, the inhibitors of XMRV are reverse transcriptase inhibitors (*e.g.,* AZT, (zidovudine, Retrovir) and protease inhibitors. More than one XMRV therapeutic compound can be used concurrently, if desired.

The XMRV therapeutic compound that is a nucleic acid is used in the treatment of a disease associated with XMRV. In one embodiment, the disease is a cancer in which the etiology of the cancer is attributable to the presence of XMRV in an individual (*e.g.,* prostate, cervical, uterine cancer). The term, "treatment" as used herein, refers not only to ameliorating symptoms associated with the disease, but also preventing or delaying the onset of the disease, inducing an immune response to the disease and also lessening the severity or frequency of symptoms of

the disease. The therapy is designed to inhibit or downregulate activity of an XMRV polypeptide in an individual. For example, an XMRV therapeutic compound can be administered in order to downregulate or decrease the expression or availability of the XMRV nucleic acid molecule or variants thereof. In one embodiment, the invention is directed to a method of treating cancer (*e.g.*, prostate cancer) in an individual wherein XMRV is present in the individual, comprising administering to the individual an effective amount of an agent that inhibits XMRV. In another embodiment, the invention is directed to a method of detecting asymptomatic (early stage) cancer in an individual wherein XMRV is present in the individual, comprising detecting the presence of an XMRV in the individual, wherein the presence of XMRV in the individual is indicative of early stage cancer in the individual. In yet another embodiment, the invention is directed to a method of identifying an individual at risk for developing cancer, comprising determining whether an XMRV is present in the individual, wherein if XMRV is present in the individual then the individual is at risk for developing cancer.

The XMRV therapeutic compound(s) are administered in a therapeutically effective amount (i.e., an amount that is sufficient to treat the disease, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease). The amount that will be therapeutically effective in the treatment of a particular individual's disorder or condition will depend on the symptoms and severity of the disease, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The invention will be further described by the following non-limiting examples.

EXEMPLIFICATION

EXAMPLE 1 General Overview of Methods Used to Identify Gammaretrovirus in Prostate Tissue

Methods and Results

1. Genotyping, processing of prostate tissues, and isolation of RNA.

Men scheduled to undergo prostatectomies at the Cleveland Clinic Foundation were genotyped for the R462Q (1385G->A) mutation in *RNASEL* using an Amplification Refractory Mutation System (ARMS) assay on DNA isolated from PBMC. The analysis employs a PCR-based assay that uses allele-specific forward primer sequences, capable of detecting homozygous wild-type (GG), heterozygous (GA), or homozygous mutant (AA) mutations in *RNASEL* (Casey G., *et al.*, *Nat Genet. 32*(4):581-3, 2002). Immediately after prostatectomies, tissue cores were taken from both the transitional zone (the site of benign prostatic hyperplasia, BPH) and the peripheral zone (where cancer generally occurs) and frozen on dry ice and stored in liquid nitrogen or at -70°C (by the Department of Laboratory Medicine, Cleveland Clinic). Immediately after cores were removed from the freshly removed prostates, the remaining prostate tissue was placed in 10% neutral buffered formalin for fixation. The fixed tissue was processed and embedded in paraffin for later histological use. Thus far, 150 patients that underwent prostatectomy have been genotyped, consisting of 73 (48.7%) with 1385GG (homozygous wild type); 62 (41.3%) with 1385GA (heterozygous); and 15 (10%) with 1385AA (homozygous mutant). A blood specimen was also collected from these men and processed into plasma and frozen at -70°C.

Transitional and peripheral cores were received on dry ice directly from the CCF anatomic pathology laboratory where they were being stored in liquid nitrogen. Once obtained, the tissue was transferred from dry ice immediately to TRIzol reagent, homogenized to completion using a power homogenizer, and processed for RNA isolation according to the manufacturer's instructions (Invitrogen). The prostate tissue RNA was then subjected to DNase I digestion. To recover the maximum amount of RNA after DNase digestion, the extracted phenol was back-extracted twice with RNase-free TE buffer. The extracted RNA was precipitated overnight at - 20°C. Poly A^+ RNA was isolated from the DNase digested total RNA using the Oligotex®

mRNA Midi Kit (Qiagen) as instructed by the manufacturer. The poly A+ RNA was then measured using the RIBOgreen quantitation kit (Molecular Probes). The samples were kept frozen at -70°C, until they were shipped on dry ice for Virochip microarray analysis.

2. Probing Virochips with cDNAs derived from prostate RNA.

Briefly, the prostate tissue total RNA and polyA$^+$ RNA were reverse transcribed into cDNA using an oligo-dT primer for first-strand synthesis. Cy5 fluorophores were incorporated into the reaction mix in the presence of unlabeled nucleotides. The resulting labeled cDNA was purified by centrifuging the sample through a Centricon-30 micro-concentrator (Amicon). The purified probe was added to the Virochip microarray and allowed to hybridize for at least 12 hours. The hybridized microarray was then washed using a stringent salt solution to remove any unbound or non-specific probe from the array. The slides were then analyzed within 2-3 hours using a scanner to visualize the fluorescent signals of probe hybridization to the array. The array hybridizations used Cy5-labeled amplified probes from either prostate tissue total RNA, polyA+ RNA, or water (control). A reference signal was generated by using a Cy3-labeled reverse complement version of a single defined 70-mer present in each spot on the microarray. Positive signals were assessed by Cy5 intensity relative to that of the controls.

Results obtained after hybridization, when using total RNA isolated from the prostate cancer tissue, was a positive signal in 9 of 14 patients genotyped as homozygous mutant for RNase L (1385AA). The virus signal was detected in 0 of 10 heterozygous (13 85GA) tissues examined and in 2 of 13 homozygous wild-type RNase L (1385GG) prostates examined, thus indicating that RNase L suppresses the virus from replicating (Table 1). The CyS-labeled probe bound to Murine Leukemia Virus (MLV) DNA.

3. Isolation and sequence analysis of full-length the viral cDNA.

To establish the full identity of the candidate virus, viral RNA was recovered from a prostate sample by hybridization/selection. This material was subsequently amplified, cloned and sequenced. Initially, the largest clone

spanned approximately 1.0kb; this fragment encompassed the 3' UTR conserved motif and extended into the most 3' coding region of the viral genome. The complete cloning of the entire 8,188 nt viral genomic RNA (SEQ ID NO: 1) from patient 35 was subsequently determined (Table 2). The viral genome is that of a canonical gammaretrovirus with *gag, pro-pol* and *env* genes (FIG. 3). All three open reading frames (ORF) are intact, therefore, it has the potential to generate infectious virus. Gammaretroviruses have C-type morphology, assemble at the plasma membrane with a central, symmetrical, spherical core, and contain the largest number of known members of the *retroviridae,* including murine leukemia virus (MLV), feline leukemia virus (FeLV) and gibbon ape leukemia virus (GALV) (Goff, S., *et al., Field's Virology*, fourth edition, (Knipe, D.M. and Howley, P.M., eds.). Lippincott Williams & Wilkins. New York. 2001, pp. 1871-1939). The most significant match in the full length viral genome database is to a xenotropic murine type C retrovirus known as DG-75 (GI 9628654) (93% identity at the nucleotide level) (FIG. 4). DG-75 retrovirus was described as an exogenous contaminant of an EBV-negative, B-lymphoblastoid cell line of the same name (Raisch KP., *et al., Virology 250*(1):135-9, 1998; Raisch KP., *et al., Virology 308*(1):83-91, 2003). An early passage of the DG-75 cell line (HAD subline), was found to be free from retroviruses, and the origin of the DG-75 virus is unknown. The divergence of the newly isolated virus (referred to herein as HXV for human xenotropic virus, or in the alternative, XMRV for xenotropic murine leukemia virus (MLV) related virus) from DG-75 virus indicates that two viruses are distinct, but related. The similarity index between the HXV_{35} and DG-75 coding sequences of gag, pro-pol, and env are 96.3%, 96.3%, and 93.8%, respectively (Lipman-Pearson Alignment) (Tables 3, 4 and 5). The HXV and DG-75 branch is most closely related to MCF1233, a C57BL-derived MLV that causes T and B lymphomas in an MHC-associated manner (Sijts EJ, *et al., Virus Res 34*(3):339-49, 1994). MCF1233 has an ecotropic backbone with polytropic sequences in the 5'-region. The next most closely related branch contains the classical ecotropic MLV strains, Moloney, Rauscher and Friend. Separate sub-branches of related viruses include KoRv, a virus isolated from koalas that clusters with gibbon ape leukemia virus (GALV) (Hanger JJ, *et al., J Virol*

74(9):4264-72, 2000). All of these MLV family members are related to endogenous *MLV* of *Mus Dunni.*

The HXV_{35} genome has at its 5' (nt 1 to 69) and 3' (nt 8116 to nt 8184) termini a 69-nt repeat (R) region. Downstream of the 5' R region is the 76-nt U5 region followed by the primer binding site (PBS) (Table 6). The HXV_{35} PBS is complementary to the last 18-nt of proline tRNA (Itin and Keshet, *J. Virol.* 54(1):236-239 (1985)), and is thus different from the DG-75 PBS complementary to glutamine or theonine tRNAs (Raisch KP., *et al.*, *Virology 308*(1):83-91, 2003). The *gag pro-pol* region is interrupted by a single UAG stop codon (at nucleotide 2223) that separates *gag* from *pro pol, a* conserved feature in both gamma- and epsilon-retroviruses (Goff, S., *et al.*, *Field's Virology*, fourth edition, (Knipe, D.M. and Howley, P.M., eds.). Lippincott Williams & Wilkins. New York. 2001. pp. 1871-1939, 2001). There is readthrough of the stop codon 5% to 10% of the time providing for the synthesis of the pro-pol polypeptide that is processed into protease (PR), reverse transcriptase (RT) and integrase (IN). The gag gene encodes a 536 amino acid polypeptide that is processed into the Matrix (M), p12 protein, Capsid (CA) and Nucleocapsid (NC) proteins (Table 3). The *env* open reading frame, transcribed as a spliced mRNA, encodes a 645 aa precursor of the envelope glycoproteins, Surface Subunit (SU) and the Transmembrane Subunit (TM) (Table 5). TM contains the transmembrane and the hydrophobic fusion segments that functions in the fusion of viral and cellular membranes. SU is the major determinant of host range and the receptor-binding site. There are hypervariable sequences in the SU protein responsible for selectively binding to the host cell surface receptor. In the SU protein, the variable region A functions in receptor recognition and variable region B stabilizes the virus with its specific receptor (Battini JL, *et al.*, *Proc Natl Acad Sci U S A. 96*(4):1385-90, 1992; Fass D., *et al.*, *Science 277*(5332):1662-6, 1997). These variable regions in HXV_{35} are nearly identical in the xenotropic strain DG-75 and are distinct from those in amphotropic and ecotropic MLV s (Table 7). The human cell-surface receptor for xenotropic MLV strains is XPR-1 (xenotropic and polytropic receptor 1), containing multiple transmembrane spanning domains (Battini JL, *et al.*, *Proc Natl Acad Sci U S A. 96*(4):1385-90, 1992). XPR-1 is thus the putative cell-surface receptor for HXV.

2-5A synthetase is activated by viral dsRNA or by stem structures in otherwise single-stranded RNA. For example, the HIV-1 TAR RNA, is capable of activating 2-5A synthetase *in vitro* (Maitra, RK, *et al., Virology*, 1994, 204(2):823-7). In addition, RNase L is able to potently suppress replication of HIV-1 (Maitra, RK, *et al., J Virol.*, 1998, 72(2):l 146-52). The MFOLD predicted secondary structure of genomic HXV_{35} RNA is shown (FIG. 5) (Zuker M, *et al., RNA,* 1998, 4(6):669-79). There are extensive regions of internal base-pairing folding into regions of double-stranded stem structures. HXV_{35} RNA will be analyzed to determine if there is sufficient double-stranded character to activate 2-5A synthetase.

To verify the presence of HXV in patient tissue, a frozen prostate core (from patient VP35 previously shown to be positive for the virus by both Virochip analysis and RT-PCR, Table 1) was obtained from the CCF surgical pathology lab in a biohazard plastic container and sent directly to the UCSF group for analysis. PCR was performed on genomic DNA isolated from the prostate tissue (case VP35) and confirmed the presence of HXV DNA by agarose gel electrophoresis and sequencing. These results confirmed that the HXV was present in the human prostate cancer tissue. In addition, nested RT-PCR parameters were developed and used to both confirm initial retrovirus positive tissues and to screen for the presence of the retrovirus in the remaining human prostate cancer tissue. Two nested PCR conditions were designed. The first reaction uses primers that are specific for a region (400bp) in the *gag* portion of the virus

PCRV-GAG-Outside Forward, 5' CGCGTCTGATTTGTTTTGTT 3' (SEQ ID NO:34);

PCRV-GAG-Outside Reverse, 5' CCGCCTCTTCTTCATTGTTC 3' (SEQ ID NO:35);

PCRV-GAG-Inside Forward, 5' TCTCGAGATCATGGGACAGA 3'(SEQ ID NO:36);

PCRV-GAG-Inside Reverse, 5' AGAGGGTAAGGGCAGGGTAA 3'(SEQ ID NO:37),

while the second uses primers that amplify 7200bp of the entire viral genome

(HEMI-nested)
Env 7600 Outside Forward, 5'CGCTTGGTCCAGTTTGTAAAA 3'(SEQ ID NO:38);
Env 227 Reverse, 5' TGGGGAACTTGAAACTGAGG 3'(SEQ ID NO:39);
Env 7200 Inside Forward, 5' CTAGTGGCCACCAAACAATTC 3'(SEQ ID NO:40).

7600 Outside Forward and 227 Reverse are the outside oligonucleotide primers for the hemi-nested PCR in the env-LTR region. 7200 Outside Forward is the nested primer. Gel electrophoresis of 3 nested RT-PCRs from different VP patients found to be homozygous mutant (1385AA, R462Q) for *RNASEL* demonstrate that the nested RT-PCR using the *gag* region was able to detect the virus in 1 (VP10) of the 3 patients, while the HEMI-nested primers amplified non-specific products (FIG. 6). Sequencing analysis of the PCR product verified the viral RNA to be from HXV.

4. Identification of an HXV-related virus in the human prostate cancer cell line, LNCaP.

To determine if the common human prostate cancer cell lines (PC3, LNCaP and DU 145) or normal prostate epithelial cells (PrEC, Clonetics Co.) contained HXV or a closely-related virus, RT-PCR was performed on RNA from these cells using primers specific for a conserved 700bp region within the *env* protein encoding region of HXV (FIG. 7). RT was performed using random hexamer primers (Applied Biosystems). PCR was subsequently performed on the cDNA produced using primers specific for the conserved 700bp region of HXV (Virus forward, 5' GTT TAT GGC CAG TTT GGA AA 3' (SEQ ID NO:41); Virus reverse, 5' GCC TTA TGG TGG GGT CTT TC 3' (SEQ ID NO:42)). As a positive control, GAPDH exon 8 specific primers were used. Results showed a band of the correct size for an HXV-related *env* product from only LNCaP cells (Virus 700bp) when analyzed by agarose gel electrophoresis. The GAPDH DNA product (391bp) was present in the RT-PCR reactions from all cell lines (FIG. 6).

Interestingly, the LNCaP cell line is heterozygous for an inactivating deletion mutation in RNase L (4710AAAG) and is heterozygous for R462Q. In contrast, PC3, DU 145 and PrEC have wild type RNase L (Xiang Y., et al., Cancer Res. 2003, 63(20):6795-801; Malathi K., et al., Cancer Res. 64(24):9144-51, 2004 *et al.*, 2004). The results described herein thus indicate that RNase L suppresses HXV infections or replication. The *env* PCR fragment from the LNCaP was cloned into the pGEM®-T Easy Vector (Promega) and sequenced to determine sequence similarity to the virus sequence found in the human prostate tissue samples. After sequence alignment of a 675 nt segment of the LNCaP virus env gene, using BLAST, the LNCaP virus was found to be 97% homologous at both the nucleotide and amino acid levels to the prototype virus, HXV_{35} (Table 8). The same plasmid containing the purified PCR fragment was sequenced another three times to determine if there were errors in the DNA sequencing analysis. It was thus confirmed that the virus from LNCaP cells differs approximately 3-4% through this particular stretch of 700 bp. It is likely that the inactivation of the RNase L, as a result of this mutation, allows the HXV-related virus to infect and replicate in LNCaP cells.

To determine if retrovirus particles with reverse transcriptase activity were being released into the media of these virus infected LNCaP cells, the tissue culture media of the infected LNCaP cells were assayed at 2, 4 and 8 days incubation for reverse transcriptase activity. This assay uses a synthetic homopolymeric polyriboadenylic acid [poly(rA)] as a template, and oligodeoxythymidylic acid [oligo(dT)] as primer. The tissue culture media was incubated with this primer-template and α-^{32}P-dTTP; the resulting dTMP incorporation was monitored by spotting reaction aliquots onto DEAE paper and washing away unincorporated dTTP. Aliquots of undiluted or diluted LNCaP media were spotted onto dry DEAE paper and dried for 30 min under a heat lamp. Mock reactions containing no media were used as negative controls. The paper was washed three times in 2X SSC, rinsed briefly with 95% EtOH twice and dried under a heat lamp. The paper was wrapped in plastic and left to expose x-ray film (Kodak) at -70°C for 12 hr. Results demonstrate that the LNCaP tissue culture media contains active reverse transcriptase while the mock controls showed only unincorporated a α^{32}P-dTTP as background

(FIG. 8). It has also demonstrated that media from the infected LNCaP cells are capable of infecting both uninfected LNCaP and DU145 cells, though the amount of virus present in the DU145 cells was lower than that in LNCaP after infection as indicated by RT-PCR analysis using primers specific for the retrovirus. DU145 is homozygous wild type for RNase L (Xiang Y., *et al., Cancer Res.,* 2003, 63(20):6795-801). The difference in the amount of virus present in these cells after infection may be due to the mutations in RNase L found in the LNCaP cell line not present in the DU145 cells. The RNase L enzymatic deficiency in the LNCaP may allow the virus to escape the anti-viral affect of RNase L, while virus load in the DU145 cells is decreased owing to these cells having fully functional RNase L.

5. Identification of HXV nucleic acid in prostate cancer tissue by fluorescence *in situ* hybridization (FISH) methods.

To directly demonstrate the presence of HXV DNA in prostates, FISH was performed on human prostate tissues collected by prostatectomy, and subsequently fixed in formalin and embedded in paraffin. The LNCaP cell line, confirmed to have a quasispecies of the virus by RT-PCR and sequencing analyses, were used as a positive control, while the PC3 and DU145, which were found negative for the virus, were used as negative controls. Cytoblocks were prepared from the three cell lines. Approximately 10^9 cells were washed with Hanks balanced salt solution (HBSS) without phenol red, or Ca^{++} & Mg^{++} (GIBCO) and resuspended gently, but completely, with 10% neutral buffered formalin. The cell suspension was then fixed overnight at 4°C. The cells were then centrifuged and washed twice with HBSS. The supernatant was aspirated and the cells were resuspended with one drop of HBSS. The cell suspension was then pipetted into the well of a cytoblock cassette. The fixed cell culture cytoblocks were sent to the histology lab to be processed within 24 hr. The processed cytoblocks were then embedded into paraffinblocks, cut at 4-6μm thick sections onto super-frost slides and baked for at least 4 hr at 60-65°C to ensure the cells adhered to the slides.

The HXV_{VP35} FISH probe was generated using a 2.15kb segment of the viral genome Virus 2345 forward, 5' ACC CCT AAG TGA CAA GTC TG 3' (SEQ ID NO:43); Virus 4495 reverse, 5' CTG GAC AGT GAA TTA TAC TA 3' (SEQ ID

NO:44) that was cloned into the pGEM®-T Easy Vector. The recombinant vector was restricted using *EcoRI* to release the 2.15kb viral cDNA fragment and purified (Qiagen) for FISH probe generation. The purified 2.15kb viral cDNA insert was used in a nick translation reaction (Vysis Inc.) as described in the manufacturer's instructions to a produce fluorescently labeled probe. The probe size, approximately 250bp, was determined on an agarose gel. To the sample, Human COT1-DNA (Vysis Inc.) and human placental DNA (Sigma) was added as blocking agents. The probe was then precipitated and resuspended in nuclease-free water. To determine incorporation, the fluorescent emission of the supernatant from the precipitation step was compared to that of the resuspended probe. The average SpectrumGreen-dUTP incorporation was determined to be 19%.

Freshly baked LNCaP, DU145 and PC3 slides cut at ~4 μm sections were first deparaffinized through a series of xylene washes. The deparaffinized slides were then rehydrated through a series of decreasing ethanol concentrations. The rehydrated tissue was subjected to target retrieval and rinsed in H_20. Proteinase K (Dako) was applied directly to slides for 10 min at room temperature. The tissue was rinsed again in H_20 and dehydrated through solutions with increasing ethanol concentrations. The remaining steps were performed in reduced light to minimize probe photobleaching. Onto freshly dried slides, probe mix [3μl probe + 7μl Hybridization buffer (Vysis Inc.)] was applied. Coverslips were placed on the slides which were debubbled and sealed with rubber cement. The probe and target DNA were codenatured using a Vysis Hybrite (Vysis). Hybridization occurred at 37°C overnight in a humidified, light tight chamber. The next day, coverslips were removed by soaking the slides in 2X SSC. The slides were then agitated for 3 sec, and incubated for 1 min in a 2X SSC wash at 57°C. The slideswere then rinsed with 2X SSC at room temperature gently, followed by distilled water, and allowed to air dry. Vectashield Mounting Medium with DAPI (Vectashield Inc.) counterstain was applied to the dried tissue and coverslips were added.

Results show positive fluorescent green signals were seen in both the cytoplasm and nucleus of the LNCaP cells, while no signal was observed in the DU145 or PC3 cells (FIG. 9). These results correlate with the RT-PCR data which showed that the virus was present in the LNCaP cells, and not the DU145 or PC3 cell

line. Intense staining observed within the cytoplasm indicated that some probe may be binding viral RNA as well as DNA. After performing an RNase pretreatment of the tissue, a significant amount of the labeling was diminished in the cytoplasm, confirming RNA hybridization, and indicating that the labeling in the cytoplasm may be probe binding to RNA and unintegrated viral DNA. A DNase digestion following the RNase digestion completely obliterated the fluorescent signals both in the cytoplasm and nucleus, specifying probe hybridization to viral DNA (not shown). DNase or RNase pretreatment of the human prostate tissue was not preformed in the subsequent experiments. As a result, the possibility of the probe hybridizing to any viral sequence (RNA or DNA) in the patient tissues was maintained.

Results of initial experiments using the human prostate cancer tissue microarray (TMA) slides (FIG. 10) demonstrate approximately 1% of cells having specific hybridization for the HXV DNA probe, and those positives are present in patients found to be positive for the virus by RT-PCR by the UCSF group (Table 9). The slides were examined in a blinded study, allowing unbiased investigation of the FISH experiments. An arbitrary cutoff value was assigned for quantitating the FISH results. The guidelines for monitoring positive FISH results were as follows: <1 positive cell/500 cells counted= -; 1-2 positive cells/500 cells counted= +/-; 3-4 positive cells/500 cells counted = +; and 5-6 positive cells/500 cells counted = ++. The positive FISH results correlated with RT-PCR results by the UCSF group. Although positive hybridizations were seen in 5 out of 8 tissues from patients found to be HXV positive by RT-PCR, one tissue (VP3 1) was determined to be "++" for FISH, without being positive for the virus by RT-PCR. Likely explanations of obtaining only 5 out of 8 positives using FISH could be differences in the frequency of the virus within the specific tissue specimen being analyzed compared to that of the tissue used for RT-PCR analysis, resulting in a viral number below the detectable limit of the FISH method. Alternately, a technical problem may have also been encountered during tissue harvesting and processing, which caused RNA or DNA degradation.

6. Detection of HXV with novel affinity-purified, anti-HXV peptide antibodies.

To detect HXV gag protein in infected cells and tissues, novel antibodies were generated (Open Biosystems, Inc.). A hydropathy plot was generated from HXV35 gag protein, from which the following peptides were chosen as immunogen (FIG. 11):

MA: DVKKRRWVTFCSAE (SEQ ID NO:7) (antibody 401)

CA: EAGKAVRGNDGRPTQL (SEQ ID NO:8) (antibody 402)

NC: KDCPKKPRGPRGPR (SEQ ID NO:9) (antibody 403)

Antibodies were generated in rabbits and subsequently affinity purified by binding to and elution from the immobilized proteins (according to protocols developed at Open Biosystems, Inc.). The antibodies to MA (401) and NC (403) were successful in detecting gag from the HXV-related infected $LNCaP_R$ cells (FIG. 12).

Conclusions

Described herein is the discovery of the novel gammaretrovirus, HXV, present in tumor-bearing prostates. The percentage of prostate containing HXV is significantly higher in patients with a homozygous R462Q variant of RNase *L (HPC1)* (60%) than in patients that are wild type or heterozygous (<10%). The potential involvement of HXV in this disease is thus based primarily on a higher rate of occurrence of HXV in prostates of patients with homozygous germline mutations in the gene for RNase L, a candidate prostate cancer susceptibility gene *(HPC1)*. Beyond this correlation, the evidence that HXV infections contribute to prostatic carcinogenesis is as follows. As a group, retroviruses are responsible for a wide-range of different diseases including immunodeficiency, leukemia and neurological disease (Goff, S., Retroviridae: The Retroviruses and Their Replication, Chapter 57 in "Field's Virology", fourth edition. (Knipe, D.M. and Howley, P.M., eds.). Lippincott Williams & Wilkins. New York, pp. 1871-1939, 2001). Nevertheless, many of the simple retroviruses are relatively benign and are even widely used as backbones of

gene therapy vectors. A relatively small number of retroviruses such as the lentivirus, HIV-1, and some avian retroviruses are cytopathic. Others, namely the acute transforming retroviruses, contain host genes that cause aggressive tumors in the absence of latency. But most retroviruses are not cytopathic and have minimal effects on cellular replication or physiology. These retroviruses, unlike many other viruses, use only a small proportion of the cell's capacity to replicate. *In vivo* low level viremia is often obtained and is persistent for the life of the animal. However, even when they are not cytopathic, retroviruses cause disease by insertional mutagenesis that alters control of cell division or survival. These DNA insertion events can activate endogenous proto-oncogenes and lead to tumorigenesis. The disease process can be very slow, as in the case of mouse mammary tumor virus (MMTV). HXV may be a member of the latter category of simple, replication-competent retroviruses characterized by slow growth and a long latency period. Simple retroviruses are linked primarily to leukemia or lymphoma, diseases not presently implicated in HXV infections. The number of HXV-infected prostate cells, even in homozygous RNase L (R462Q) cases, is low (on the order of 1 %) (Table 9). The potential contribution of HXV to cancer could be owing to a slow infection resulting in proliferative inflammatory atrophy, a suspected precursor to prostatic intraepithelial neoplasia and carcinoma (Nelson WG., *et al.*, *N Engl J Med*, *349*(4):366-81, 2003). The infected cells, while few in number, could be producing growth factors, cytokines or other factors that are indirectly contributing to cell proliferation (Brightman BK, *et al.*, J Virol., 1990 Sep;64(9):4582-4). Regardless of the mechanism, it is likely that any carcinogenesis caused by HXV would occur as a multistep process that occurs over the course of many years. The fact that prostate cancer is a disease of aging that is usually not apparent until after age 65 is consistent with a slow-virus causing chronic or recurrent inflammation.

The general overview described in Example 1 is even more fully detailed in Examples 2 and 3 as follows.

EXAMPLE 2 Identification of a Distinctive Gammaretrovirus Genome in Prostate Tumors of Patients Homozygous for R462Q *RNASEL* Variant

RNase L is an important effector of the innate antiviral response. Mutations or variants that impair function of RNase L, particularly R462Q, have been proposed as susceptibility factors for prostate cancer. As shown herein, a viral infection likely contributes to prostate cancer in individuals harboring the R462Q variant. Randomly amplified cDNA from prostate tumors was assayed for the presence of viral sequences by hybridization to a DNA microarray composed of oligonucleotides corresponding to the most conserved sequences of all known viruses. The presence of retroviral sequences was revealed by microarray in 7 of 11 R462Q-homozygous (QQ) cases, and in one among 8 heterozygous (RQ) and homozygous wild-type (RR) cases. Full-length viral genomes were cloned and sequenced from the tumor tissue of two QQ cases. The virus is closely related to xenotropic murine leukemia viruses (MuLVs), but its sequence is clearly distinct from all known members of this group. Based on recovered sequence, a specific RT-PCR assay was developed and testing of tumor tissue was expanded to a total of 86 cases. Eight of 20 QQ cases (40%) were found to be positive, compared to only one sample (1.5%) among 66 RQ and RR cases. Comparison of *gag* and *pol* sequences from different tumor isolates indicated infection with the same virus in all cases, yet sequence variation was consistent with the infections being independently acquired. These data provide the first demonstration that xenotropic MuLV-related viruses can produce an authentic human infection, and strongly implicate RNase L activity in the prevention or clearance of infection in vivo. These findings also demonstrate a relationship between exogenous infection and cancer development in genetically susceptible individuals.

Materials and Methods

Genotyping of patients and prostate tissue processing

All human samples used in this study were obtained according to protocols approved by the Cleveland Clinic's Institutional Review Board. Men scheduled to undergo prostatectomies at the Cleveland Clinic were genotyped for the R462Q (1385G->A) RNASEL variant using a premade TAQMAN genotyping assay (Applied Biosystems, Foster City, CA, USA; Assay c_935391_1) on DNA isolated from peripheral blood mononuclear cells (PBMC). Five nanograms of genomic DNA were assayed according to the manufacturer's instructions, and analyzed on an Applied

Biosystems 7900HT Sequence Detection System instrument. Immediately after prostatectomies, tissue cores were taken from both the transitional zone (the site of benign prostatic hyperplasia, BPH) and the peripheral zone (where cancer generally occurs), snap-frozen in liquid nitrogen and then stored at -80°C. Remaining prostate tissue was fixed in 10% neutral buffered formalin, processed and embedded in paraffin for later histological analyses. Frozen tissue cores were transferred from dry ice immediately to TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), homogenized with a power homogenizer or manually using a scalpel followed by a syringe, and total RNA was isolated according to the manufacturer's instructions. The prostate tissue RNA was then subjected to RNase-free DNase I (Ambion, Austin, TX, USA) digestion for 30 minutes at 37°C. The sample was then extracted with phenol and the RNA was precipitated with isopropanol overnight at -20°C followed by centrifugation at 12,000 g for 30 minutes at 4°C. Poly-A RNA was isolated from the DNase digested total RNA using the Oligotex mRNA Midi Kit (Qiagen USA, Valencia, CA, USA) as instructed by the manufacturer. The poly-A RNA concentration was measured using the RIBOgreen quantitation kit (Molecular Probes, Invitrogen, Carlsbad, CA, USA), and the samples were stored at -80°C.

Microarray screening

Virochip microarrays used in this study were identical to those previously described (Wang D, *et al.*, 2002, *Proc Natl Acad Sc. USA 99*: 15687-15692; Wang D, *et al.* , 2003, *PLoS Biol* 1: E2; Urisman A, *et al.*, 2005, *Genome Biol 6*: R78) Prostate tumor RNA samples were amplified and labeled using a modified Round A/B random PCR method and hybridized to the Virochip microarrays as reported previously (Wang D, *et al.* , 2003, *PLoS Biol* 1: E2). Microarrays were scanned with an Axon 4000B scanner (Axon Instruments, Union City, CA, USA) and gridded using the bundled GenePix 3.0 software. Microarray data have been submitted to the NCBI GEO database (GSE3607). Hybridization patterns were interpreted using E-Predict as previously described (Urisman A, *et al.*, 2005, *Genome Biol 6*: R78) (Table 12). To make Figures 22A-22B, background-subtracted hybridization intensities of all retroviral oligonucleotides (205) were used to cluster samples and the oligonucleotides. Average linkage hierarchical clustering with Pearson correlation as

the similarity metric was carried out using Cluster (v. 2.0) (Eisen, MB., *PNAS 95*:14863-14868 (1998)) .Cluster images were generated using Java TreeView (version 1.0.8) (Saldanha, AJ., 2004, *Bioinformatics 20*: 3246-3248).

Genome cloning and sequencing

Amplified and labeled cDNA from the VP35 tumor sample was hybridized to a hand-spotted microarray containing several retroviral oligonucleotides, which had high hybridization intensity on the Virochip during the initial microarray screening. Nucleic acid hybridizing to two of the oligonucleotides (9628654_317 rc derived from MTCR: TTC GCT TTA TCT GAG TAC CAT CTG TTC TTG GCC CTG AGC CGG GGC CCA GGT GCT CGA CCA CAG ATA TCC T (SEQ ID NO:45); and 9626955_16 rc derived from Spleen focus-forming virus: TCG GAT GCA ATC AGC AAG AGG CTT TAT TGG GAA CAC GGG TAC CCG GGC GAC TCA GTC TGT CGG AGG ACT G (SEQ ID NO:46)) was then individually eluted off the surface of the spots and amplified by PCR with Round B primers. Preparation of the hand-spotted array, hybridization, probe recovery, and PCR amplification of the recovered material were carried out. The recovered amplified DNA samples were then cloned into pCR2.1-TOPO TA vector (Invitrogen), and the resulting libraries were screened by colony hybridization with the corresponding above oligonucleotides as probes. Hybridizations were carried out using Rapid-Hyb buffer (Amersham, Piscataway, NJ, USA) according to the manufacturer's protocol at 50 °C for 4 hours. Eight positive clones were sequenced, of which two (one from each library; clones Kl and K2R1 in Figure 23A) were viral and had 94-95% nucleotide identity to MTCR.

To sequence the remainder of the VP35 genome as well as the entire genome from the VP42 tumor, fragments of the genome were amplified by PCR using either amplified (Round B) or unamplified (Round A) cDNA prepared for original Virochip screening. This was accomplished first using a combination of primers derived from the sequence of MTCR (GenBank: NC 001702) and earlier recovered clones of XMRV; all primers are listed in Table 11. The amplified fragments were cloned into pCR2.1-TOPO TA vector (Invitrogen) and sequenced using M13 sequencing primers. Genome assembly was carried out using CONSED version 13.84 for Linux (Gordon, D., *et al.,* 1998, *Genome Res 8*: 195-202). Assembled genome sequences of XMRV

VP35 and VP42 have been submitted to GenBank (accessions DQ241301 and DQ241302).

PCR

Screening of tumor samples by gag nested RT-PCR was carried out according to Protocol S3. PCR fragments in all positive cases were gel purified using QIAEX II gel extraction kit (Qiagen), cloned into pCR2.1-TOPO TA vector (Invitrogen), and sequenced using M13 sequencing primers. *Pol* PCR was carried out using amplified cDNA (Round B material) as the template. Sequence of the primers used for amplification (2670F, 3870R, 3810F, and 5190R) are listed in Table 11. Amplified products were gel purified using QIAEX II gel extraction kit (Qiagen), and purified products were directly used for sequencing.

Phylogenetic analysis

The neighbor joining tree of full-length genomes (Figure 24) was generated as follows. Genomes of XMRV VP35 (GenBank: DQ241301) and VP42 (GenBank: DQ241302), MTCR (GenBank: NC 001702), MuLV DG-75 (GenBank: AF221065), MuLV MCF1233 (GenBank: U13766), AKV MuLV (GenBank: J01998), Friend MuLV (GenBank: NC 001362), Rauscher MuLV (GenBank: NC 001819), Moloney MuLV (GenBank: NC 001501), Feline leukemia virus (GenBank: NC 001940), Gibbon ape leukemia virus (GenBank: NC 001885), and Koala retrovirus (GenBank: AF151794) were first manually edited to make all genomes the same length, i.e. R to R. The edited sequences were then aligned with ClustalX version 1.82 for Linux (Thompson, JD., *et al.*, 1997, *Nucleic Acids Res 25*: 4876-4882; Jeanmougin, F., *et al.*, 1998, *Trends Biochem Sci 23*: 403-405) using default settings. The tree was generated based on positions without gaps only; Kimura correction for multiple base substitutions (Kimura, M., 1980, *J Mol Evol 16*: 111-120) and bootstrapping with N=1000 were also used.

The neighbor-joining trees of *gag* and *pol* fragments from different patients (Figures 27A-27B) were generated as above, except only MuLV genomes were included.

The neighbor-joining trees of Gag-Pro-Pol (Figure 29A) and Env (Figure 29B) polyproteins were based on alignments of protein sequences extracted from the GenBank records of the above 12 genomes and were generated as above, except gaps were included, and Kimura correction was not used.

Abbreviations

2-5A – 5'-phosphorylated 2'-5' oligoadenylate; CAT-I – Cationic amino acid transporter ; FLV – Feline leukemia virus; GALV – Gibbon ape leukemia virus; GRE – Glucocorticoid response element; HPC – Hereditary prostate cancer; IFN – Interferon; KoRV – Koala retrovirus; MCF1233 – Mink cell focus-inducing 1233 murine leukemia virus; MuLV – Murine leukemia virus; MTCR – Murine type C retrovirus; NZB-9-1 – New Zealand Black 9-1 xenotropic retrovirus; OAS – 2'-5' oligoadenylate synthetases; QQ – RNASEL homozygous R462Q; QR – RNASEL heterozygous R462Q; RNase L – Ribonuclease L; RR – RNASEL homozygous wild-type; SCLC – Human small cell lung cancer; SYG 1 – Suppressor of yeast gpal; VRA – Variable region A; VRB – Variable region B; XMRV – Xenotropic MuLV-related virus; XPR1 – Xenotropic and polytropic retrovirus receptor

Results

Detection of XMRV by microarray-based screening

To search for potential viruses in prostate cancer tumors, a DNA microarray-based strategy designed was employed to screen for viruses from all known viral families (Wang D, *et al.,* 2002, *Proc Natl Acad Sc. USA 99*: 15687-15692; Wang D, *et al.* , 2003, *PLoS Biol* 1: E2). Total or polyadenylated RNA extracted from tumor tissue was first amplified and fiuorescently labeled in a sequence-nonspecific fashion. The amplified and labeled fragments, which contained host as well as potential viral sequences, were then hybridized to a DNA microarray (Virochip) bearing the most conserved sequences of ~950 fully-sequenced NCBI reference viral genomes (~11,000 70-mer oligonucleotides).

The Virochip was used to screen RNA samples isolated from prostate tumors of 19 individuals (Figure 22A). A positive hybridization signal suggestive of a gammaretrovirus was detected in 7 of 11 tumors from patients homozygous for the

R462Q RNASEL variant (QQ). In contrast, no virus was detected in 3 tumors from RQ heterozygotes, and only 1 of 5 tumors from RR individuals was positive. Clustering of the microarray oligonucleotide intensities (Figure 22A) revealed a similar hybridization pattern in all positive cases. Furthermore, a computational analysis using E-Predict, a recently described algorithm for viral species identification (Urisman, A., *et al.*, 2005, *Genome Biol 6*: R78), assigned highest probabilities to several closely related mammalian gammaretroviruses, suggesting that the same or similar virus was present in all positive tumors (Table 12). Thus, Virochip detected the presence of a probable gammaretrovirus in nearly half of the QQ tumor samples and only one non-QQ sample.

Characterization of XMRV genome

To further characterize the virus its entire genome was recovered from one of the tumors (VP35) (Figure 23A-23C). To obtain viral clones, a direct microarray recovery technique described previously was first employed (Wang, D., *et al.*, 2003, *PLoS Biol 1*: E2). Briefly, amplified nucleic acid from the tumor tissue, which hybridized to viral microarray oligonucleotides, was eluted from two specific spots. The eluted DNA was re-amplified, and plasmid libraries constructed from this material were screened by colony hybridization using the spots' oligonucleotides as probes. The array oligonucleotides used in this case derived from the LTR region of Murine Type-C Retrovirus (MTCR; GenBank: NC_001702) and Spleen focus-forming virus (GenBank: NC 001500; (Clark, SP., *et al.*, 1983, *Proc Natl Acad Sci USA 80*: 5037-5041). The largest recovered fragment was 415 nucleotides in length, and had 96% nucleotide identity to the LTR region of MTCR, a MuLV identified in the genome of a mouse myeloma cell line (Heinemeyer T; unpublished). These findings established that the virus in question was indeed a gammaretrovirus, and likely a relative of murine leukemia viruses. To clone and sequence the rest of the viral genome, tumor cDNA was used to PCR-amplify overlapping segments using primers derived from MTCR; gaps were closed using primers from earlier recovered clones (Figures 23A-23C). Using a similar strategy, the full sequence of the virus from a second tumor, VP42, was also determined. The two genomes share >98% nucleotide

identity overall and >99% amino acid (aa) identity for predicted open reading frames (ORFS), and thus represent the same virus.

The full genome of the virus (Figures 23A-23C and 28) is 8185 nucleotides long and is distinct from all known isolates of MuLV. The genome is most similar to the genomes of MuLV DG-75 cloned from a human B-lymphoblastoid cell line (GenBank: AF221065, (Raisch, KP., *et al.*, 2003, *Virology 308*: 83-91) and of MTCR, with which it shares 94 and 93% overall nucleotide sequence identity, respectively. Phylogenetic trees constructed using available mammalian type C retroviral genomes (Figures 24 and 29A-29B) showed that the newly identified virus is more similar to xenotropic and polytropic than to ecotropic genomes. Based on these findings the provisional name "Xenotropic MuLV-related virus" (or XMRV) for this agent was proposed.

Translation of the XMRV genomic sequence using ORF Finder (Wheeler, DL., *et al.*, 2003, *Nucleic Acids Res 31*: 28-33) identified two overlapping ORFs coding for the full-length Gag-Pro-Pol and Env polyproteins. No exogenous coding sequences, such as viral oncogenes, could be detected in the XMRV genome. The predicted Gag-Pro-Pol polyprotein is 1733 aa long and has the highest aa identity with MuLV DG-75 (95%) (Figure 29A). An amber (UAG) stop codon separates the 536 aa Gag and 1197 aa Pro-Pol ORFs, analogous to other MuLVs in which a translational read-through is required to generate the full-length Gag-Pro-Pol polyprotein (reviewed in Wills, NM., *et al.*, 1991, *Proc Natl Acad Sci USA 88*: 6991-6995).

Similar to other MuLVs (Clark, SP., *et al.*, (1983), *Proc Natl Acad Sci USA 80*: 5037-5041; Raisch, KP., *et al.*, (2003), *Virology 308*: 83-91; Herr, W., 1984, *J Virol 49*: 471-478; O'Neill, RR., *et al.*, (1985), *J Virol 53*: 100-106; Perryman, S., 1991, *Nucleic Acids Res 19*: 6950; Shinnick, TM., *et al.*, (1981), *Nature 293*: 543-548; Sijts, EJ., *et al.*, (1994), *Virus Res 34*: 339-349), the Env polyprotein of XMRV is in a different reading frame compared to Gag-Pro-Pol. The Env protein sequence is 645 aa long, and has the highest amino acid identity with the Env protein of an infectious MuLV isolated from a human small cell lung cancer (SCLC) line NCI-417 (GenBank: AAC97875; (Antoine, M., *et al.*, (1998), *Virus Genes 17*: 157-168)) and MuLV NZB-9-1 (GenBank: K02730; (O'Neill, RR., Buckler *et al.*, (1985), *J Virol 53*: 100-106)), 95% and 94%, respectively. Conserved splice donor (AGGTAAG (SEQ ID

NO:47), position 204) and acceptor (CACTTACAG (SEQ ID NO:48), position 5479) sites involved in the generation of *env* subgenomic RNAs (Coffin, JM., *et al.,* (1997), Retroviruses. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press) were found in the same relative locations as in other MuLV genomes. A multiple sequence alignment of XMRV Env and corresponding protein sequences of other gammaretroviruses (Figure 25) showed that within two highly variable regions (VRA and VRB) known to be important for cellular tropism (Battini, JL., *et al.,* (1992), *J Virol 66*: 1468-1475; Tailor, CS., *et al.*, (2003), *Curr Top Microbiol Immunol 281*: 29-106), XMRV shares high aa identity with MuLV DG-75 and MuLV NZB-9-1 xenotropic envelopes (87% for VRA and 78% for VRB). Based on this finding, it was predicted that the cellular receptor for XMRV is XPR1 (SYGI), the recently identified receptor for xenotropic and polytropic MuLVs (Battini, JL., et al., (1999), Proc Natl Acad Sci USA 96: 1385-1390; Tailor CS, et al., (1999), Proc Natl Acad Sci USA 96: 927-932; Yang, YL., et al. ,(1999), Nat Genet 21: 216-219).

The long terminal repeat (LTR) of XMRV is 535 nucleotides long and has highest nucleotide identity with the LTRs of MTCR (96%) and MuLV NZB-9-1 (94%). The XMRV LTRs contain known structural and regulatory elements typical of other MuLV LTRs (Coffin, JM., *et al.,* (1997), Retroviruses. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; Temin, HM., (1981), *Cell 27*: 1-3) (Figure 28). In particular, the CCAAT (SEQ ID NO:49) box, TATAAAA (SEQ ID NO:50) box, and AATAAA (SEQ ID NO:51) polyadenylation signal sequences were found in U3 at their expected locations. U3 also contains a glucocorticoid response element (GRE) sequence AGA ACA GAT GGT CCT (SEQ ID NO:52). Essentially identical sequences are present in genomes of other MuLVs. These elements have been shown to activate LTR-directed transcription and viral replication in vitro in response to various steroids including androgens (Celander, D., *et al.,* (1988), *J Virol 62*: 1314-1322; Speck, NA., *et al.,* (1987), *Mol Cell Biol* 7: 1101-1110; DeFranco, D., *et al.,* (1986), *Mol Cell Biol* 6: 993-1001; Miksicek, R., *et al.,* (1986), *Cell 46*: 283-290). In addition, presence of an intact GRE is thought to be the determinant of higher susceptibility to FIS-2 MuLV infection in male compared to female NMRI mice (Bruland, T., *et al.,* (2003), *J Gen Virol 84*: 907-916; Bruland, T., *et al.,* (2001), *J Gen Virol 82*: 1821-1827).

The 5' *gag* leader of XMRV, defined as the sequence extending from the end of U5 to the ATG start codon of *gag*, consists of a conserved non-coding region of ~200 nucleotides, containing a proline tRNA primer binding site as well as sequences required for viral packaging (Adam, MA., *et al.,* (1988), *J Virol 62*: 3802-3806; Fisher, J., *et al.,* (1998), *Virology 244*: 133-145) and the initiation of translation (Berlioz, C., *et al.,* (1995), *J Virol 69*: 2214-2222; Vagner, S., *et al.*, (1995), *J Biol Chem 270*: 20376-20383). The non-coding region is followed by a ~270 nucleotide region extending from the conserved CTG alternative start codon of *gag*. This region represents the most divergent segment of the genome compared to other MuLVs (Figures 26 and 23B). Unlike ecotropic MuLVs, where translation from this codon adds a ~90 aa N-terminal leader peptide in frame with the rest of the Gag protein, thus generating a glycosylated form of Gag (Prats, AC., *et al.,* (1989), *J Mol Biol 205*: 363-372), XMRV has a stop codon 53 aa residues downstream from the alternative start. Interestingly, both MuLV DG-75 and MTCR gag leader sequences are also interrupted by stop codons, and therefore are not expected to produce full-length glyco-Gag. Furthermore, a characteristic 24-nucleotide deletion was present in this region of the XMRV genome, which is not found in any known exogenous MuLV isolate or endogenous retroviral element in the sequenced mouse genome. In cell culture, expression of intact glyco-Gag is not essential for viral replication (Fan, H., *et al.,* (1983), *Proc Natl Acad Sci USA 80*: 5965-5969; Schwartzberg, P., *et al.,* (1983), *J Virol 46*: 538-546). However, lesions in this region have been associated with interesting variations in pathogenetic properties in vivo (Chun, R., *et al.,* (1994), *J Biomed Sci 1*: 218-223 ; Corbin, A., *et al.,* (1994), *J Virol 68*: 3857-3867; Fujisawa, R., *et al.,* (1997), *J Virol 71*: 5355-5360; Munk, C., *et al.*, (2003), *Virology 313*: 44-55; Portis, JL., *et al.,* (1996), *Virology 226*: 384-392). For example, an alteration in 10 nucleotides affecting 5 residues in the N-terminal peptide of glyco-Gag was found to be responsible for a 100-fold difference in the frequency of neuroinvasion observed between CasFrKP and CasFrKP41 MuLV strains (Fujisawa, R., *et al.,* (1998), *J Virol 72*: 5619-5625). In addition, insertion of an octanucleotide resulting in a stop codon downstream of the CUG start codon prevented severe early hemolytic anemia and prolonged latency of erythroleukemia in mice infected with Friend MuLV (Corbin, A., *et al.,* (1994), *J Virol 68*: 3857-3867). While the

pathogenetic significance of the lesions in XMRV glyco-Gag is not known, the high degree of sequence divergence indicates that this region is under positive selective pressure, and therefore, likely relevant to the establishment of infection within the human host.

Association of XMRV infection and R462Q RNASEL genotype

To further examine the association between presence of the virus and the R462Q (1385G->A) RNASEL genotype, a specific nested RT-PCR assay based on the virus sequence recovered from one of the tumor samples (VP35, see above) was developed. The primers in this assay (Figures 28A-28B) amplify a 380-nucleotide fragment from the divergent 5' leader and the N-terminal end of gag. The RT-PCR was positive in 8 (40%) of 20 examined tumors from homozygous (QQ) individuals. In addition, one tumor from a homozygous wild-type (RR) patient was positive among 52 RR and 14 RQ tumors examined (Figure 22B and Table 10). Interestingly, this case was associated with the highest tumor grade among all XMRV-positive cases (Table 11 in Molinaro et al, 2005). PCR specific for the mouse GAPDH gene was negative in all samples, arguing strongly against the possibility that the tumor samples were contaminated with mouse nucleic acid. Collectively, these data demonstrate a strong association between the homozygous (QQ) R462Q RNASEL genotype and presence of the virus in the tumor tissue ($p<0.00002$ by two-tail Fisher's exact test).

XMRV sequence diversity in samples from different patients

To examine the degree of XMRV sequence diversity in different patients, the amplified fragments from all 9 samples, which were positive by the nested gag RT-PCR, were sequenced. The amplified *gag* fragments were highly similar (Figure 27A) with >98% nucleotide and >98% aa identity to each other. In contrast, the fragments had <89% nucleotide and <95% aa identity with the most related sequence of MuLV DG-75. In addition to the *gag* gene, the same patient samples were also examined for sequence variation in the *pol* gene. PCR fragments obtained with a set of primers targeting a 2500-nucleotide stretch in the *pol* gene were sequences (Figures 28A-28B). Similar to the *gag* fragments, the amplified *pol* fragments were highly similar (Figure 27B) and had >97 % nucleotide and >97 % aa identity to each other. In contrast, the

fragments had <94 % nucleotide and <95% aa identity with the most related sequence, that of MuLV DG-75.

Close clustering of the sequenced *gag* and *pol* fragments (Figures 27A-27B) indicates that all microarray and RT-PCR positive cases represent infection with the same virus. On the other hand, the degree of sequence variation in the examined fragments is higher than that expected from errors introduced during PCR amplification and sequencing. The frequency of nucleotide misincorporation by Taq polymerase has been estimated as 10^{-6} -10^{-4} ((Bracho, MA., *et al.*, (1998), *J Gen Virol 79 (Pt 12)*: 2921-2928) and references therein), compared to the observed rate of up to 2% in the *gag* and *pol* fragments. These findings indicate that the observed XMRV sequence variation is a result of natural sequence diversity, consistent with the virus being independently acquired by the affected patients, and argue against laboratory contamination as a possible source of XMRV.

Discussion

The results presented here identify XMRV infection in prostate tissue from approximately half of patients with prostate cancer who are homozygous for the R462Q variant (QQ) of RNase L, as judged by both hybridization to the Virochip microarray and by RT-PCR with XMRV-specific primers. Parallel RT-PCR studies of prostate tumors from wild-type (RR) and heterozygous (RQ) patients revealed evidence of XMRV in only 1 of 66 samples, clearly demonstrating that human XMRV infection is strongly linked to decrements in RNase L activity. This result supports the view that the R462Q RNase L variant leads to a subtle defect in innate (IFN-dependent) antiviral immunity.

As its name indicates, XMRV is closely related to xenotropic murine leukemia viruses (MuLVs). Unlike ecotropic MuLVs, such as the canonical Moloney MuLV, which grow only in rodent cells in culture, xenotropic MuLVs can grow in non-rodent cells in culture but not in rodent cell lines. Xenotropic MuLVs are thought to result from in vivo recombination events between an exogenous ecotropic virus infecting a susceptible mouse strains and numerous endogenous MuLV-like sequences present in the mouse genome. These endogenous elements are relics of ancestral retroviral integration events into the mouse germline, and most

have suffered inactivating deletions and other rearrangements over evolutionary time. Some, though, are full-length and are expressed in certain mouse backgrounds (Levy, JA, (1973), *Science 182*: 1151-1153; Levy, JA, (1978), *Curr Top Microbiol Immunol 79*: 111-213). The recombination invariably involves substitution of the 5' end of the *env* gene encoding the N-terminal region of the mature SU glycoprotein ((Evan, LH., *et al.,* (2003), *J Virol 77*: 10327-10338) and references therein). This region specifies receptor preference of the SU glycoprotein, and thus determines the host range of the recombinant virus (Battini, JL., *et al.,* (1992), *J Virol 66*: 1468-1475; Ott, D., *et al.,* (1992), *J Virol 66*: 4632-4638). Unlike ecotropic MuLVs, which can only recognize a receptor (CAT-1) specific to mouse and rat species (Albritton, LM., *et al.,* (1989), *Cell 57*: 659-666; Kim, JW., *et al.,* (1991), *Nature 352*: 725-728; Wang, H., *et al.,* (1991), *Nature 352*: 729-731), xenotropic strains recognize a protein known as XP RI or SYGI. XPR1 is expressed in all higher vertebrates, including mice, but polymorphisms in the murine gene render it unable to mediate xenotropic MuLV entry (Battini, JL., *et al.,* (1999), *Proc Natl Acad Sci USA 96*: 1385-1390; Tailor CS, *et al.,* (1999), *Proc Natl Acad Sci USA 96*: 927-932; Yang, YL., *et al.* ,(1999), *Nat Genet 21*: 216-219). Thus, xenotropic MuLVs have a potential to infect a wide variety of mammalian species, including humans.

Xenotropic MuLVs have occasionally been detected in cultured human cell lines. For example, MuLV DG-75 was cloned from a human B-lymphoblastoid cell line (Raisch, KP., *et al.*, (2003), *Virology 308*: 83-91), and an infectious xenotropic MuLV was detected in a human small cell lung cancer (SCLC) line NCI-417 (Antoine, M., *et al.,* (1998), *Virus Genes 17*: 157-168). Although laboratory contamination, either in culture or during passage of cell lines in nude mice, cannot be ruled out as a possible source in these cases, such contamination cannot explain our results. The evidence for this is as follows: (i) XMRV was detected in primary human tissues; (ii) no murine sequences (e.g. GAPDH) could be detected in our materials by PCR; and (iii) infection was predominantly restricted to human samples with the QQ RNASEL genotype; (iv) polymorphisms were found in the XMRV clones recovered from different patients consistent with independent acquisition of the virus by these individuals. Finally, it is shown in Example 3 (Molinaro et al, 2005) that viral transcripts and antigens can be detected in infected QQ prostate tissue by fluorescence

in situ hybridization and immunohistochemistry, respectively, providing additional evidence for infection in vivo. Taken together, the above evidence argues strongly against laboratory contamination with virus or cloned DNA material as the source of XMRV infection in the analyzed samples. The findings described herein are examples of authentic infection of humans with a xenotropic MuLV-like agent.

The XMRV sequence is not found in human genomic DNA (as represented in sequence databanks), indicating that it must have been acquired exogenously by infection in positive subjects. From what reservoir, and by what route such infections were acquired is unknown. It seems unlikely that direct contact with feral mice could explain the observed distribution of infection in our cohort, since there is no reason to believe that rodent exposure would vary according to RNASEL genotype. It is possible that infection is more widespread than indicated by the present studies, especially if, as seems likely, individuals with the wild-type RNase L clear infection more promptly than those with the QQ genotype. But if so, a cross-species transfer model of XMRV infection would require improbably high levels of rodent exposure for a developed society like our own. Thus, although the viral sequence suggests that the ultimate reservoir of XMRV is probably the rodent, the proximate source of the infection seems unlikely to be mice or rats.

The data described herein do indicate that XMRV is not functioning by encoding a dominantly-acting oncogene, as XMRV is a simple retrovirus composed solely of *gag*, *pol* and *env* sequences, and has no acquired host-derived sequences in its genome. Moreover, the single cell analyses of Example 3 show that the viral genome is not present in the cancer cells themselves, but appears to target stromal cells whose identities are still under examination. This renders unlikely another model of retroviral oncogenesis — namely, host oncogene activation by insertion into the cellular genome of prostatic epithelial cells as the proximal cause of clonal expansion of these cells.

EXAMPLE 3 XMRV Infection in Tissues and Cell Lines from Prostate Cancers with *RNASEL/HPC1* Mutations

RNase L is a unique antiviral protein activated by 5'-phosphorylated, 2'-5'-oligoadenylates. Example 2 describes the identification of the genome of

a novel gammaretrovirus, named xenotropic MuLV related virus (XMRV), in prostate cancer cases homozygous for a reduced activity variant of RNase L (R462Q). Shown herein by fluorescence in situ hybridization and immunohistochemistry that XMRV nucleic acid and protein can be detected in about 1% of cells in prostate tissues from cases infected with XMRV and homozygous for the RNase L variant. The infected cells are prostatic stromal cells, predominantly fibroblasts and hematopoietic elements, in regions adjacent to the carcinoma. Screening of cell lines derived from prostate cancer revealed that a single clone of LNCaP cells, which also bears mutations in RNase L, harbors an XMRV-like genome closely related to those found in tumors in vivo. This clone expresses genomic and subgenomic viral transcripts, and releases infectious particles into the medium; these particles can be serially propagated in several cell lines of human but not murine origin. The availability of replication-competent XMRV should facilitate the study of viral replication, its link to RNase L variants and its relationship to prostatic and other diseases.

Materials and Methods

Genotyping of patients and prostate tissue processing

Men scheduled to undergo prostatectomy with curative intent at the Cleveland Clinic were genotyped for the R462Q (1385G->A) RNASEL variant using a premade TAQMAN genotyping assay (Applied Biosystems, assay c_935391_1) on DNA isolated from perpheral blood mononuclear cells. Immediately after radical prostatectomy, tissue cores were taken from the peripheral zone (where most cancer occurs) and frozen in liquid nitrogen for subsequent RNA isolation (Urisman, A., *et al.,* (2005), *PLOS Pathogens*). For histologic analysis, freshly excised prostate tissue was fixed in 10% neutral buffered formalin, processed and embedded in paraffin. Tissue microarrays were used for some experiments (see legend to Figure 31). All of these studies were performed under a Cleveland Clinic institutional review board approved protocol.

Antibodies

Monoclonal antibody to SFFV Gag protein was produced from R187 cells (ATCC; CRL-1912) grown in DMEM (Media Core, Cleveland Clinic Foundation, Cleveland, OH)with 10% ultra-low IgG FBS (Invitrogen) until confluent. Conditioned media was collected every three days from confluent cultures. Five ml of conditioned media per preparation was centrifuged at 168 x g for 5 min at 4°C. Supernatant was filtered through a 0.22 μm syringe filter unit (Millipore Corp.) and concentrated 16-fold in an Amicon ultrafiltration unit with a 100 kDa molecular weight cutoff membrane (Millipore Corp.). Sodium azide was added to a final concentration of 0.02%. Rabbit polyclonal antibody to the conserved MuLV NC peptide sequence: KDCPKKPRGPRGPR (SEQ ID NO:53) conjugated to keyhole limpet hemocyanin was prepared by Open Biosystems, Inc., Huntsville, AL. The antibody was affinity purified by a protocol including linking the peptide to sepharose, binding then eluting the antibody from the column. Concomitant XMRV FISH/cytokeratin immunofluorescence was performed using a mouse anti-cytokeratin AE1/AE3 (20:1 mixture) monoclonal antibody (Chemicon) capable of recognizing normal and neoplastic cells of epithelial origin.

Cell Culture

Cell lines, LNCaP-R (W. Heston, Cleveland), LNCaP-FGC (ATCC Cat# CRL-1740), DU145 (ATCC Cat# HTB-81) and PC3 (ATCC Cat# CRL-1435) were grown in RPMI 1640 medium with 2 mM L-glutamine, fetal bovine serum, 10%; 200 units penicillin G and 200 μg/ml streptomycin. Normal prostate epithelial cells (PrEC) were obtained from Clonetics Corporation (San Diego, CA) and were maintained in PrEGM supplemented with a mixture of various growth factors (SingleQuots) (Clonetics); fetal bovine serum, 10%.

Cytoblock Preparation

Approximately 10^9 cells (LNCaP-R, LNCaP-FGC, DU145 and PC3) were washed with Hanks balanced salt solution (HBSS) without phenol red, Ca++ or Mg++ (Invitrogen) and resuspended gently, but completely, with 10% neutral buffered formalin. The cell suspensions were fixed overnight at 4°C, centrifuged and washed

twice with HBSS. The supernatant was aspirated and the cells were resuspended in one drop of HBSS. Cell suspensions were pipetted into a cytoblock cassette (Thermo Electron Corp.). The fixed cell culture cytoblocks were processed and embedded into paraffin blocks within 24 hr. The embedded cytoblocks were cut into ~4 μm thick sections placed onto charged slides and baked for at least 4 hr at 60-65°C for immunofluorescence.

RNA Isolation and RT-PCR

Total RNA was isolated from cells using TRIzol reagent (Invitrogen). The RNA was treated with DNase I (RNase-free) (Ambion), acid phenol:chloroform extracted and
precipitated for RT-PCR analysis. First strand cDNA synthesis was performed using 1μg RNA and random hexamer primers with the TaqMan® Reverse Transcription Reagents
kit (Applied Biosystems). PCR was performed on the first strand cDNA using primers specific for a 700 bp env region of XMRV-35; forward primer-7050, 5' GTT TAT GGC CAG TTT GGA AA 3' (SEQ ID NO:41), and reverse primer-7750, 5' GCC TTA TGG TGG GGT CTT TC 3' (SEQ ID NO:42). GAPDH exon 8 specific primers were used as a positive control; forward primer, 5' TGC CAT CAC TGC CAC CCA GA 3' (SEQ ID NO:54), and reverse primer, 5' CTT GAC AAA GTG GTC GTT GA 3' (SEQ ID NO:55).

FISH

The XMRV-35 FISH probe cocktail was generated using both 2.15kb and 1.84 kb segments of the viral genome obtained by PCR with forward primer-2345, 5' ACC CCT AAG TGA CAA GTC TG 3' (SEQ ID NO:43)with reverse primer-4495, 5' CTG GAC AGT GAA TTA TAC TA 3' (SEQ ID NO:44) and forward primer-4915, 5' AAA TTG GGG CAG GGG TGC GA 3' (SEQ ID NO:56)with reverse primer-6755, 5' TTG GAG TAA GTA CCT AGG AC 3' (SEQ ID NO:57), both cloned into pGEM®-T (Promega). The recombinant vectors were digested with EcoRI to release the viral cDNA fragments, which were purified after gel electrophoresis (Qiagen). The purified viral cDNA inserts were used in nick translation reactions to produce

SpectrumGreen™ dUTP fluorescently labeled probe according to manufacturer's instructions (Vysis Inc.). Freshly baked slides of prostatic tissues or tissue microarray arrays with ~4 μm thick tissue sections were deparaffinized, rehydrated, and subjected to Target Retrieval (Dako) for 40 min at 95°C. Slides were cooled to room temperature and rinsed in H2O. Proteinase K (Dako) at 1:5000 in Tris-HCl pH 7.4 was applied directly to slides for 10 min at room temperature. Adjacent tissue sections were also probed with SpectrumGreen™ dUTP fluorescently labeled KSHV-8 DNA (nts 85820-92789) as a negative control or, as a positive control with SpectrumGreen™ and SpectrumOrange™ labeled TelVysion™ DNA Probe cocktail (Vysis Inc.), specific for different arms of human chromosome 1 as a positive control to ensure the tissue was completely accessible to FISH. FISH slides were examined using a Leica DMR microscope (Leica Micro-Systems, Heidelberg, Germany), equipped with a Retiga EX CCD camera (Q-Imaging, Vancouver, British Columbia, Canada). FISH images were captured using a Leica TCS SP2 laser scanning confocal with a 63X oil objective numerical aperature (N.A.) 1.4 (Leica Micro-Systems, Heidelberg, Germany) microscope. XMRV nucleic acids were visualized using maximum intensity projections of optical slices acquired using a 488 nm argon-laser (emission at 500 to 550 nm). TelVysion™ DNA Probes were visualized using maximum intensity projections of optical slices acquired using a 488 nm argonlaser (emission at 500 to 550 nm) and 568 nm krypton-argon-laser (emission at 575 to 680 nm). DAPI was visualized using maximum intensity projections of optical slices acquired using a 364 nm UV-laser (emission at 400 to 500 nm). Slides were subsequently washed in 2X SSC [0.3 M sodium chloride and 0.03 M sodium citrate (pH 7.0)] to remove coverslips, and H&E stained for morphological evaluation.

IHC

IHC on human tissues was performed on a Benchmark Ventana Autostainer (Ventana Inc.). Unstained, formalin fixed, paraffin embedded prostate sections were placed on electrostatically charged slides and deparaffinized followed by a mild cell conditioning achieved through the use of Cell Conditioner #2 (Ventana Inc.). The concentrated R187 monoclonal antibody against SFFV p30 Gag was dispensed manually onto the sections at 10 μg per ml (Ventana Inc.) and allowed to incubate for

32 min at 37°C. Endogenous biotin was blocked in sections using the Endogenous Biotin Blocking Kit (Ventana Inc.). Sections were washed, and biotinylated ImmunoPure Goat Anti-Rat IgG (Pierce) was applied at a concentration of 4.8 μg/ml for 8 min. To obtain Gag protein localization, the Ventana Enhanced Alkaline Phosphatase Red Detection Kit (Ventana Inc.) was used. Sections were briefly washed in distilled water and counterstained with Hematoxylin II (Ventana Inc.) for approximately 6 min. Sections were washed, dehydrated in graded alcohols, incubated in xylene for 5 min and coverslips were added with Cytoseal (Microm Int.). Negative controls were performed as above except without the addition of the R187 monoclonal antibody.

Concomitant XMRV FISH/cytokeratin IHC was performed on slides of prostate tissue from patient VP62. First, sections were immunostained for cytokeratin AE1/AE3 using the Alexa Fluor 594 Tyramide Signal Amplification Kit (Molecular Probes) exactly as described below except Protease II (Ventana Inc.) was used for 3 min at room temperature and goat anti-mouse IgG-horseradish peroxidase (Molecular Probes) was added. Slides were placed in Target Retrieval solution (Dako) for 40 min at 95°C. FISH for XMRV was performed as described above except in the absence of proteinase K treatment. After FISH, the slides were mounted with Mounting Medium plus DAPI (Vectashield Inc.) and examined using fluorescence microscopy. Immunofluorescence images were captured using a Texas red filter with a Leica DMR microscope (Leica Micro-Systems, Heidelberg, Germany), equipped with a Retiga EX CCD camera (QImaging, Vancouver, British Columbia, Canada).

Immunofluoresence of the LNCaP-R, LNCaP-FGC, DU145 and PC3 cytoblock sections was performed manually using the Alexa Fluor 594 Tyramide Signal Amplification Kit (Molecular Probes). Briefly, unstained, formalin fixed, paraffin embedded cytoblock sections cut at ~4 μm were placed on electrostatically charged slides, baked at 65°C for at least 4 hr, deparaffinized in xylene and rehydrated through decreasing alcohol concentrations. Slides were incubated in Protease III (Ventana Inc.) for 3 min at room temperature and washed in phosphate buffered saline (PBS) in peroxidase quenching buffer (PBS + 3% H_2O_2) for 60 min at room temperature, incubated with 1% blocking reagent (10 mg/ml BSA in PBS) for 60 min at room temperature. The slides were incubated with the antibody against XMRV-35

NC (Gag) peptide (Open Biosystems) at a concentration of 0.25 μg/ml diluted in 1% blocking reagent for 60 min at room temperature and rinsed three times in PBS. Goat anti-rabbit IgG-horseradish peroxidase (Molecular Probes) was added and incubated for 60 min at room temperature. The slides were rinsed three times in PBS. The tyramide solution was added to the slides for 10 min at room temperature and the slides were rinsed 3X in PBS. The slides were mounted with Mounting Medium plus DAPI (Vectashield Inc.) and examined using fluorescence microscopy.

Virus Infections and RT Assays

LNCaP-FGC cells were plated at 20% confluency and washed with PBS. Five hundred μl LNCaP-R supernatant, centrifuged at 3000 g for 15 min and filtered twice through a 0.22 μm filter, was added to the cells diluted 1:2 in RPMI with 8 μg/ml polybrene (Sigma) without FBS or antibiotics for 3 hr. Virus was removed, and cells were replenished with RPMI, 90%; fetal bovine serum, 10%; 200 Units Penicillin/Streptomycin. RT activity was measured after incubating at 37°C for 1 hr as described (Telesnitsky, A., *et al.,* (1995), *Methods Enzymol 262*: 347-362). All reactions were performed with α-^{32}P-dTTP, and aliquots of tissue culture media were collected each day post-infection and tested for RT activity. Quantitation was by phosphorimage analysis using a Storm Scanner 840 (GE Healthcare), and software ImageQuant V5.2 (Molecular Dynamics). PBS was used as a negative control, and 0.4 units of MLV-RT (Invitrogen) were used as a positive control in the RT assays.

Northern blots

RNA was separated on a 1% agarose formaldehyde gel and then transferred to nylon membranes using Turbo Blot kits (Schleicher and Schuell) according to the manufacturer's instructions. The transferred membranes were rinsed in 2x SSC and autocrosslinked (UV Stratalinker 2400; Stratagene). The blots were prehybridized in Ultrahyb (Ambion) and then hybridized with ^{32}P-labeled probes. DNA probes were generated using RediPrimeII (Amersham Biosciences) according to the manufacturers' instructions.

Southern hybridization

Cells growing in 75-cm^2 flasks were trypsinized and lysed in sodium dodecyl sulfate buffer (100 mM Tris, 150 mM NaCl, 10 mM EDTA, 0.1% sodium dodecyl sulfate) containing 100 μg of proteinase K per ml for 1hr at 55°C followed by RNaseA treatment, 30 min at 37oC. DNA was extracted with phenol-chloroform, ethanol precipitated, and digested with PstI. 20 αg of DNA for each sample was separated by agarose gel electrophoresis, transferred to a nylon membrane (alkalic transfer), neutralized with 2xSSC, crosslinked and hybridized to a radiolabeled XMRV VP35 U3 LTR probe corresponding to position 7780-7991. The membrane was washed and exposed to Kodak XAR5 film for 1-12 hr.

Genome cloning and sequencing

Genome cloning and sequencing of XMRV-LNCaP RV were performed as described in (Urisman, A., *et al.,* (2005), *PLOS Pathogens*).

Phylogenetic analysis

The neighbor-joining tree of full-length genomes was generated as follows. Genomes of XMRV LNCaP-R (Genebank: DQ272467), VP35 (GenBank: DQ241301) and VP42 (GenBank: DQ241302), MTCR (GenBank: NC_001702), MuLV DG-75 (GenBank: AF221065), MuLV MCF1233 (GenBank: U13766), AKV MuLV (GenBank: J01998), Friend MuLV (GenBank: NC_001362), Rauscher MuLV (GenBank: NC_001819), Moloney MuLV (GenBank: NC_001501), Feline leukemia virus (GenBank: NC_001940), Gibbon ape leukemia virus (GenBank: NC_001885), and Koala retrovirus (GenBank: AF151794) were aligned with ClustalX version 1.83 using default settings. The tree was generated as described by Urisman, A., *et al.,* (2005), *PLOS Pathogens*. The neighbor-joining trees of Gag-Pro-Pol and Env polyproteins were based on alignments of protein sequences extracted from the GenBank records of the above 12 genomes and were generated as described in Urisman, A., *et al.,* (2005), *PLOS Pathogens*.

Cloning and Mapping of Integration Sites

Twenty micrograms of genomic DNA was digested with Pst I and extracted with phenol:chloroform (1:1, v/v), followed by ethanol precipitation. Pst I cleaves once in the viral genome at nucleotide position 7,150 and was used to produce DNA fragments containing the right LTR and the neighboring cellular DNA. The digested DNA was annealed with 0.1 μM of a biotinylated primer B-7151F (5' Bio-TEGGGAGTTGGAACAGGGACTACA (SEQ ID NO:58); Operon), which is complementary to nucleotide positions 7,151-7,171, about 600 bp upstream of the right LTR. The annealed primer was then extended in a final volume of 300 μl using 10 units *pfuUltra* DNA polymerase and 0.2 mM dNTPs in 1 x PfuUltra buffer (Stratagene). The reaction mixture was heated to 94^0C for 5 min, cooled down to 56^0C for 5 min, and then kept at 72^0C for 20 min. After chain elongation, the free biotinylated primer was removed by E. coli exonuclease I digestion, and the sample was treated with phenol:chloroform extraction and ethanol precipitation. The biotinylated DNA was isolated using the Dynabeads kilobase BINDER kit (DYNAL Biotech) as described by the manufacturer. The isolated DNA was digested with *Nsp*I and washed with 2 x 800 μl buffer A (10 mM Tris-HCl, pH7.5, 1M NaCl, 1 mM EDTA, 200 μg/ml bovine serum albumin) and 2 x 800 μl 1 x T4 DNA ligation buffer. The DNA was then ligated to the Nsp-linker using T4 DNA ligase (Invitrogen) for 3 hr at 16^0C with occasional tapping. The Nsp-Linker was prepared by annealing 20 μM Link-A (5' CGGATCCCGCATCATATCTCCAGGTGTGACAGTTT (SEQ ID NO:59)) with 20 μM Link-Nsp-S (5'AACCTGGAGATATGATGCGGGATCCGCATG (SEQ ID NO:60)). The excess Nsp-linkers were removed by washing the Dynabeads with 2 x 800 μl buffer A, followed with 2 x 800 μl buffer B (5 mM Tris-HCl, pH7.5, 0.1 mM EDTA). The proviral DNA junctions were amplified by PCR using 0.5 μM of U38F (5'-CGTGTTCCCAATAAAGCCTT (SEQ ID NO:61)) and NspL-R (5'-TAACCTGGAGATATGATGCGGGA (SEQ ID NO:62)) as the forward and reverse primers, respectively. The reaction mixture, which contained *Pfu*Ultra DNA polymerase, 0.2 mM of dNTPs, and 1 x PfuUltra buffer, was heated to 94^0C for 2 min, then cycled 27 times at 94^0C for 30 s, 56^0C for 40 s, and 72^0C for 8 min, and followed with a final extension at 72^0C for 15 min. The amplified DNA was cloned using the

Zero Blunt PCR Cloning Kit (Invitrogen) and sequenced. The chromosomal sequences adjacent to the viral LTR were mapped onto the human genome using UCSC Genome Browser on Human May 2004 Assembly.

Abbreviations

FISH – Fluorescence in situ hybridization; H&E – Hematoxylin and eosin; HPC-Hereditary prostate cancer; IFN – Interferon; IHC – Immunohistochemistry; FLV – Feline leukemia virus; GALV – Gibbon ape leukemia virus; KoRV – Koala retrovirus; MCF1233 – Mink cell focus-inducing 1233 murine leukemia virus; MuLV – Murine leukemia virus; MTCR – Murine type C retrovirus; N.A. – numerical aperature; OAS – 2'-5' oligoadenylate synthetases; PBS – Phosphate buffered saline; PCR – Polymerase chain reaction; PIA – Proliferative inflammatory atrophy; PIN – Prostatic intraeplithelial neoplasia; 2-5A – 5'-phosphorylated 2'-5' oligoadenylate; QQ – RNASEL homozygous R462Q; QR – RNASEL heterozygous R462Q; RNase L – Ribonuclease L; RT - Reverse transcriptase; RR – RNASEL homozygous wild-type 462R; SNP – Single nucleotide polymorphism; VRA – Variable region A; VRB – Variable region B; XMRV – Xenotropic MuLV-related virus

Results

XMRV nucleic acid is present in tumor-bearing prostate tissue

To localize XMRV within human prostatic tissues, and to measure the frequency of the infected cells, *in situ* molecular techniques were used. XMRV nucleic acid was visualized using fluorescence in situ hybridization (FISH) on formalin-fixed prostate tissues. A SpectrumGreen fluorsescently labeled FISH probe cocktail spanning all viral genes was prepared using cDNA derived from the XMRV isolate cloned from patient VP35 (Materials and Methods). Distinct FISH-positive cells were observed in the tumors positive for XMRV by RT-PCR (e.g. VP62 and VP88) (Figure 30). To identify cell types associated with the positive FISH signal, the same sections were subsequently stained with hematoxylin and eosin (H&E). Most FISH-positive cells were stromal fibroblasts, although occasional infected hematopoietic cells were also seen. While the XMRV nucleic acid was usually present within nuclei, indicating integrated proviral DNA, some cells showed cytoplasmic

staining adjacent to the nucleus, suggestive of pre-integration complexes in non-dividing cells (Figure 31, panel A). An example of an XMRV-infected leukocyte is shown adjacent to a prostatic gland stained (red) with cytokeratin AE1/AE3 murine monoclonal antibody cocktail specific for epithelial cells (Wernert, N., *et al.,* (1987), *Pathol Res Pract 182*: 617-626)(Figure 31, panels E&F). The infected cell in the stroma is negative for cytokeratins AE1/AE3, confirming its non-epithelial origin. The indented nucleus and the dark and condensed chromatin are consistent with a stromal hematopoietic cell.

Frequency of XMRV-infected prostatic cells

FISH was used to obtain a minimal estimate of the frequency of XMRV-infected prostatic cells. The XMRV FISH probes were compared with two FISH probes specific for subtelomeric regions of the p and q arms of chromosome 1 (labeled with SpectrumGreen and SpectrumOrange, respectively) (Figure 32). Whereas two XMRV/FISH positive cells were observed in a field of VP88 prostatic tissue (green signals in Figure 32, panel A), essentially every cell in an adjacent section was labeled with the chromosome 1-specific probes (red and green signals in Figure 32, panel B). Because of the low frequency of XMRV positive cells, negative controls were performed using a probe targeting Kaposi's sarcoma-associated herpesvirus (KSHV) DNA (nts 85820-92789) which did not label any cells in prostate specimens VP88 and VP51, but this probe did efficiently label 293T cells transfected with KSHV DNA. Additional XMRV FISH experiments were performed on a tissue microarray containing duplicates of fourteen different prostate cancer tissue specimens (Table 13). Five homozygous RNase L 462Q (QQ) cases (VP29, 31, 42, 62, and 88) showed 5 to 10 XMRV/FISH positive cells each (about 1% of prostate cells observed). Patient sample VP79, also a QQ case, contained 2 positive cells (0.4% of total cells examined). All of the XMRV FISH positive cells observed were stromal cells. In contrast, three RR tissue samples and two RQ tissue samples showed one or no (<0.15%) FISH positive cells. Two of the QQ cases, VP35 and VP90, positive by gag RT-PCR (Urisman, A., *et al.,* (2005), *PLOS Pathogens*) showed only one FISH positive cell each (Table 13). Conversely, one case, VP31, was FISH positive, but gag

RT-PCR negative. These results could be due to heterogeneity in virus copy numbers between specific regions of the prostate sampled.

Presence of XMRV in prostatic tissues as determined by immunohistochemistry

To identify cells expressing XMRV proteins, the presence of Gag protein was investigated using a monoclonal antibody against spleen focusing forming virus (SFFV); this antibody is reactive against Gag proteins from a wide range of different ecotropic, amphotropic and xenotropic MuLV strains (Chesebro, B., *et al.*, (1983), *Virology 127*:134-148). Using this antibody, positive signal by IHC was observed in prostatic tissues of XMRV-positive cases VP62 and VP88, both QQ (Figure 33). An enhanced alkaline phosphatase red detection method allowed Gag detection in the same cells with both fluorescence (Figure 33, panels A, B, E&F) and bright field (Figure 33 panels C, D, G&H) microscopy. The Gag expressing cells were observed in prostatic stromal cells with a distribution and frequency similar to that detected by FISH (Figure 33). In contrast, no Gag positive cells were observed in VP51 prostatic tissue, which is of RR genotype (Figure 33, panels I&J).

A related gammaretrovirus in a prostate cancer cell line

Over the years, several cell lines have been derived from human prostate cancers. Two of these, PC3 and DU145, are wild-type with respect to RNASEL, while the widely-studied LNCaP line is heterozygous both for an inactivating deletion mutation in RNASEL (471ΔAAAG) and for the R462Q variant (Rennert, H., *et al.*, (2002), *Am J Hum Genet 71*: 981-984; Xiang, Y., *et al.*, (2003), *Cancer Res 63*: 6795-6801). Once the relation between XMRV infection and RNASEL mutations was established (Urisman, A., *et al.*, (2005), *PLOS Pathogens*), evidence of infection with XMRV-like agents was investigated in these cell lines; additionally, a line of normal prostatic epithelial cells (PrEC) were investigated . Two clones of LNCaP were studied, one of which, LNCaP-FGC, was freshly obtained for this purpose from the ATCC repository; the other (LNCaP-R) had been serially passaged in the laboratory. [LNCaP-R is the name for an isolate originally obtained from ATCC (as LNCaP-FGC) and maintained at the Cleveland Clinic, Department of Cancer Biology (laboratory of W. Heston)]. RT-PCR was performed on RNA from these cell lines

using primers specific for a conserved 700 bp region within the env protein encoding region of XMRV VP35 (Figure 34, panel A). No PCR products were detected in most cell lines, including all those with wild-type RNASEL. Notably, however, one of the two tested clones of LNCaP (LNCaP-R) was positive for a band of the expected size, while the other (LNCaP-FGC) was negative. The positive control GAPDH amplimer (391 bp) was present at similar levels after RT-PCR reactions from each of the cell lines.

The genome of the LNCaP-R retrovirus

The entire retroviral genome from LNCaP-R cells was recovered as overlapping cDNA fragments applying the same RT-PCR strategy used to recover XMRV genome from prostate tumor samples (Figure 31, panel A and Urisman, A., *et al.,* (2005), *PLOS Pathogens*). Briefly, total RNA from the LNCaP-R clone was reverse transcribed using random hexamer oligonucleotides, followed by PCR with XMRV-specific PCR primers (Urisman, A., *et al.,* (2005), *PLOS Pathogens*). The amplified PCR fragments were then cloned and sequenced.

The deduced LNCaP-R retrovirus genome (GenBank: DQ272467) is 8185 nt long and is most similar to the two XMRV genomes (XMRV VP35 and XMRV VP42) derived from prostate tumors (Urisman, A., *et al.,* (2005), *PLOS Pathogens*), with which it shares 94% nucleotide identity (Figure 34, panel B [tree and similarity plots]). The recovered genome also shares high nucleotide identity (92%) with two other xenotropic MuLV genomes: Murine type C retrovirus (GenBank: NC_001702, Heinemeyer T., unpublished) and MuLV DG-75 (GenBank: AF221065, (Raisch, KP., *et al.,* (2003), *Virology 308*: 83-91)). Based on these findings, we assigned the virus a provisional name of XMRV LNCaP-R.

The genome of XMRV LNCaP-R contains two overlapping ORFs, encoding the full-length Gag-Pro-Pol and the Env polyproteins (Fig 35A). Similar to the tumor-derived relatives, XMRV LNCaP-R is a canonical simple retrovirus lacking accessory viral regulatory genes or host-derived oncogene sequences. The Gag protein is 536 aa long and shares its highest aa identity with XMRV VP35 (98%) (Fig. 34, panel D). Upstream of gag AUG is a ca. 300 nt region known as the 5' gag leader, which in most ecotropic MuLVs encodes a minor glycosylated form of Gag (glyco-Gag)

expressed from an alternative CUG start codon (Prats, AC., *et al.,* (1989), *J Mol Biol 205*: 363-372). The 5' gag leader was found to be the most divergent region in the tumor-derived XMRV genomes as compared to other MuLVs (Urisman, A., *et al.,* (2005), *PLOS Pathogens*). Just as in XMRV VP35 and VP42, this region is interrupted by a stop codon 53 aa downstream from the CUG initiation codon of glyco-Gag and bears a signature 24-nt deletion characteristic of XMRV (Fig 34, panel C) and not found in any other known gammaretroviral genome. (Note: since the glyco-gag protein is dispensable for retroviral replication (Fan, H., *et al.*, (1983), *Proc Natl Acad Sci USA 80*: 5965-5969; Schwartzberg P, *et al.,* (1983), *J Virol 46*: 538-546), these lesions are not expected to disrupt XMRV infectivity; see below). Similarly, all regulatory sequences present in the tumor-derived XMRV genomes were also found in the same positions in the genome of XMRV LNCaP-R. These include a binding site for a prolyl-tRNA, which functions as the primer for reverse transcription during viral replication (Adam, MA., *et al.,* (1988), *J Virol 62*: 3802-3806; Fisher, J., *et al.,* (1998), *Virology 244*: 133-145; Berlioz, C., *et al.,* (1995), *J Virol 69*: 2214-2222; Vagner, S., *et al.*, (1995), *J Biol Chem 270*:20376-20383); splice donor and acceptor sites involved in the generation of *env* subgenomic RNAs (see below); TATAAA (SEQ ID NO:50) and CCAAT (SEQ ID NO:49) boxes involved in transcription initiation (Temin, HM, (1981), *Cell 27*: 1-3; Coffin, JM., *et al.,* (eds.) (1997) Retroviruses. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press); a glucocorticoid response element (GRE); and AATAAA (SEQ ID NO:51) polyadenylation signal.

However, despite its overall similarity to the characterized XMRVs, the LNCaP-R isolate is nonetheless distinct from these tumor-derived isolates. At the level of the whole genome, the isolate is not as closely related to the XMRV VP35 and VP42 sequences as the latter two are to each other. For the predicted pro-pol region (1084 aa encoding the protease, reverse transcriptase and integrase activities), aa identity with the tumor-derived XMRVs is lower than in other regions (92%), and is similar to that seen with MuLV DG-75 or MTCR. In addition, portions of the *env* region show significant divergence from the characterized XMRVs (see Fig 34, panel D for a similarity plot comparing XMRV35 with XMRV LNCaP R). The *env* region nonetheless is most similar to the xenotropic/polytropic gammaretroviruses

indicating that the virus should display a host range that includes human cells (see below).

Viral gene expression in XMRV LNCaP-R

As noted above, conserved splice donor (AGGTAAG (SEQ ID NO:47), position 204) and acceptor (CACTTACAG (SEQ ID NO:48), position 5479) sites involved in the generation of *env* subgenomic RNAs were found in the same position as in XMRV (Urisman, A., *et al.,* (2005), *PLOS Pathogens*). Transcripts for both the complete, unspliced 8.2 kb transcript encoding *gag* and *pro-pol*, and the spliced *env* transcript (3.2 kb) were confirmed in LNCaP-R cells by Northern analysis using an LTR probe (nucleotide positions 7780 to 7991), which detects both messages (Figure 35B). As predicted, a gag-specific probe (nucleotide positions 603 to 957) detects only the full length genomic RNA (Figure 35B); env mRNA is not detectable with this probe because gag sequences have been removed by splicing. Consistent with the expression of these transcripts, a large proportion of LNCaP-R cells stained positively with anti-Gag antibody, while LNCaP-FGC cells did not (Fig 35C).

LNCaP-R cells produce infectious XMRV

The presence of genomic and subgenomic mRNAs strongly implied the existence of integrated proviral DNA in LNCaP-R cells. To search for this directly, genomic DNA, cleaved with the restriction enzyme PstI (which cleaves once, within the env region) was extracted and examined. The resulting fragments were examined by Southern blotting with a probe corresponding to the U3 region (positions 7780-7991). As shown in Fig 36A, a complex array of bands, suggestive of multiple insertion sites, were observed in LNCaP-R but not in the virus-negative lines. PCR-based cloning of several host-viral junctions affirmed that multiple distinct integration sites on different human chromosomes are present in LNCaP-R DNA (Fig 36C).

The pattern of viral integrants in LNCaP-R implied that multiple *de novo* infections had occurred within this subline, indicating the production of infectious virus. To directly demonstrate this, whether supernatants from LNCaP-R could transmit infection to other lines was determined. Supernatant from LNCaP-R cells was transferred to LNCaP-FGC cells and incubated for 2 hr; following this, the cells were

washed, fresh medium added and supernatant sampled daily for the ensuing week. Fig 37A shows a progressive rise in reverse transcriptase activity in the recipient culture medium during this time period, indicative of virus replication and spread.

To examine the host range of XMRV-LNCaP R, human (DU145, LNCaP-FGC and 293T) or murine (3T3) cells were inoculated with LNCaP-R supernatants; 24 or 36 hr later, RNA and DNA were prepared and examined by blot-hybridization. A Northern blot of infected human (LNCaP-FGC and 293T cells) and murine 3T3 cells showed genomic and subgenomic transcripts in the human lines, but no XMRV mRNA is seen in the 3T3 cells (Fig. 37B). Southern blot analyses of viral DNA in the recipient human cells (LNCaP-FGC and DU145) (Fig 37C) showed a diffuse smear of bands, indicative of multiple independent integrations, in inoculated, but not uninoculated, human lines. (Mouse 3T3 cells were used here only as a positive hybridization control, and revealed multiple integration sites derived from endogenous mouse retroviruses; this high background prevents effective use of Southern blotting for XMRV proviruses in this line). The finding that human but not mouse cells are vulnerable to infection is consistent with the xenotropism predicted from the sequence of the viral envelope protein.

Discussion

XMRV is a novel gammaretrovirus originally detected in prostate tissue of patients with prostate cancer and genomic mutations in RNASEL (Urisman, A., *et al.*, (2005), *PLOS Pathogens*). XMRV is the first example of a xenotropic retrovirus infection of human tissue, and the epidemiologic link to RNASEL lesions strongly implies an important role for RNase L in the control of infection. These findings are supported by the *in situ* analyses of prostate tissue described herein, which reinforce that infection is found primarily in subjects homozygous for the R462Q RNase L variant. Importantly, we now show that the infection is not present in the carcinoma cells themselves, but rather in a subset of stromal cells, chiefly fibroblasts and blood elements. These findings provide the boundary conditions within which we can frame the two major questions posed by XMRV: (i) how does RNase L activity influence XMRV infection? And (ii) what, if any, is the relationship between XMRV infection and prostatic cancer?

RNase L action and XMRV infection

The data provided herein and that of Urisman and coworkers (Urisman, A., *et al.,* (2005), *PLOS Pathogens*) provide strong empiric evidence that down-mutations in RNASEL are important in the acquisition or persistence of XMRV infection in vivo (Urisman, A., *et al.,* (2005), *PLOS Pathogens*). Although there is considerable evidence from animal studies and cell culture that RNase L is an important antiviral protein, most studies have focused on viruses with RNA-based replication cycles, including picornaviruses (Zhou, A., *et al.*, (1997), *Embo J 16*: 6355-6363; Flodstrom-Tullberg, M., *et al.*, (2005), *J Immunol 174*: 1171-1177), paramyxoviruses (Behera, AK., *et al.,* (2002), *J Biol Chem 277*: 25601-25608), alphaviruses (Ryman, KD., *et al.,* (2002), *Viral Immunol 15*: 53-76) or retroviruses (Smith, JA., *et al.,* (2005), *J Virol 79*: 2240-2250). Relatively few studies have focused on the role of the 2-5A/RNase L system in retrovirus infections. Type I interferons clearly inhibit retroviral replication, but these cytokines activate many downstream effectors in addition to RNase L, and can create blocks at many stages of retroviral replication, including reverse transcription, translation, viral assembly and release (Pitha, PM., (1994) *Antiviral Res 24*: 205-219; Poli, G., *et al.,* (1994), *Antiviral Res 24*: 221-233; Friedman, RM., *et al.,* (1974) *Proc Natl Acad Sci USA 71*: 3542-3544). Nonetheless, experimental overexpression of OAS (Schroder, HC., *et al.,* (1992), *Int J Biochem 24*: 55-63) or RNase L (Maitra, RK., *et al.,* (1998), *J Virol 72*: 1146-1152) in cultured cells impairs HIV replication, and suppression of RNase L activity by overexpression of an RNase L inhibitor also modestly enhances HIV growth in culture (Martinand, C., *et al.*, (1999), *J Virol 73*: 290-296). In HIV infection, OAS can be activated by structured RNA regions within the 5' UTR and TAR; OAS can thus be thought of as one cellular "sensor" for HIV (Maitra, RK., *et al.*, (1994), *Virology 204*: 823-827). Regions of XMRV RNA detected by OAS, or how RNase L activation affects XMRV replication is unclear, but recovery of an infectious XMRV family member from LNCaP-R cells opens both questions to experimental study. The barrier to XMRV infection posed by RNase L is relative, not absolute: DU145, which have no lesions in RNASEL, can be infected by XMRV under conditions of high multiplicity of infection (MOI) in vitro (Figures 37A-37C).

This should not be entirely surprising, as it is in keeping with other known restriction factors for retroviral replication, like Fv-1 restriction of MuLV and more recently identified restrictors of HIV and SIV, all of which can be overcome by high MOI (Goff, SP., (2004), *Mol Cell 16*: 849-859; Bieniasz, PD., (2003), *Trends Microbiol 11*: 286-291). Since most *in vivo* infections are established initially under low MOI conditions, it is restriction at this MOI level that is presumably selected for during viral evolution.

From the results examining human prostate cancer cell lines described herein, several conclusions can be drawn. First, the absence of viral DNA in the genomic DNA of most human cells indicates that XMRV is not an endogenous retrovirus, but an exogenously acquired agent. Second, although LNCaP cells were reportedly established from a clonal tumor, the integration sites do not appear to be clonal. This indicates that the infection of the cell line postdated the establishment of the clonal line; the multiplicity of integrants presumably reflects horizontal spread of infection within the line. This interpretation is also consistent with the fact that the LNCaP-FGC clone is negative for infection. Since the *in situ* analyses of prostate tumors (Figures 30-33) indicate that carcinoma cells are not infected *in vivo*, this infection must have occurred in vitro. Therefore, two possible scenarios for such infection: (i) during explantation of the original tumor, a small number of tumor cells might have acquired infection by spread from stromal cells; or (ii) infection may have occurred in the laboratory during serial passage of the line. If LNCaP-FGC cells were clonally purified prior to deposition at ATCC, then possibility (ii) is more likely.

XMRV infection and prostate cancer

The findings described herein that XMRV infection is targeted to stromal cells and not to carcinoma cells has major implications for considering the relation of XMRV infection to prostate cancer. This finding, and the fact that the XMRV genome harbors no host-derived oncogenes, rules out two classical models for retroviral oncogenesis: direct introduction of a dominantly acting oncogene and insertional activation of such a gene. It is emphasized that the epidemiologic described herein links XMRV infection to the RNASEL genotype but does not mandate any etiological link to prostate cancer. While its exclusion from the carcinoma cells makes direct

oncogenesis by XMRV improbable, more indirect contributions of the virus to the tumor can certainly be envisioned. Prostate cancer is a disease with a long natural history, and many histologic changes occur in the gland prior to the supervention of overt malignancy. Recent work emphasizes that prostate cancers are very commonly accompanied by evidence of chronic prostatic inflammation, and a lesion called proliferative inflammatory atrophy (PIA) is often found in premalignant stages of the disease (Nelson, WG., *et al.,* (2003), *N Engl J Med 349*:366-381). It is speculated that byproducts of this inflammation (e.g. free radicals and oxidative damage) can trigger injury and regeneration in the prostatic epithelium. This enhanced proliferation allows opportunities for replicative errors to engender mutations; those that deregulate growth then have a selective advantage. PIA is often found adjacent to high grade prostatic intraepithelial neoplasia (HGPIN) or early cancer, and accumulating evidence suggests an identifiable genetic pathway between PIA, HGPIN, and cancer (Nelson, WG., *et al.,* (2003), *N Engl J Med 349*:366-381). In support of the infection/inflammation hypothesis are observations that variants or epigenetic events in other genes involved in innate immunity (the TLR family), control of the inflammatory response (MSR1 and MIC-1), antioxidant activity (PON1 and GSTP1), or DNA repair in response to oxidative stress (OGG1, CHEK2, and BRCA2) have also been reported to predispose men to prostate cancer (Zheng, SL., *et al.*, (2004), *Cancer Res 64*: 2918-2922; Xu, J., *et al.*, (2002), *Nat Genet 32*: 321-325; Lindmark, F., *et al.*, (2004), *J Natl Cancer Inst 96*: 1248-1254; Marchesani, M., *et al.,* (2003), *J Natl Cancer Inst 95*: 812-818; Xu. J,, *et al.*, (2002), *Cancer Res 62*:2253-2257; Dong, X., *et al.*, (2003), *Am J Hum Genet 72*: 270-280; Edwards, SM., *et al.*, (2003), *Am J Hum Genet 72*: 1-12). While the cause of the inflammation in PIA is unknown, infection is an obvious potential trigger, and a persistent viral infection restricted to the stroma would be well-positioned to contribute to such a process. In this view, one reason for the link between RNASEL mutations and prostate cancer would be the inability of an RNase L-deficient innate immune system to terminate a stromal XMRV infection; the resulting persistent infection would then contribute to a chronic inflammatory state whose end result can be PIA. (We note that XMRV need not be the sole infectious precipitant in such a scenario).

The finding that XMRV primarily affects stromal cells raises still another

potential mechanism for contributing to prostatic neoplasia. Recent work has shown that stromal cells have an active role in promoting tumorigenesis of adjacent epithelial cells by producing various cytokines and growth factors that serve as proliferative signals (Tlsty, TD., *et al.,* (2001), *Curr Opin Genet Dev 11*: 54-59). In particular, cancer associated fibroblasts stimulate growth of human prostatic epithelial cells and alter their histology *in vivo* (Olumi, AF., *et al.*, (1999), *Cancer Res 59*: 5002-5011). It is conceivable that XMRV-infected prostatic stromal cells could produce and secrete growth factors, cytokines or other factors that stimulate cell proliferation in surrounding epithelia. Such a paracrine mechanism could still function quite efficiently even with the relatively small number of XMRV-infected cells that characterize the lesion.

Finally, it is noted that the identification of an exogenous infection like XMRV could help explain why not all genetic studies have consistently identified RNase L as a prostate cancer susceptibility factor. If such an infection were linked, however indirectly, to prostate cancer risk, and if the prevalence of infection is not uniform in different populations, populations with low XMRV prevalence might be expected to show no association of RNASEL lesions to prostate cancer.

Table 1. Detection of HXV in Prostates (Masterlist)

Legend: MR = Murine Retrovirus

Lab ID	J-Number	Study number	1385 SNP	Received?	ArrayID (Total)	Array (Total)	ArrayID (Poly A)	Array (Poly A)	RT PCR (7200F-227R) total RNA	RT PCR (7200F-227R) poly A RNA	Nested PCR (7600F-227R) template: RT PCR	Specific PCR LTR/env/pol murine type C retrovirus gen.DNA	Mouse GAPDH PCR Rdb total and poly A RNA	Nested Gag RT-PCR neg	Souther Blot of the nexted Gag RT-PCR (column O)
VP10		3-03-0291A/D	AA	X	7-247	MR	7-244	neg	pos	pos	pos	neg		neg	pos
VP10	J8	3-03-0291A/D	AA	X	6-151	neg	6-108	neg	neg	neg	neg			neg	neg
VP107		3-04-0661A/B	AA		7-249	neg	7-246	neg	neg	neg	neg			neg	neg
VP24		3-03-0434A/D	AA								pos				
VP27		3-03-0452A/D	AA	X	7-248	neg	7-245	neg			neg			neg	neg
VP27	J9	3-03-0452A/D	AA	X	6-152	neg	6-108	neg			pos		neg	pos	pos
VP29		3-03-0473B/D	AA	X	5-169	neg	5-174	MR			pos		neg	pos	pos
VP31		3-03-0481A/D	AA	X	5-171	neg	5-176	neg			neg		neg	pos	pos

VP35	3-03-0530A	AA	X	5-172	MR	5-177	MR			neg	pos	neg	pos
VP42	3-03-0647A/B	AA	X	5-173		5-178	MR			pos		pos	pos
VP62	3-03-1040A/B	AA	X		neg					pos		neg	neg
VP79	3-04-0116A1	AA	X							pos			
VP88	3-04-0381A/B	AA	X										
VP90	3-04-0430A/B	AA	X										
VP94	3-04-0286A/B	AA											
VP104	no label	AG											
VP33	3-03-0518A	AG											
VP37	3-03-0556A	AG											
VP40	3-03-0629A/D	AG											
VP41	3-03-0631A	AG											

VP44		3-03-03702B/D	AG																
VP45		3-03-0707	AG	X	5-190	neg		5-195	neg	neg	neg	neg	neg					neg	neg
VP46		3-03-0742A/B	AG	X	5-191	neg		5-196	neg	neg	neg	neg	neg					neg	neg
VP49		3-03-0791A1	AG	X	5-192	neg		5-197	neg	neg	neg	neg	neg					neg	neg
VP55		3-03-0898C/D	AG																
VP81		3-04-0149A1	AG																
VP82		3-04-0210A/B	AG																
VP83		3-04-0237A/B	AG																
VP89		3-04-0428A/B	AG																
VP92		?	AG																
VP04	J3	3-03-0237	GA	X	6-112	neg		6-103	neg										
VP05	J4	3-03-0244A/D	GA	X	6-113	neg		6-104	neg										

VP07	J6	3-03-00252A/D	GA	X	6-115	neg	6-106	neg											
VP08	J7	3-03-0262A/D	GA	X	6-150	neg	6-107	neg											
VP09		3-03-0271A/D	GA																
VP100		3-04-0496A/B	GA																
VP108		3-04-0704A/B	GA																
VP11		3-03-0307A/D	GA																
VP111		3-04-0755A/B	GA																
VP112		3-04-0759A/B	GA																
VP115		3-04-0780A/B	GA																
VP14		3-03-0329A	GA																
VP15		3-03-0330A1	GA																
VP18		3-03-0367C/D	GA																

VP22		3-03-0444A/D	GA												
VP23		3-03-0432B/D	GA												
VP56		3-03-0907A1	GA												
VP57		3-03-0934C/D	GA												
VP61		3-03-1011D1	GA												
VP63		3-03-1057A/C	GA												
VP64		3-03-1060A1	GA												
VP65		3-03-1074A/B	GA												
VP68		3-03-10192A/B	GA												
VP70		3-03-1114A/B	GA	X						neg	neg			neg	neg
VP71		3-04-0014A1	GA												
VP73		3-04-0018A/B	GA												

VP74		3-04-0041A/B	GA	X					neg	neg		neg	neg		neg
VP77		3-04-0051A/B	GA	X					neg	neg		neg	neg		neg
VP78		no label	GA												
VP97		3-04-0460A/B	GA												
VP01			GG												
VP02	J1	3-03-0217A	GG	X	6-110	neg	6-101	neg							
VP03	J2	3-03-0235	GG	X	6-111	neg	6-102	neg							
VP06	J5	3-03-0248A/D	GG	X	6-114	neg	6-105	neg							
VP101		3-04-0497A/B	GG												
VP102		3-04-0522A/B	GG												
VP103		3-04-0570A/B	GG												
VP105		no label	GG												
VP106		3-04-0660A/B	GG												
VP109		3-04-0705A/B	GG												

VP110		3-04-0718A/B	GG												
VP113		3-04-0770A/B	GG												
VP114		3-04-0776A/B	GG												
VP116		3-04-0791A/B	GG												
VP117		3-04-0795A/B	GG												
VP118		3-04-0803A/B	GG												
VP119		3-04-0806A/B	GG												
VP12		3-03-0312A/D	GG												
VP13		3-03-0324A/D	GG												
VP16		3-03-0357B/C	GG												
VP17		3-03-0358A/D	GG												
VP19		3-03-0362C/D	GG												
VP20		3-03-0378A/D	GG												

VP21	3-03-0389A/D	GG																	
VP25	3-03-0443A/D	GG																	
VP26	3-03-0455A/D	GG																	
VP28	3-03-0459A/D	GG																	
VP30	3-03-0487A/D	GG	X	5-170	neg	5-175	neg	neg	neg		neg	neg							
VP32	3-03-0497A/D	GG																	
VP34	3-03-0526A	GG																	
VP36	3-03-0528A	GG																	
VP38	3-03-0587A/D	GG																	
VP39	3-03-0589A/D	GG																	
VP43	3-03-0662A/D	GG																	
VP47	no label	GG	?																
VP48	3-03-0765A-D	GG																	

VP50		3-030817A/C	GG	X	5-193	neg	5-198	neg	neg	neg		neg	neg
VP51		3-03-0820A/B	GG	X	5-194	neg	5-199	neg	neg	neg		neg	neg
VP52		3-03-0834B/D	GG										
VP53		3-03-0835B/D	GG										
VP54		3-03-0837C/D	GG										
VP58		3-03-0959C/D	GG										
VP59		3-03-0960C/D	GG										
VP60		3-03-0994A/B	GG										
VP66		3-03-1078A/B	GG	X					neg	neg		neg	pos
VP67		3-03-1090A/B	GG										
VP69		3-03-1105A1	GG										
VP72		3-04-0007A/B	GG	X					neg	neg		neg	neg

VP75		3-04-0046A/B	GG															neg	
VP76		3-04-0049A/B	GG														neg		
VP80		3-04-0152A1	GG													neg	neg		
VP84		3-04-0250A1	GG	X											neg	neg			
VP85		3-04-0248A/B	GG											neg	neg			pos	
VP86		3-04-0256A/B	GG	X													pos		
VP87		3-04-0380A/B	GG																
VP91		?	GG																
VP93		3-04-0269A/B	GG																
VP95		3-04-0324A/B	GG																
VP96		3-04-0457A1	GG																
VP98		3-04-0465A/B	GG																
VP99		3-04-0479A/B	GG																

Table 2. Sequence of HXV_{35}.

>HXV_{35}: PCRV_complete_genome_from_RdB (Edit1, 06-30-2004; Edit 2, 02-28-05)

GCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTT
GCTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAGGGTCTCCTCAGAGTGATT
GACTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCCGC
CCAGGGACCACCGACCCACCGTCGGGAGGTAAGCCGGCCGGCGATCGTTTTGTCTTTGT
CTCTGTCTTTGTGCGTGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAAT
CTGTACTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTT
CGTATTCCCGGCCGCAGCCCAGGGAGACGTCCCAGCGGCCTCGGGGGCCCGTTTTGTGG
CCCATTCTGTATCAGTTAACCTACCCGAGTCGGACTCTTTGGAGTGGCTTTGTTGGGGG
ACGAGAGACAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTACGCCGA
AACCGCGCCGCGCGTCTGATTTGTTTTGTTGTTCTTCTGTTCTTCGTTAGTTTTCTTCT
GTCTTTAAGTGTTCTCGAGATCATGGGACAGACCGTAACTACCCCTCTGAGTCTAACCT
TGCAGCACTGGGGAGATGTCCAGCGCATTGCATCCAACCAGTCTGTGGATGTCAAGAAG
AGGCGCTGGGTTACCTTCTGTTCCGCCGAATGGCCAACTTTCAATGTAGGATGGCCTCA
GGATGGTACTTTTAATTTAGGTGTTATCTCTCAGGTCAAGTCTAGAGTGTTTTGTCCTG
GTCCCCACGGACACCCGGATCAGGTCCCATATATCGTCACCTGGGAGGCACTTGCCTAT
GACCCCCCTCCGTGGGTCAAACCGTTTGTCTCTCCTAAACCCCCTCCTTTACCGACAGC
TCCCGTCCTCCCGCCCGGTCCTTCTGCGCAACCTCCGTCCCGATCTGCCCTTTACCCTG
CCCTTACCCTCTCTATAAAGTCCAAACCTCCTAAGCCCCAGGTTCTCCCTGATAGCGGC
GGACCTCTCATTGACCTTCTCACAGAGGATCCCCCGCCGTACGGAGTACAACCTTCCTC
CTCTGCCAGGGAGAACAATGAAGAAGAGGCGGCCACCACCTCCGAGGTTTCCCCCCCTT
CTCCCATGGTGTCTCGACTGCGGGGAAGGAGAGACCCTCCCGCAGCGGACTCCACCACC
TCCCAGGCATTCCCACTCCGCATGGGGGGAGATGGCCAGCTTCAGTACTGGCCGTTTTC
CTCCTCTGATTTATATAATTGGAAAAATAATAACCCTTCCTTTTCTGAAGATCCAGGTA
AATTGACGGCCTTGATTGAGTCCGTCCTCATCACCCACCAGCCCACCTGGGACGACTGT
CAGCAGTTGTTGGGGACCCTGCTGACCGGAGAAGAAAAGCAGCGGGTGCTCCTAGAGGC
TGGAAAGGCAGTCCGGGGCAATGATGGACGCCCCACTCAGTTGCCTAATGAAGTCAATG
CTGCTTTTCCCCTTGAGCGCCCCGATTGGGATTACACCACTACAGAAGGTAGGAACCAC
CTAGTCCTCTACCGCCAGTTGCTCTTAGCGGGTCTCCAAAACGCGGGCAGGAGCCCCAC
CAATTTGGCCAAGGTAAAAGGGATAACCCAGGGACCTAATGAGTCTCCCTCAGCCTTTT
TAGAGAGACTCAAGGAGGCCTATCGCAGGTACACTCCTTATGACCCTGAGGACCCAGGG
CAAGAAACCAATGTGTCCATGTCATTCATCTGGCAGTCTGCCCCGGATATCGGGCGAAA
GTTAGAGCGGTTAGAAGATTTAAAGAGCAAGACCTTAGGAGACTTAGTGAGGGAAGCTG
AAAAGATCTTTAATAAGCGAGAAACCCCGGAAGAAAGAGAGGAACGTATCAGGAGAGAA
ATAGAGGAAAAAGAAGAACGCCGTAGGGCAGAGGATGAGCAGAGAGAGAGAGAAAGGGA
CCGCAGAAGACATAGAGAGATGAGCAAGCTCTTGGCCACTGTAGTTATTGGTCAGAGAC
AGGATAGACAGGGGGGAGAGCGGAGGAGGCCCCAACTTGATAAGGACCAATGCGCCTAC
TGCAAAGAAAAGGGACACTGGGCTAAGGACTGCCCAAAGAAGCCACGAGGGCCCCGAGG
ACCGAGGCCCCAGACCTCCCTCCTGACCTTAGGTGACTAGGGAGGTCAGGGTCAGGAGC
CCCCCCCTGAACCCAGGATAACCCTCAAAGTCGGGGGGCAACCCGTCACCTTCCTGGTA
GATACTGGGGCCCAACACTCCGTGCTGACCCAAAATCCTGGACCCCTAAGTGACAAGTC
TGCCTGGGTCCAAGGGGCTACTGGAGGAAAGCGGTATCGCTGGACCACGGATCGCAAAG
TACATCTGGCTACCGGTAAGGTCACCCACTCTTTCCTCCATGTACCAGACTGCCCCTAT
CCTCTGCTAGGAAGAGACTTGCTGACTAAACTAAAAGCCCAAATCCACTTCGAGGGATC

AGGAGCTCAGGTTGTGGGACCGATGGGACAGCCCCTGCAAGTGCTGACCCTAAACATAG
AAAATAAGTATCGGCTACATGAGACCTCAAAAGAGCCAGATGTTCCTCTAGGGTCCACA
TGGCTTTCTGATTTTCCCCAGGCCTGGGCGGAAACCGGGGGCATGGGACTGGCAGTTCG
CCAAGCTCCTCTGATCATACCTCTGAAGGCAACCTCTACCCCCGTGTCCATAAAACAAT
ACCCCATGTCACAAGAAGCCAGACTGGGGATCAAGCCCCACATACAGAGGCTGTTGGAC
CAGGGAATACTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTACCCGTTAAGAA
ACCAGGGACTAATGATTATAGGCCTGTCCAGGATCTGAGAGAAGTCAACAAGCGGGTGG
AAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTCCCACCGTCC
CACCAGTGGTACACTGTGCTTGATTTAAAGGATGCCTTTTTCTGCCTGAGACTCCACCC
CACCAGTCAGCCTCTCTTCGCCTTTGAGTGGAGAGATCCAGAGATGGGAATCTCAGGAC
AACTGACCTGGACCAGACTCCCACAGGGTTTCAAAAACAGTCCCACCCTGTTTGATGAG
GCACTGCACAGAGACCTAGCAGATTTCCGGATCCAGCACCCAGACTTGATCCTGCTACA
GTACGTGGATGACTTACTGCTGGCCGCCACTTCTGAGCAAGACTGCCAACGAGGTACTC
GGGCCCTATTACAAACCCTAGGGAACCTCGGGTATCGGGCCTCGGCCAAGAAAGCCCAA
ATTTGCCAGAAACAGGTCAAGTATCTGGGGTATCTCCTAAAAGAGGGACAGAGATGGCT
GACTGAGGCCAGAAAAGAGACTGTGATGGGGCAGCCCACTCCGAAGACCCCTCGACAAC
TAAGGGAGTTCCTAGGGACGGCAGGCTTCTGTCGCCTCTGGATCCCTGGGTTTGCAGAA
ATGGCAGCCCCCTTGTACCCTCTTACCAAAACGGGGACTCTGTTTAATTGGGGCCCAGA
CCAGCAAAAGGCCTATCAAGAAATCAAACAGGCTCTTCTAACTGCCCCCGCCCTGGGAT
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GGCGTCCTAACGCAAAAACTGGGACCTTGGCGTCGGCCTGTGGCCTACCTGTCCAAAAA
GCTAGACCCAGTGGCAGCTGGGTGGCCCCCTTGCCTACGGATGGTAGCAGCCATTGCCG
TTCTGACAAAGGATGCAGGCAAGCTAACTATGGGACAGCCGCTAGTCATTCTGGCCCCC
CATGCGGTAGAAGCACTGGTCAAACAACCCCCTGACCGTTGGCTATCCAATGCCCGCAT
GACCCACTATCAGGCAATGCTCCTGGATACAGACCGGGTTCAGTTCGGACCGGTGGTGG
CCCTCAACCCGGCCACCCTGCTCCCCCTACCGGAAAAGGAAGCCCCCCATGACTGCCTC
GAGATCTTGGCTGAGACGCACGGAACCAGACCGGACCTCACGGACCAGCCCATCCCAGA
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CTGGAGCAGCGGTGACTACTGAGACCGAGGTAATCTGGGCGAGGGCTCTGCCGGCTGGA
ACATCCGCCCAACGAGCCGAACTGATAGCACTCACCCAAGCCTTAAAGATGGCAGAAGG
TAAGAAGCTAAATGTTTACACTGATAGCCGCTATGCCTTCGCCACGGCCCATGTCCATG
GAGAAATATATAGGAGGCGAGGGTTGCTGACCTCAGAAGGCAGAGAAATTAAAAACAAG
AACGAGATCTTGGCCTTGCTAAAAGCTCTCTTTCTGCCCAAACGACTTAGTATAATTCA
CTGTCCAGGACATCAAAAAGGAAACAGTGCTGAGGCCAGAGGCAACCGTATGGCAGATC
AAGCAGCCCGAGAGGCAGCCATGAAGGCAGTTCTAGAAACCTCTACACTCCTCATAGAG
GACTCAACCCCGTATACGCCTCCCCATTTCCATTACACCGAAACAGATCTCAAAAGACT
ACGGGAACTGGGAGCCACCTACAATCAGACAAAAGGATATTGGGTCCTACAAGGCAAAC
CTGTGATGCCCGATCAGTCCGTGTTTGAACTGTTAGACTCCCTACACAGACTCACCCAT
CCGAGCCCTCAAAAGATGAAGGCACTCCTCGACAGAGAAGAAAGCCCCTACTACATGTT
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ATGCCAGCAAAGCCAAAATTGGGGCAGGGGTGCGAGTACGCGGACATCGGCCAGGCACC
CATTGGGAAGTTGATTTCACGGAAGTAAAGCCAGGACTGTATGGGTACAAGTACCTCCT
AGTGTTTGTAGACACCTTCTCTGGCTGGGTAGAGGCATTCCCGACCAAGCGGGAAACTG
CCAAGGTCGTGTCCAAAAAGCTGTTAGAAGACATTTTTCCGAGATTTGAAATGCCGCAG
GTATTGGGATCTGATAACGGGCCTGCCTTCGCCTCCCAGGTAAGTCAGTCAGTGGCCGA
TTTACTGGGAATCGATTGGAAGTTACATTGTGCTTATAAACCCCAGAGTTCAGGACAGG
TAGAAAGAATAAATAAAACAATTAAGGAGACTTTAACCAAATTAACGCTTGCATCTGGC
ACTAAAGACTGGGTACTCCTACTCCCCTTAGCCCTCTACCGAGCCCGGAATACTCCGGG

CCCCCACGGACTGACTCCGTATGAAATTCTGTATGGGGCACCCCCGCCCCTTGTCAATT
TTCATAATCCTGAAATGTCAAAGTTAACTAATAGTCCCTCTCTCCAAGCTCACTTACAG
GCCCTCCAAGCAGTACAACAAGAGGTCTGGAAGCCGCTGGCCGCTGCTTATCAGGACCA
GCTAGATCAGCCAGTGATACCACACCCCTTCCGTGTCGGTGACGCCGTGTGGGTACGCC
GGCACCAGACTAAGAACTTAGAACCTCGCTGGAAAGGACCCTACACCGTCCTGCTGACA
ACCCCCACCGCTCTCAAAGTAGACGGCATCTCTGCGTGGATACACGCCGCTCACGTAAA
GGCGGCGACAACTCCTCCGGCCGGAACAGCATGGAAAGTCCAGCGTTCTCAAAACCCCT
TAAAGATAAGATTAACCCGTGGGGCCCCCTGATAATTATGGGGATCTTGGTGAGGGCAG
GAGCCTCAGTACAACGTGACAGCCCTCACCAGGTCTTTAATGTCACTTGGAAAATTACC
AACCTAATGACAGGACAAACAGCTAATGCTACCTCCCTCCTGGGGACGATGACAGACAC
TTTCCCTAAACTATATTTTGACTTGTGTGATTTAGTTGGAGACAACTGGGATGACCCGG
AACCCGATATTGGAGATGGTTGCCGCTCTCCCGGGGGAAGAAAAAGGACAAGACTATAT
GATTTCTATGTTTGCCCCGGTCATACTGTATTAACAGGGTGTGGAGGGCCGAGAGAGGG
CTACTGTGGCAAATGGGGATGTGAGACCACTGGACAGGCATACTGGAAGCCATCATCAT
CATGGGACCTAATTTCCCTTAAGCGAGGAAACACTCCTAAGGGTCAGGGCCCCTGTTTT
GATTCCTCAGTGGGCTCCGGTAGCATCCAGGGTGCCACACCGGGGGGTCGATGCAACCC
CCTAGTCCTAGAATTCACTGACGCGGGTAAAAGGGCCAGCTGGGATGCCCCCAAAACAT
GGGGACTAAGACTGTATCGATCCACTGGGGCCGACCCGGTGACCCTGTTCTCTCTGACC
CGCCAGGTCCTCAATGTAGGGCCCCGCGTCCCCATTGGGCCTAATCCCGTGATCACTGA
ACAGCTACCCCCCTCCCAACCCGTGCAGATCATGCTCCCCAGGCCTCCTCGTCCTCCTC
CTTCAGGCGCGGCCTCTATGGTGCCTGGGGCTCCCCCGCCTTCTCAACAACCTGGGACG
GGAGACAGGCTGCTAAACCTGGTAGAAGGAGCCTACCAAGCCCTCAACCTCACCAGTCC
CGACAAAACCCAAGAGTGCTGGCTGTGTCTAGTATCGGGACCCCCCTACTACGAAGGGG
TGGCCGTCCTAGGTACTTACTCCAACCATACCTCTGCCCCGGCTAACTGCTCCGTGACC
TCCCAACACAAGCTGACCCTGTCCGAAGTGACCGGGCAGGGACTCTGCATAGGAGCAGT
TCCCAAAACCCATCAGGCCCTGTGTAATACCACCCAGAAGACGAGCGACGGGTCCTACT
ATTTGGCCTCTCCCGCCGGGACCATTTGGGCTTGCAGCACCGGGCTCACTCCCTGTCTA
TCTACTACTGTGCTTAACTTAACCACTGATTACTGTGTCCTGGTTGAACTCTGGCCAAA
GGTAACCTACCACTCCCCTAATTATGTTTATGGCCAGTTTGGAAAGAAAACTAAATATA
AAAGAGAGCCGGTGTCATTAACTCTGGCCCTGCTGTTGGGAGGACTTACTATGGGCGGC
ATAGCTGCAGGAGTTGGAACAGGGACTACAGCCCTAGTGGCCACCAAACAATTCGAGCA
GCTCCAGGCAGCCATACATACAGACCTTGGGGCCTTAGAAAAATCAGTCAGTGCCCTAG
AAAAGTCTCTGACCTCGTTGTCTGAGGTGGTCCTACAGAACCGGAGGGGATTAGATCTA
CTGTTCCTAAAAGAAGGAGGATTATGTGCTGCCCTAAAAAAAGAATGCTGTTTTTACGC
GGACCACACTGGCGTAGTAAGAGATAGCATGGCAAAGCTAAGAGAAAGGTTAAACCAGA
GACAAAAATTGTTCGAATCAGGACAAGGGTGGTTTGAGGGACTGTTTAACAGGTCCCCA
TGGTTCACGACCCTGATATCCACCATTATGGGCCCTCTGATAGTACTTTTATTAATCCT
ACTCTTCGGACCCTGTATTCTCAACCGCTTGGTCCAGTTTGTAAAAGACAGAATTTCGG
TAGTGCAGGCCCTGGTTCTGACCCAACAGTATCACCAACTCAAATCAATAGATCCAGAA
GAAGTGGAATCACGTGAATAAAAGATTTTATTCAGTTTCCAGAAAGAGGGGGGAATGAA
AGACCCCACCATAAGGCTTAGCACGCTAGCTACAGTAACGCCATTTTGCAAGGCATGGA
AAAGTACCAGAGCTGAGTTCTCAAAAGTTACAAGGAAGTTTAATTAAAGAATAAGGCTG
AATAACACTGGGACAGGGGCCAAACAGGATATCTGTAGTCAGGCACCTGGGCCCCGGCT
CAGGGCCAAGAACAGATGGTCCTCAGATAAAGCGAAACTAACAACAGTTTCTGGAAAGT
CCCACCTCAGTTTCAAGTTCCCCAAAAGACCGGGAAATACCCCAAGCCTTATTTAAACT
AACCAATCAGCTCGCTTCTCGCTTCTGTACCCGCGCTTTTTGCTCCCCAGTCCTAGCCC
TATAAAAAAGGGGTAAGAACTCCACACTCGGCGCGCCAGTCATCCGATAGACTGAGTCG
CCCGGGTACCCGTGTTCCCAATAAAGCCTTTTGCTGTTTGCAAAAA

Table 3. Alignment of HXV35 and DG-75 Gag Polypeptides

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>536) HXV35 gag (1>536)	Seq2(1>536) DG75 gag (1>536)	Similarity Index	Gap Number	Gap Length	Consensus Length
		96.3	0	0	536
		96.3	0	0	536

```
        v10       v20       v30       v40       v50       v60       v70       v80       v90       v100
MGQTVTTPLSLTLQHWGDVQRIASNQSVDVKKRRWVTFCSAEWPTFNVGWPQDGTFNLGVISQVKSRVFCPGPHGHPDQVPYIVTWEALAYDPPPWVKPF
MGQTVTTPLSLTL:HWGDVQRIASNQSVDVKKRRWVTFCSAEWPTF:VGWPQDGTFNL::I QVKS:VF.PGPHGHPDQVPYIVTWEALAYDPPPWVKPF
MGQTVTTPLSLTLEHWGDVQRIASNQSVDVKKRRWVTFCSAEWPTFDVGWPQDGTFNLDIILQVKSKVFSPGPHGHPDQVPYIVTWEALAYDPPPWVKPF
        ^10       ^20       ^30       ^40       ^50       ^60       ^70       ^80       ^90       ^100

        v110      v120      v130      v140      v150      v160      v170      v180      v190      v200
VSPKPPPLPTAPVLPPGPSAQPPSRSALYPALTLSIKSKPPKPQVLPDSGGPLIDLLTEDPPPYGVQPSSSARENNEEEAATTSEVSPPSPMVSRLRGRR
VSPKPPPLPTAPVLPPGPSAQPPSRSALYPALT SIK:KPPKPQVLPD:GGPLIDLLTEDPPPYG.QPSSSAR.N:EEEAA:TSEVSPPSPMVSRLRGRR
VSPKPPPLPTAPVLPPGPSAQPPSRSALYPALTPSIKTKPPKPQVLPDNGGPLIDLLTEDPPPYGAQPSSSARGNDEEEAAATSEVSPPSPMVSRLRGRR
        ^110      ^120      ^130      ^140      ^150      ^160      ^170      ^180      ^190      ^200

        v210      v220      v230      v240      v250      v260      v270      v280      v290      v300
DPPAADSTTSQAFPLRMGGDGQLQYWPFSSSDLYNWKNNNPSFSEDPGKLTALIESVLITHQPTWDDCQQLLGTLLTGEEKQRVLLEAGKAVRGNDGRPT
DPPAADST:SQAFPLRMGGDGQLQYWPFSSSDLYNWKNNNPSFSEDPGKLTALIESVLITHQPTWDDCQQLLGTLLTGEEKQRVLLEA KAVRGNDGRPT
DPPAADSTSSQAFPLRMGGDGQLQYWPFSSSDLYNWKNNNPSFSEDPGKLTALIESVLITHQPTWDDCQQLLGTLLTGEEKQRVLLEARKAVRGNDGRPT
        ^210      ^220      ^230      ^240      ^250      ^260      ^270      ^280      ^290      ^300
```

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     v310       v320       v330       v340       v350       v360       v370       v380       v390       v400
QLPNEVNAAFPLERPDWDYTTTEGRNHLVLYRQLLLAGLQNAGRSPTNLAKVKGITQGPNESPSAFLERLKEAYRRYTPYDPEDPGQETNVSMSFIWQSA
QLPNEVNAAFPLERPDWDYTTTEGRNHLVLYRQLLLAGLQNAGRSPTNLAKVKGITQGPNESPSAFLERLKEAYRRYTPYDPEDPGQETNVSMSFIWQSA
QLPNEVNAAFPLERPDWDYTTTEGRNHLVLYRQLLLAGLQNAGRSPTNLAKVKGITQGPNESPSAFLERLKEAYRRYTPYDPEDPGQETNVSMSFIWQSA
     ^310       ^320       ^330       ^340       ^350       ^360       ^370       ^380       ^390       ^400

     v410       v420       v430       v440       v450       v460       v470       v480       v490       v500
PDIGRKLERLEDLKSKTLGDLVREAEKIFNKRETPEERERERIRREIEEKERRRAEDEQRERERDRRRHREMSKLLATVVIGQRQDRQGGERRRPQLDKD
PDIGRKLERLEDLKSKTLGDLVREAEKIFNKRETPEERERERIRRE.EEKERRRAEDEQRE:ERDR:RHREMSKLLATVV GQRQDRQGGERRRPQLDKD
PDIGRKLERLEDLKSKTLGDLVREAEKIFNKRETPEERERERIRRETEEKERRRAEDEQREKERDRKRHREMSKLLATVVSGQRQDRQGGERRRPQLDKD
     ^410       ^420       ^430       ^440       ^450       ^460       ^470       ^480       ^490       ^500

     v510       v520       v530
QCAYCKEKGHWAKDCPKKKPRGPRGPRPQTSLLTLGD (SEQ ID NO:3)
QCAYCKEKGHWAKDCPKKKPRGPRGPRPQTSLLTLGD (SEQ ID NO:63)
QCAYCKEKGHWAKDCPKKKPRGPRGPRPQTSLLTLGD (SEQ ID NO:64)
     ^510       ^520       ^530
```

Table 4. Alignment of HXV35 and DG-75 PRO-POL Polypeptides.

```
Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12
Seq1(1>1196)              Seq2(1>1197)          Similarity   Gap      Gap      Consensus
HXV35 pro-pol 2 2 8 05    DG75 pro-pol          Index        Number   Length   Length
(1>1196)                       (2>1197)         96.5         0        0        1196
(1>1196)                       (2>1197)         96.5         0        0        1196


       v10       v20       v30       v40       v50       v60       v70       v80       v90      v100
GGQGQEPPPEPRITLKVGGQPVTFLVDTGAQHSVLTQNPGPLSDKSAWVQGATGGKRYRWTTDRKVHLATGKVTHSFLHVPDCPYPLLGRDLLTKLKAQIHF
GGQGQEPPPEPRITLKVGGQPVTFLVDTGAQHSVLTQNPGPLSDKSAWVQGATGGKRYRWTTDRKVHLATGKVTHSFLHVPDCPYPLLGRDLLTKLKAQIHF
GGQGQEPPPEPRITLKVGGQPVTFLVDTGAQHSVLTQNPGPLSDKSAWVQGATGGKRYRWTTDRKVHLATGKVTHSFLHVPDCPYPLLGRDLLTKLKAQIHF
       ^10       ^20       ^30       ^40       ^50       ^60       ^70       ^80       ^90      ^100

      v110      v120      v130      v140      v150      v160      v170      v180      v190      v200
EGSGAQVVGPMGQPLQVLTLNIENKYRLHETSKEPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLD
EGSGAQVVGPMGQPLQVLTLNIE:,YRLHETS.EPDV:LGSTWLSDFPQAWAETG MGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLD
EGSGAQVVGPMGQPLQVLTLNIEDEYRLHETSTEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLD
      ^110      ^120      ^130      ^140      ^150      ^160      ^170      ^180      ^190      ^200

      v210      v220      v230      v240      v250      v260      v270      v280      v290      v300
QGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQL
QGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQL
QGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQL
      ^210      ^220      ^230      ^240      ^250      ^260      ^270      ^280      ^290      ^300

      v310      v320      v330      v340      v350      v360      v370      v380      v390      v400
TWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSEQDCQRGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEA
TWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDD:LLAATSE DCQ:GTRALL TLGNLGYRASAKKAQ:CQKQVKYLGYLLKEGQRWLTEA
TWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDILLAATSELDCQQGTRALLLTLGNLGYRASAKKAQLCQKQVKYLGYLLKEGQRWLTEA
      ^310      ^320      ^330      ^340      ^350      ^360      ^370      ^380      ^390      ^400
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    v410        v420        v430        v440        v450        v460        v470        v480        v490        v500
RKETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQ
RKETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTILFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQ
RKETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQ
      ^410        ^420        ^430        ^440        ^450        ^460        ^470        ^480        ^490        ^500

    v510        v520        v530        v540        v550        v560        v570        v580        v590        v600
KLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTDRVQFGPVVALNPATL
KLGPWRRPVAYLSKKLDPVAA WPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTDRVQFGPVVALNPATL
KLGPWRRPVAYLSKKLDPVAARWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTDRVQFGPVVALNPATL
      ^510        ^520        ^530        ^540        ^550        ^560        ^570        ^580        ^590        ^600

    v610        v620        v630        v640        v650        v660        v670        v680        v690        v700
LPLPEKEAPHDCLEILAETHGTRPDLTDQPIPDADYTWYTDGSSFLQEGQRRAGAAVTTETEVIWARALPAGTSAQRAELIALTQALKMAEGKKLNVYTD
LPLPEK.APHDCLEILAETHGTRPDLTDQPIPDAD.TWYTDGSSFLQEGQRRAGAAVTTETEVIWARALPAGTSAQRAELIALTQALKMAEGKKLNVYTD
LPLPEKGAPHDCLEILAETHGTRPDLTDQPIPDADHTWYTDGSSFLQEGQRRAGAAVTTETEVIWARALPAGTSAQRAELIALTQALKMAEGKKLNVYTD
      ^610        ^620        ^630        ^640        ^650        ^660        ^670        ^680        ^690        ^700

    v710        v720        v730        v740        v750        v760        v770        v780        v790        v800
SRYAFATAHVHGEIYRRRGLLTSEGREIKNKNEILALLKALFLPKRLSIIHCPGHQKGNSAEARGNRMADQAAREAAMKAVLETSTLLIEDSTPYTPPHF
SRYAFATAHVHGEIYRRRGLLTSEGREIKNK:EILALLKALFLPKRLSIIHCPGHQKGNSAEARGNRMADQAAREAA::::  ETSTLLIEDSTPYTP:HF
SRYAFATAHVHGEIYRRRGLLTSEGREIKNKSEILALLKALFLPKRLSIIHCPGHQKGNSAEARGNRMADQAAREAAIRTSPETSTLLIEDSTPYTPSHF
      ^710        ^720        ^730        ^740        ^750        ^760        ^770        ^780        ^790        ^800

    v810        v820        v830        v840        v850        v860        v870        v880        v890        v900
HYTETDLKRLRELGATYNQTKGYWVLQGKPVMPDQSVFELLDSLHRLTHPSPQKMKALLDDREESPYYMLNRDRTIQYVTETCTACAQVNASKAKIGAGV
HYTETDLKRLRELGATYNQ.KGYWVLQGKPVMPDQ VFELLDSLHRLTH SPQKMKALLDDREESPYYMLNRDRT:QYV:E:CTACAQVNASKAKIGAGV
HYTETDLKRLRELGATYNQIKGYWVLQGKPVMPDQFVFELLDSLHRLTLPSPQKMKALLDDREESPYYMLNRDRTLQYVAESCTACAQVNASKAKIGAGV
      ^810        ^820        ^830        ^840        ^850        ^860        ^870        ^880        ^890        ^900
```

```
    v910       v920       v930       v940       v950       v960       v970       v980       v990       v1000
RVRGHRPGTHWEVDETEVKPGLYGYKYLLVFVDTFSGWVEAFPTKRETAKVVSKKLLEDIFPRFEMPQVLGSDNGPAFASQVSQSVADLLGIDWKLHCAY
R:RGHRPGTHWE:DETEVKPGLYGYKYLLVFVDTFSGWVEAFPTKRETAKVV:KKLLE:IFPRF.MPQVLGSDNGPAF.SQVS:SVADLLGIDWKLHCAY
RIRGHRPGTHWEIDFTEVKPGLYGYKYLLVFVDTFSGWVEAFPTKRETAKVVTKKLLEEIFPRFGMPQVLGSDNGPAFVSQVSHSVADLLGIDWKLHCAY
     ^910       ^920       ^930       ^940       ^950       ^960       ^970       ^980       ^990       ^1000


    v1010      v1020      v1030      v1040      v1050      v1060      v1070      v1080      v1090      v1100
KPQSSGQVERINKTIKETLTKLTLASGTKDWVLLLPLALYRARNTPGPHGLTPYEILYGAPPPLVNFHNPEMSKLTNSPSLQAHLQALQAVQQEVWKPLA
:PQSSGQVER:N:TIKETLTKLTLA:GT:DWVLLLPLALYRARNTPGPHGLTPYEILYGAPPPLVNFH:PEMSKLTNSPSLQAHLQALQAVQ:EVWKPLA
RPQSSGQVERMNRTIKETLTKLTLAAGTRDWVLLLPLALYRARNTPGPHGLTPYEILYGAPPPLVNFHDPEMSKLTNSPSLQAHLQALQAVQREVWKPLA
    ^1010      ^1020      ^1030      ^1040      ^1050      ^1060      ^1070      ^1080      ^1090      ^1100


    v1110      v1120      v1130      v1140      v1150      v1160      v1170      v1180      v1190
AAYQDQLDQPVIPHPFRVGDAVWVRRHQTKNLEPRWKGPYTVLLTTPTALKVDGISAWIHAAHVKAATTPPAGTAWKVQRSQNPLKIRLTRGAP (SEQ ID NO.4)
AAYQDQLDQPVIPHPFRVGDAVWVRRHQTKNLEPRWKGPYTVLLTTPTALKVDGISAWIHAAHVKAATTPPAGTAWKVQRSQNPLKIRLTRGAP (SEQ ID NO.65)
AAYQDQLDQPVIPHPFRVGDAVWVRRHQTKNLEPRWKGPYTVLLTTPTALKVDGISAWIHAAHVKAATTPPAGTAWKVQRSQNPLKIRLTRGAP (SEQ ID NO.66)
    ^1110      ^1120      ^1130      ^1140      ^1150      ^1160      ^1170      ^1180      ^1190
```

Table 5. Alignment of HXV35 and DG-75 ENV Polypeptides.

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>645)	Seq2(1>644)	Similarity	Gap	Gap	Consensus
HXV35 env	DG75 env	Index	Number	Length	Length
(1>645)	(1>644)	93.8	1	1	645
(1>645)	(1>644)	93.8	1	1	645

```
        v10       v20       v30       v40       v50       v60       v70       v80       v90       v100
MESPAFSKPLKDKINPWGPLIIMGILVRAGASVQRDSPHQVFNVTWKITNLMTGQTANATSLLGTMTDTFPKLYFDLCDLVGDNWDDPEPDIGDGCRSPG
ME:PAFSKPLKDKINPWGPLI:MGILVRAGASVQRDSPHQ:FNVTW::TNLMTGQTANATSLLGTMTDTFPKLYFDLCDLVGD WDDPEPDIGDGCR:PG
MEGPAFSKPLKDKINPWGPLIVMGILVRAGASVQRDSPHQIFNVTWRVTNLMTGQTANATSLLGTMTDTFPKLYFDLCDLVGDYWDDPEPDIGDGCRTPG
        ^10       ^20       ^30       ^40       ^50       ^60       ^70       ^80       ^90       ^100

        v110      v120      v130      v140      v150      v160      v170      v180      v190      v200
GRKRTRLTDFYVCPGHTVLTGCGGPREGYCGKWGCETTGQAYWKPSSSWDLISLKRGNTPKGQGPCFDSSVGSGSIQGATPGGRCNPLVLEFTDAGKRAS
GR:RTRLTDFYVCPGHTV .GCGGP EGYCGKWGCETTGQAYWKPSSSWDLISLKRGNTPK:QGPC:DSSV:SG :QGATPGGRCNPLVLEFTDAG::AS
GRKRTRLTDFYVCPGHTVPIGCGGPGEGYCGKWGCETTGQAYWKPSSSWDLISLKRGNTPKDQGPCYDSSVSSG-VQGATPGGRCNPLVLEFTDAGRKAS
        ^110      ^120      ^130      ^140      ^150      ^160      ^170      ^180      ^190

        v210      v220      v230      v240      v250      v260      v270      v280      v290      v300
WDAPKTWGLRLYRSTGADPVTLFSLTRQVLNVGPRVPIGPNPVITEQLPPSQPVQIMLPRPPRPPPSGAASMVPGAPPPSQQPGTGDRLLNLVEGAYQAL
WDAPK.WGLRLYRSTGADPVT FSLTRQVLNVGPR:PIGPNPVIT:QLPPSQPVQIMLPRPP:PPPS::.SMVPGAPPPSQQPGTGDRLLNLVEGAYQAL
WDAPKVWGLRLYRSTGADPVTRFSLTRQVLNVGPRIPIGPNPVITDQLPPSQPVQIMLPRPPHPPPSDTVSMVPGAPPPSQQPGTGDRLLNLVEGAYQAL
^200      ^210      ^220      ^230      ^240      ^250      ^260      ^270      ^280      ^290

        v310      v320      v330      v340      v350      v360      v370      v380      v390      v400
NLTSPDKTQECWLCLVSGPPYYEGVAVLGTYSNHTSAPANCSVTSQHKLTLSEVTGQGLCIGAVPKTHQALCNTTQKTSDGSYYLASPAGTIWACSTGLT
NLTSPDKTQECWLCLVSGPPYYEGVAVLGTYSNHTSAPANCSV:SQHKLTLSEVTGQGLC:GAVPKTHQALCNTTQKTSDGSYYLA:PAGTIWAC:TGLT
NLTSPDKTQECWLCLVSGPPYYEGVAVLGTYSNHTSAPANCSVASQHKLTLSEVTGQGLCVGAVPKTHQALCNTTQKTSDGSYYLAAPAGTIWACNTGLT
^300      ^310      ^320      ^330      ^340      ^350      ^360      ^370      ^380      ^390
```

```
      v410        v420        v430        v440        v450        v460        v470        v480        v490        v500
PCLSTTVLNLTTDYCVLVELWPKVTYHSPNYVYGQFGKKTKYKREPVSLTLALLLGGLTMGGIAAGVGTGTTALVATKQFEQLQAAIHTDLGALEKSVSA
PCLSTTVLNLTTDYCVLVELWPKVTYHSP.XVYGQF.:KTKYKREPVSLTLALLLGGLTMGGIAAGVGTGTTALVATKQFEQLQAAI  .LGALEKSVSA
PCLSTTVLNLTTDYCVLVELWPKVTYHSPGYVYGQFERKTKYKREPVSLTLALLLGGLTMGGIAAGVGTGTTALVATKQFEQLQAAILQTLGALEKSVSA
^400        ^410        ^420        ^430        ^440        ^450        ^460        ^470        ^480        ^490

      v510        v520        v530        v540        v550        v560        v570        v580        v590        v600
LEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALKKECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLISTIMGPLIVLLLIL
LEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALK.ECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLISTIMGPLIVLLLIL
LEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALKEECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLISTIMGPLIVLLLIL
^500        ^510        ^520        ^530        ^540        ^550        ^560        ^570        ^580        ^590

      v610        v620        v630        v640
LFGPCILNRLVQFVKDRISVVQALVLTQQYHQLKSIDPEEVESRE (SEQ ID NO.5)
LFGPCILNRLVQFVK:RISVVQALVLTQQYHQLKSIDPE.VESRE (SEQ ID NO.67)
LFGPCILNRLVQFVKGRISVVQALVLTQQYHQLKSIDPEAVESRE (SEQ ID NO.68)
 ^600        ^610        ^620        ^630        ^640
```

Table 6. Primer-Binding Sites in HXV_{35} and DG-75

HXV35 PBS: T G G G G G C T C G T C C G G G A T (SEQ ID NO.69)
proline tRNA

DG-75 PBS (1): T G G A G G T C C C A C C G A G A T (SEQ ID NO.70)
threonine tRNA

DG-75 PBS (2): T G G A G G C C C C A G C G A G A T (SEQ ID NO.71)

Table 7. Variable Regions A and B in the SU proteins of different gammaretroviruses

Variable Region A:

HXV35	(xenotropic)	DnWDDpepdigd (SEQ ID NO.72).....GCrTPggRrR (SEQ ID NO.73)
DG-75	(xenotropic)	DyWDDpepdigd (SEQ ID NO.74).....GCrTPggRrR (SEQ ID NO.75)
MCF247	(polytropic)	DdWDEtgl (SEQ ID NO.76).....GCrTPggRkR (SEQ ID NO.77)
MKVENVA MLV	(amphotropic)	EeWDPsdqepyvgy (SEQ ID NO.78).....GCrTPggRqR (SEQ ID NO.79)
M-MLV	(ecotropic)	SyWGLeyqspfssppgppccsggsspgcsrdceepltsltpRCnTAwnRlK (SEQ ID NO. 80)

Variable Region B:

HXV35	(xenotropic)	trlyd(SEQ ID NO.81)[...]FYVCPGhtvltG (SEQ ID NO.82)
DG-75	(xenotropic)	trlyd(SEQ ID NO.83)[...]FYVCPGhtvpiG (SEQ ID NO.84)
MCF247	(polytropic)	artfd(SEQ ID NO.85)[...]FYVCPGhtvptG (SEQ ID NO.86)
MKVENVA MLV	(amphotropic)	trtfd(SEQ ID NO.87)[...]FYVCPGhtvskG (SEQ ID NO.88)
M-MLV	(ecotropic)	ldgtthksnegFYVCPGphrprEsks (SEQ ID NO.89)

Table 8. Alignment of partial sequence of LNCap derived virus.

Sequence from LNCap RNA RTPCR product. (7084-7750bp)

AAAAGAGAGCCCGGTGTCATTAACTCTGGCCCTGTCTGTTGGGAGGACTTACTATGGGCGGCATAGCTCCAGGAGTTGGAACAGGGACTACAGCCCTAGTGGCCACCAA
ACAATTCGAGCAGCTCCAGGCAGCCTACATACAGACCTTGGGGCCTTAGAAAAAATCAGTCAGTGCCCTAGAAAAGTCTCTGACCTCGTTGTCTGAGGTGGTCCTACAG
AACCGGAGGGGATTAGATCTACTGTTCCTAAAAGAAGGAGGATTATGTGCTGCCCTAAAAGAAAGAATGCTGTTTTTACGCGGACCACACTGGCGTAGTAAGAGATAGC
ATGGCAAAGCTAAGAGAAAGGTTAAACCAGAGACAAAAATTGTTCGAATCAGGACAAGGGTGGTTTGAGGGACTGTTTAACAGGTCCCCATGGTTCACGACCCTGATAT
CCACCACCATTATGGGCCCTCTGATAGTACTTTTATTAATCCTACTTTTCGGACCCTGTATTCTCAACCGCTTGGTCCAGTTTGTAAAAGACACAGAATTTCGGTAGTG
CAGGCCCTGGTTCTGACCCAGCAGTATCACCAACTCAAATCAATAGATCCAGAAGAAGTGGAATCACGTGAATAAAAGATTTTATTCAGTTTCCAGAAAGAGGGGGGAA
TGAAAGACCCCCCCATAAGGC (SEQ ID NO.6)

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>127)	Seq2(1>645)	Similarity	Gap	Gap	Consensus
LNCAP env seq	HXV35 env	Index	Number	Length	Length
(2>123)	(496>616)	97.6	1	1	122
(2>123)	(496>616)	97.6	1	1	122

```
        v10       v20       v30       v40       v50       v60       v70       v80       v90       v100
KSVSALEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALKEECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLISTTIMGPLI
KSVSALEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALK.ECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLIST IMGPLI
KSVSALEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALKKECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWPEGLFNRSPWFTTLIST-IMGPLI
    ^500      ^510      ^520      ^530      ^540      ^550      ^560      ^570      ^580      ^590

        v110      v120
VLLLILLFGPCILNRLVQFVKD (SEQ ID NO.90)
VLLLILLFGPCILNRLVQFVKD (SEQ ID NO.91)
VLLLILLFGPCILNRLVQFVKD (SEQ ID NO.92)
     ^600      ^610
```

```
Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12
Seq1(1>127)       Seq2(1>644)         Similarity     Gap        Gap        Consensus
LNCAP env seq     DG-75 env           Index          Number     Length     Length
(2>123)               (495>615)       97.6           1          1          122
(2>123)               (495>615)       97.6           1          1          122

        v10       v20        v30        v40        v50        v60        v70        v60        v90
KSVSALEKSLTSLSEVVLQNRRGLDLLPLKEGGLCAALKEECCFYADHTGVVRDSMAXLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLIST
KSVSALEKSLTSLSEVVLQNRRGLDLLPLKEGGLCAALKEECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLIST
KSVSALEKSLTSLSEVVLQNRRGLDLLFLKEGGLCAALKEECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEGLFNRSPWFTTLIST
     ^500       ^510       ^520       ^530       ^540       ^550       ^560       ^570       ^580

     v100       v110       v120
TIMGPLIVLLLILLFGPCILNRLVQFVXD (SEQ ID NO.90)
 IMGPLIVLLLILLFGPCILNRLVQFVK: (SEQ ID NO.93)
-IMGPLIVLLLILLFGPCILNRLVQFVKG (SEQ ID NO.94)
   ^590       ^600       ^610
```

Table 9. Presence of HXV in prostates as determined by RT-PCR and FISH.

VP#	RNL genotype	HXV by RT-PCR	FISH (+) cells	Cells counted	% FISH (+) cells	HXV by FISH
VP 29	AA	yes	7	659	1.062	++
VP 42	AA	yes	6	530	1.132	++
VP 62	AA	yes	10	904	1.106	++
VP 88	AA	yes	5	408	1.225	++
VP 31	AA	no	6	526	1.141	++
VP 79	AA	yes	2	464	0.431	+/-
VP 10	AA	yes	1	872	0.115	-
VP 35	AA	yes	1	849	0.118	-
VP 90	AA	yes	1	843	0.119	-
VP 27	AA	no	0	762	0.000	-
VP 45	AG	no	0	987	0.000	-
VP 46	AG	no	0	794	0.000	-
VP 30	GG	no	1	661	0.151	-
VP 50	GG	no	1	787	0.127	-
VP 51	GG	no	0	842	0.000	-

<1/500= -; 1-2/500 = +/-; 3-4/500 +; 5-6/500 ++

Table 10. XMRV screening by *gag* nested RT-PCR.

	Genotype[a]			Total
	QQ	RQ	RR	
PCR +	8	0	1	9
PCR -	12	14	51	77
Total	20	14	52	86

[a]*RNASEL* genotypes are as follows:
QQ—homozygous R462Q variant; RQ—heterozygous;
RR—homozygous wild-type.

Table 11. PCR primers used for sequencing of XMRV genomes.

Primer	**Sequence**	**Fragment size**	**XMRV nucleotide positions**
IF	5'-GCGCCAGTCATCCGATAGACT(SEQ ID NO.95)	642	1-642
NA3-136R	5'-CCCAGTGCTGCAAGGTTAGA(SEQ ID NO.96)		
550F	5'-CGCCGAAACCGCGCCGCGCGT(SEQ ID NO.97)	968	526-1494
1500R	5'-TCGTCGCCCCGGACTGCCTTTCTG(SEQ ID NO.98)		
1470F	5'-GACAGGAGAAGAAAAGCAGCG(SEQ ID NO.99)	1280	1440-2720
2730R	5'-GCTTGGCGAACTGCCAGTCCC(SEQ ID NO.100)		
2670F	5'-AGCCGGATGTTTCTCTAGGGT(SEQ ID NO.101)	1228	2631-3859
3870R	5'-GCTTGCCTGCATCTTTTGTC(SEQ ID NO.102)		
3810F	5'-AGACCCAGTGGCAGCCGGGT(SEQ ID NO.103)	1400	3780-5180
5190R	5'-TGACTTACCTGGGAGACGAAG(SEQ ID NO.104)		
5100F	5'-AACTGCCAAGGTTGTGACCAA(SEQ ID NO.105)	748	5071-5819
5842R	5'-AACTATTGGGGGCCCCACGGGTTA(SEQ ID NO.106)		
NA7-F	5'-CATGGAAAGTCCAGCGTTCT(SEQ ID NO.107)	1448	5753-7201
C9-R	5'-AGCTGCTCGAATTGTTTGGT(SEQ ID NO.108)	997	7175-8172
7200F	5'- CTAGTGGCCACCAAACAATTC(SEQ ID NO.40)		
K1-R	5'- AAGGCTTTATTGGGAACACG(SEQ ID NO.109)	411	7578-7989
7600F	5'-CGCTTGGTCCAGTTTGTAAAA(SEQ ID NO.38)		
227R	5'-TGGGGAACTTGAAACTGAGG(SEQ ID NO.39)		
100F	5'-AGGGGCCAAACAGGATAACT(SEQ ID NO.110)	127	7862-7989
227R	5'-TGGGGAACTTGAAACTGAGG(SEQ ID NO.111)		
B7-F	5'-TCTGGAAAGTCCCACCTCAG(SEQ ID NO.112)	216	7956-8172
K1-R	5'-AAGGCTTTATTGGGAACACG(SEQ ID NO.109)		

Table 12. Computational viral species predictions using E-Predict for the Virochip microarrays shown in Figures 22A-22B.

Sample	Array ID	Top prediction ($p < 0.05$)[a]	NCBI Taxonomy ID	p-value
VP10	MegaViroP7-244	NA		
VP27	MegaViroP7-245	NA		
VP29	MegaViroP5-174	Spleen focus-forming	11819	1.3E-05
VP31	MegaViroP5-176	NA		
VP35	MegaViroP5-177	Spleen focus-forming	11819	1.0E-05
VP42	MegaViroP5-178	Murine osteosarcoma	11830	1.5E-05
VP62	MegaViroP8-037	Spleen focus-forming	11819	2.0E-05
VP79	MegaViroP8-030	Murine type C	44561	2.9E-03
VP88	MegaViroP8-031	Spleen focus-forming	11819	1.4E-05
VP90	MegaViroP8-032	Spleen focus-forming	11819	2.4E-04
VP107	MegaViroP7-246	NA		
VP45	MegaViroP5-195	NA		
VP46	MegaViroP5-196	NA		
VP49	MegaViroP5-197	NA		
VP30	MegaViroP5-175	NA		
VP50	MegaViroP10-128	NA		
VP51	MegaViroP5-199	NA		
VP66	MegaViroP8-035	NA		
VP86	MegaViroP8-036	Spleen focus-forming virus	11819	8.2E-04
HeLa	MegaViroP5-179	Human papillomavirus type 18	10582	1.0E-06

[a]Microarrays were analyzed using E-Predict as described previously (Urisman, A., et al. (2005), Genome Biol 6: R78).

Table 13. Frequency of XMRV infected prostatic cells determined by FISH.

Patient	*RNASEL* Amino Acid Residue 462[a]	Total # Cells Counted[b]	FISH/XMRV Positive Cells (%)	XMRV FISH[c]	XMRV *gag* RT-PCR[d]
VP 88	QQ	408	5	++	+
VP 31	QQ	526	6	++	-
VP 42	QQ	530	6	++	+
VP 62	QQ	904	10	++	+
VP 29	QQ	659	7	++	+
VP 79	QQ	464	2	+	+
VP 10	QQ	872	1	+/-	-
VP 35	QQ	849	1	+/-	+
VP 90	QQ	843	1	+/-	+
VP 45	RQ	987	0	-	-
VP 46	RQ	794	0	-	-
VP 30	RR	661	1	+/-	-
VP 50	RR	787	1	+/-	-
VP 51	RR	842	0	-	-

[a], SNP nt1385 "A" results in glutamine (Q) at amino acid 462, and SNP nt1385 "G" correspond to an arginine (R) at residue 462; [b], includes all types of prostatic cells; [c], +/- =0.1-0.2%1 +=02-1%; ++=>1%; [d] see Urisman, A., et al., (2005), PLOS Pathogens.

All of the references cited herein are incorporated by reference in their entirety.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT/Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 19, line 23 and on page 20, line 5.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [ ]

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION

Address of depositary institution *(including postal code and country)*

American Type Culture Collection
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
30 March 2005	Not Available

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [X]

In respect of those designations for which a European patent is sought, the Applicant hereby informs the International Bureau that the Applicant wishes that, until the publication of the mention of the grant of a European patent or for 20 years from the date of filing if the application is refused or withdrawn or deemed to be withdrawn, the biological material deposited with the American Type Culture Collection under Accession No. [ ] shall be made available as provided in Rule 28(3) EPC only by the issue of a sample to an expert nominated by the requester (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the International application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL (Additional Sheet)

C. ADDITIONAL INDICATIONS (Continued)

In respect of the designation of Australia in the subject PCT application, and in accordance with Regulation 3.25(3) of the Australian Patents Regulations, the Applicant hereby gives notice that the furnishing of a sample of the biological material deposited with the American Type Culture Collection under Accession No. __N/A____ shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention and who is nominated in a request for the furnishing of a sample.

In respect of the designation of Canada in the subject PCT application, the Applicant hereby informs the International Bureau that the Applicant wishes that, until either a Canadian patent has been issued on the basis of the application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the biological material deposited with the American Type Culture Collection under Accession No. __N/A____ and referred to in the application to an independent expert nominated by the Commissioner.

CLAIMS

What is claimed is:

1. An isolated XMRV present in a prostate tumor of an individual.

2. The virus of Claim 1 wherein the individual comprises a mutation in the hereditary prostate cancer-1 (HPC1) allele encoding an RNase L gene.

3. The virus of Claim 2 wherein the mutation is a homozygous mutation.

4. The virus of Claim 3 wherein the mutation is homozygous for the RNase L mutation R462Q.

5. An isolated virus comprising a nucleic acid sequence having at least 94% identity to SEQ ID No: 1 or a complement thereof.

6. An isolated virus comprising SEQ ID No: 1 and a complement thereof.

7. An isolated virus comprising an amino acid sequence encoded by a nucleic acid sequence having at least 94% identity to SEQ ID No: 1.

8. An isolated virus comprising an amino acid sequence encoded by SEQ ID No: 1.

9. An isolated virus comprising SEQ ID No: 2.

10. An isolated polypeptide comprising an amino acid sequence having at least 97% similarity to SEQ ID No: 3.

11. An isolated polypeptide comprising an amino acid sequence having at least 97% similarity to SEQ ID No: 4.

12. An isolated polypeptide comprising an amino acid sequence having at least 94% similarity to SEQ ID No: 5.

13. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and combinations thereof.

14. A vector comprising the nucleic acid sequence of Claim 5.

15. A cell comprising the vector of Claim 14.

16. An antibody or antigen binding fragment thereof that specifically binds to a virus comprising an amino acid sequence encoded by a nucleic acid sequence having at least 94% identity to SEQ ID NO: 1.

17. The antibody or antigen binding fragment of Claim 16 wherein the antibody or antigen binding fragment binds to a gag polypeptide encoded by the virus.

18. The antibody or antigen binding fragment of Claim 17 wherein the gag polypeptide comprises SEQ ID No: 3.

19. A method of detecting XMRV in a sample comprising:

a) contacting the sample with a nucleic acid sequence that hybridizes to all of a portion of an XMRV nucleic acid sequence under conditions in which a hybridization complex can occur between the nucleic acid sequence and the XMRV nucleic acid sequence; and

b) determining whether the hybridization complex is detected in the sample,

wherein if the hybridization complex is detected, then XMRV is in the sample.

20. A method of detecting XMRV in a sample comprising:

a) contacting the sample with an antibody or antigen binding fragment thereof that specifically binds to an XMRV polypeptide under conditions in which a complex can occur between the antibody and the XMRV polypeptide; and

b) determining whether the complex is detected in the sample,

wherein if the complex is detected, then XMRV is in the sample.

21. A method of identifying an agent that inhibits an XMRV comprising:

a) contacting the XMRV with an agent to be assessed; and

b) determining whether XMRV is inhibited in the presence of the agent,

wherein, if XMRV is inhibited in the presence of the agent, then the agent inhibits XMRV.

22. The method of Claim 21 wherein the activity of the XMRV in the presence of the agent is determined by measuring the ability of the XMRV to produce retroviral particles with reverse transcriptase activity.

23. A method of inducing an immune response to an XMRV in an individual in need thereof, comprising administering to the individual an effective amount of an agent which induces an immune response to the XMRV in the individual upon administration.

24. The method of Claim 23 wherein the agent is selected from the group consisting of: a subunit of XMRV and an attenuated XMRV.

25. A method of treating prostate cancer in an individual wherein XMRV is present in the prostate of the individual, comprising administering to the individual an effective amount of an agent that inhibits XMRV.

26. A method of detecting asymptomatic (early stage) prostate cancer in an individual wherein XMRV is present in the prostate of the individual, comprising detecting the presence of anXMRV in the individual, wherein the presence of XMRV in the individual is indicative of early stage prostate cancer in the individual.

27. A method of identifying an individual at risk for developing prostate cancer , comprising determining whether an XMRV is present in the individual, wherein if XMRV is present in the individual then the individual is at risk for developing prostate cancer.

28. The method of Claim 27 wherein the individual has a mutation in the hereditary prostate cancer-1 (HCP1) allele encoding an RNase L gene.

29. The method of Claim 28 wherein the mutation is a homozygous mutation.

30. The method of Claim 29 wherein the mutation is homozygous for the RNase L mutation R462Q.

31. A kit for detecting the presence of XMRV in a sample comprising a labeled moiety that detects XMRV in a sample.

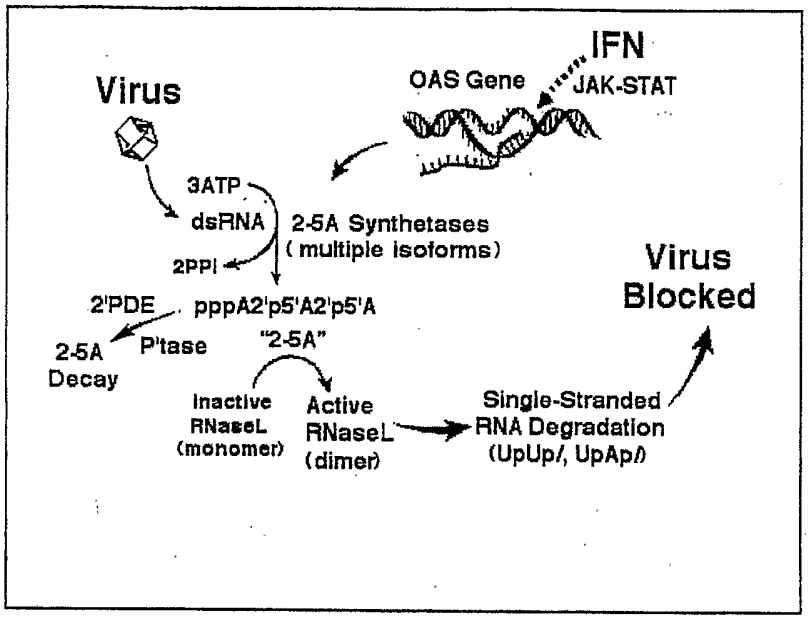
32. The kit of Claim 31 wherein the labeled moiety is a nucleic acid sequence that hybridizes to all of a portion of an XMRV nucleic acid sequence.

33. The kit of Claim 31 wherein the labeled moiety is an antibody or antigen binding fragment thereof that specifically binds to XMRV.

34. A method of producing XMRV comprising maintaining the cell of Claim 14 under conditions in which XMRV is produced.

35. The method of Claim 33 comprising isolating the XMRV produced by the cell.

Figure 1.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JAK-STAT
3ATP
dsRNA
2-5A Synthetases
(multiple isoforms)
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Blocked
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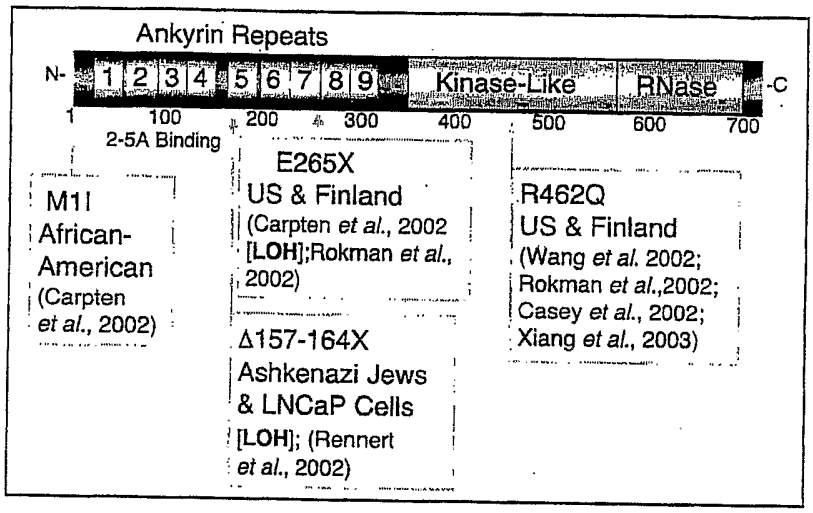
pppA2'p5'A2'p5'A
2-5A
Decay
P'tase
"2-5A"
Inactive
RNaseL
(monomer)
Active
RNaseL
(dimer)
Single-Stranded
RNA Degradation
(UpUp/, UpAp/)

Figure 2.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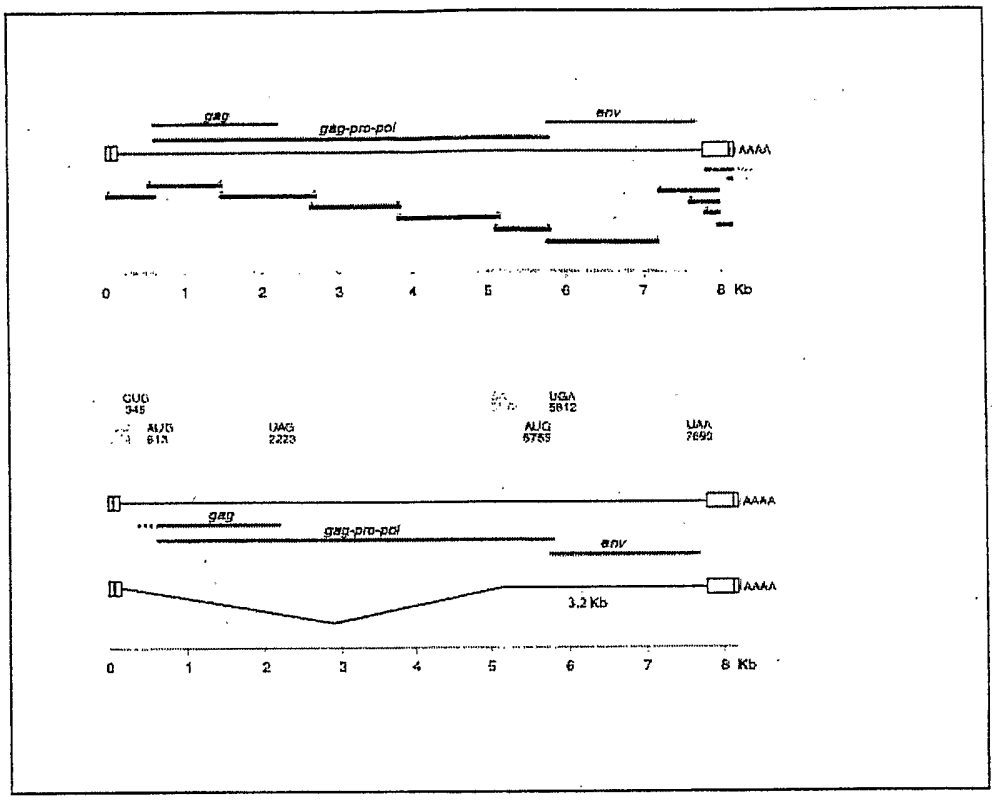
Kinase-Like
RNase
-C
1
100
200
300
400
500
600
700
2-5A Binding
M1I
African-American
(Carpten et al., 2002)
E265X
US & Finland
(Carpten et al., 2002 [LOH];Rokman et al., 2002)
Δ157-164X
Ashkenazi Jews & LNCaP Cells
[LOH]; (Rennert et al., 2002)
R462Q
US & Finland
(Wang et al. 2002; Rokman et al.,2002; Casey et al., 2002; Xiang et al., 2003)

Figure 3.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env
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CUG
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AUG
613
UAG
2223
UGA
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AUG
6755
UAA
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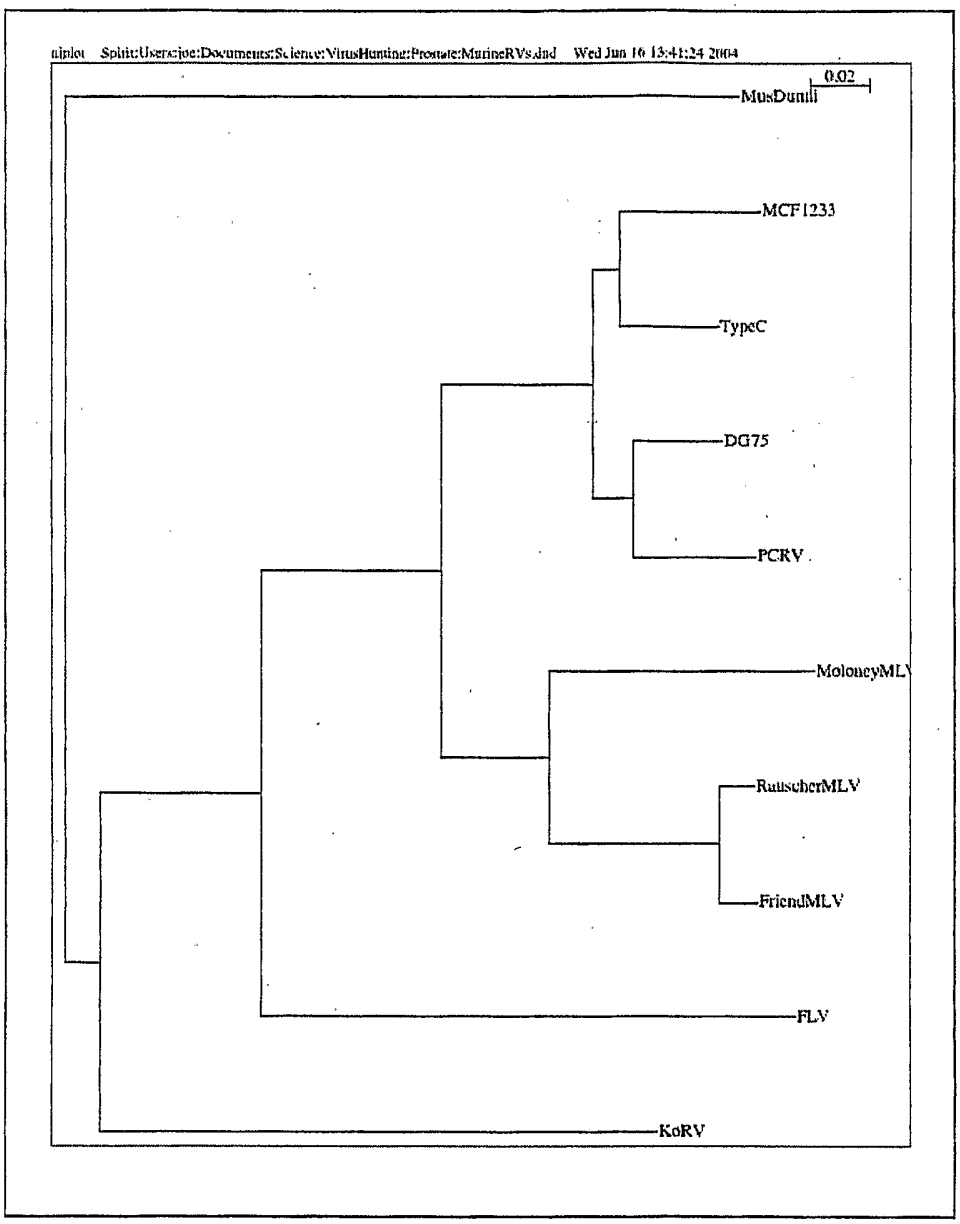
AAAA
gag
gag-pro-pol
env
AAAA
3.2 Kb
0 1 2 3 4 5 6 7 8 Kb

Figure 4.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MCF1233
TypeC
DG75
PCRV
MoloneyMLV
RauscherMLV
FriendMLV
FLV
KoRV

Figure 5.

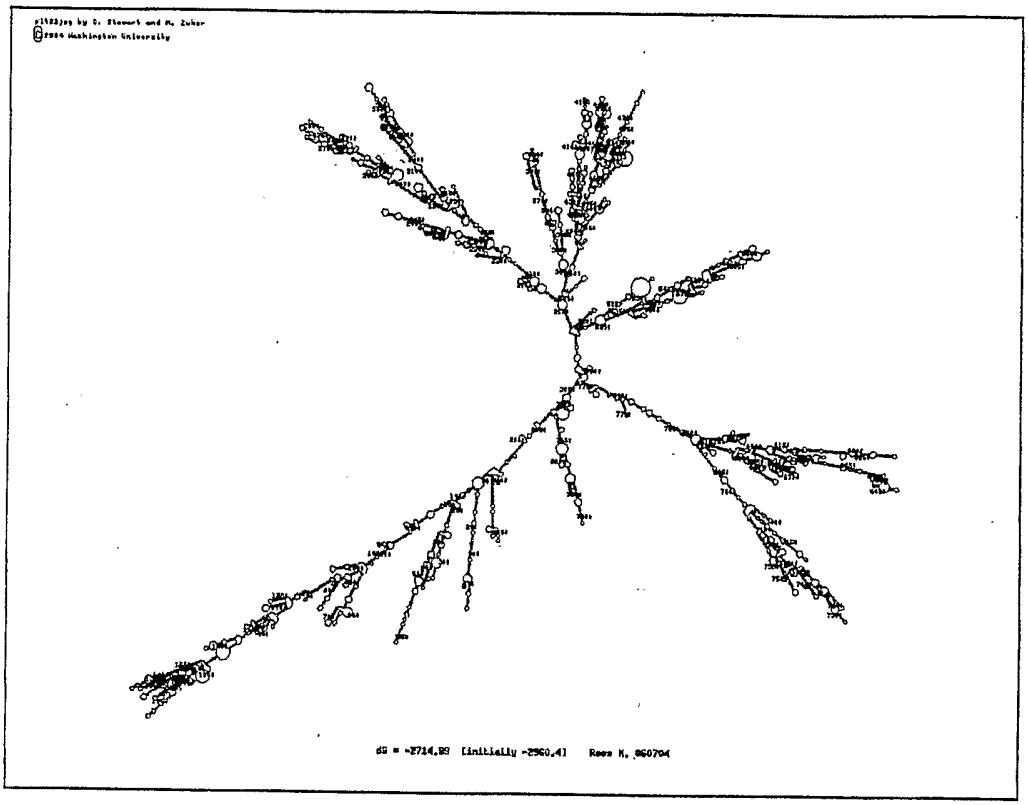
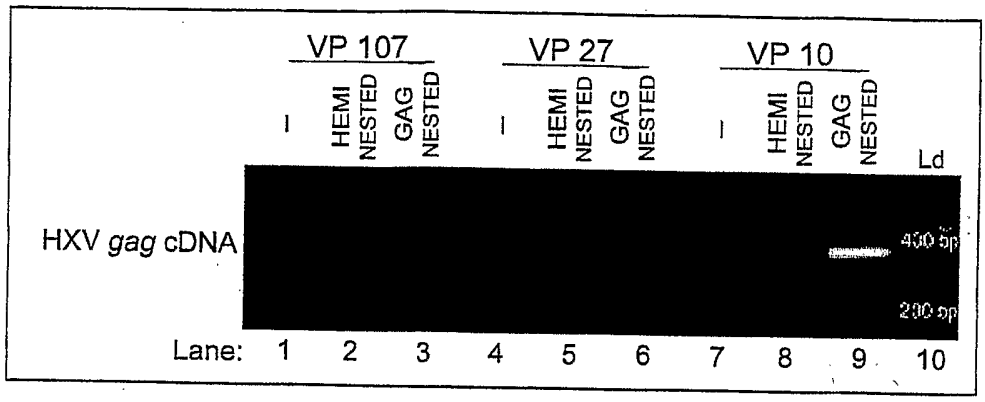


Figure 6.


VP 107
VP 27
VP 10
-
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GAG NESTED
Ld
HXV gag cDNA
400 bp
200 bp
Lane: 1 2 3 4 5 6 7 8 9 10

Figure 7.

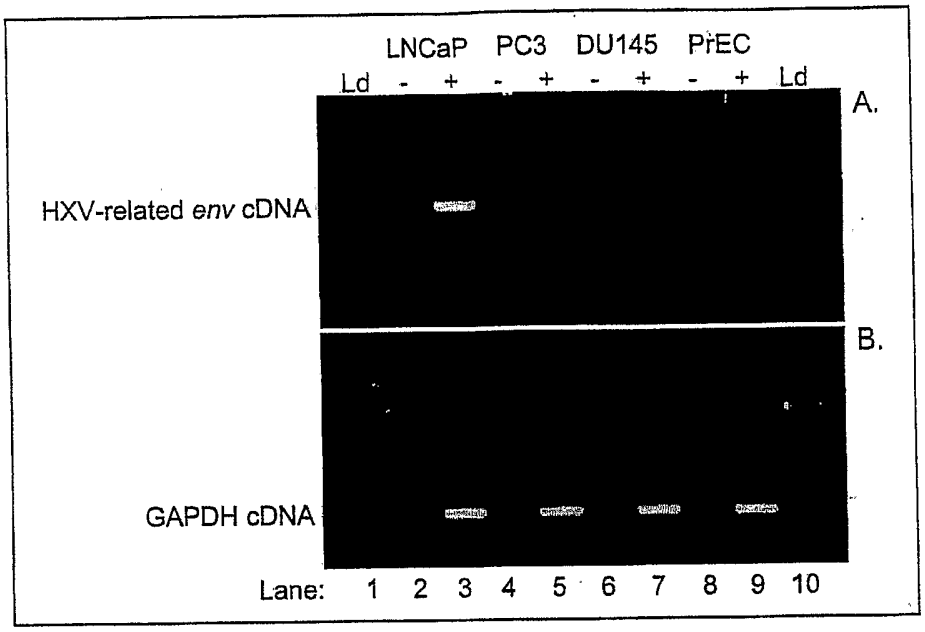

LNCaP PC3 DU145 PrEC
Ld - + - + - + - + Ld
A.
HXV-related env cDNA
B.
GAPDH cDNA
Lane: 1 2 3 4 5 6 7 8 9 10

Figure 8.

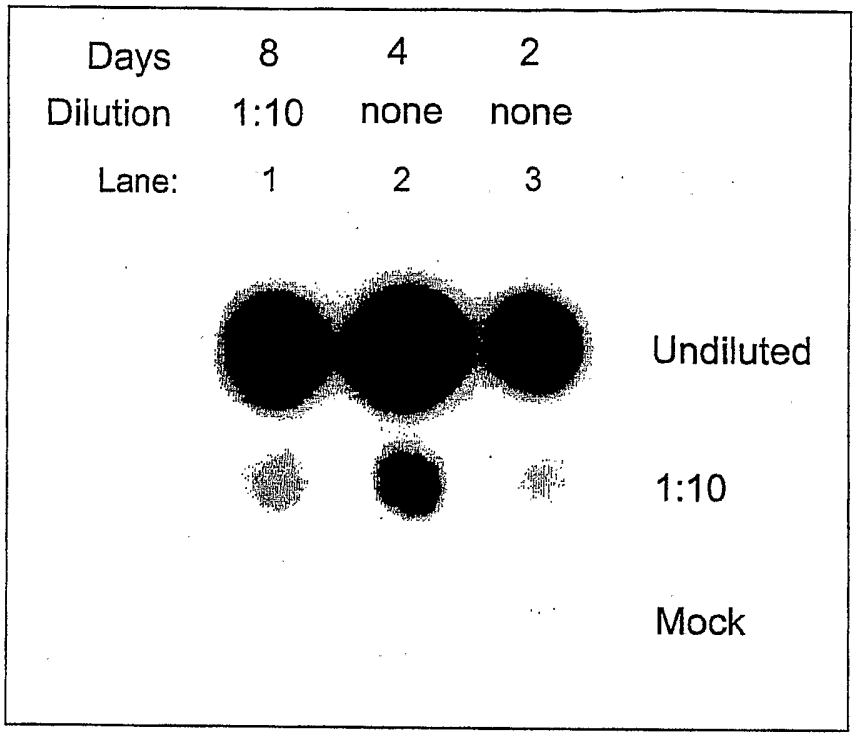


Figure 9.

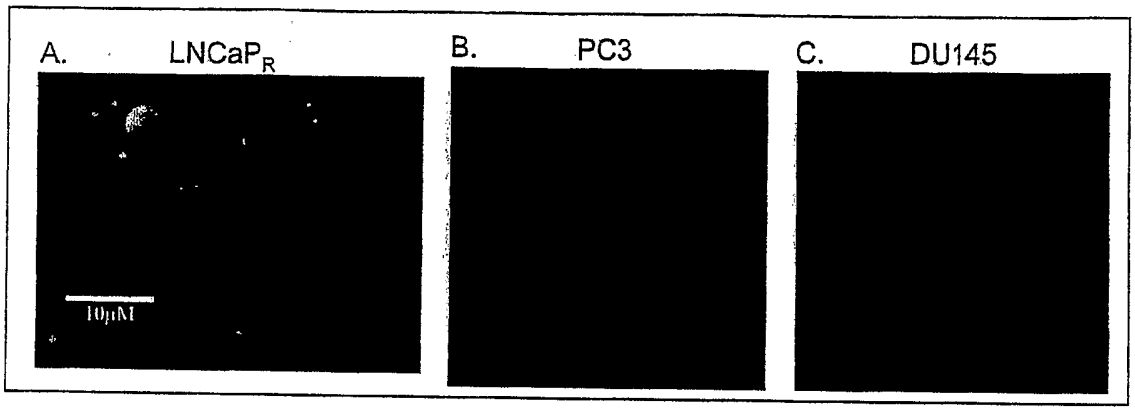


Figure 10.

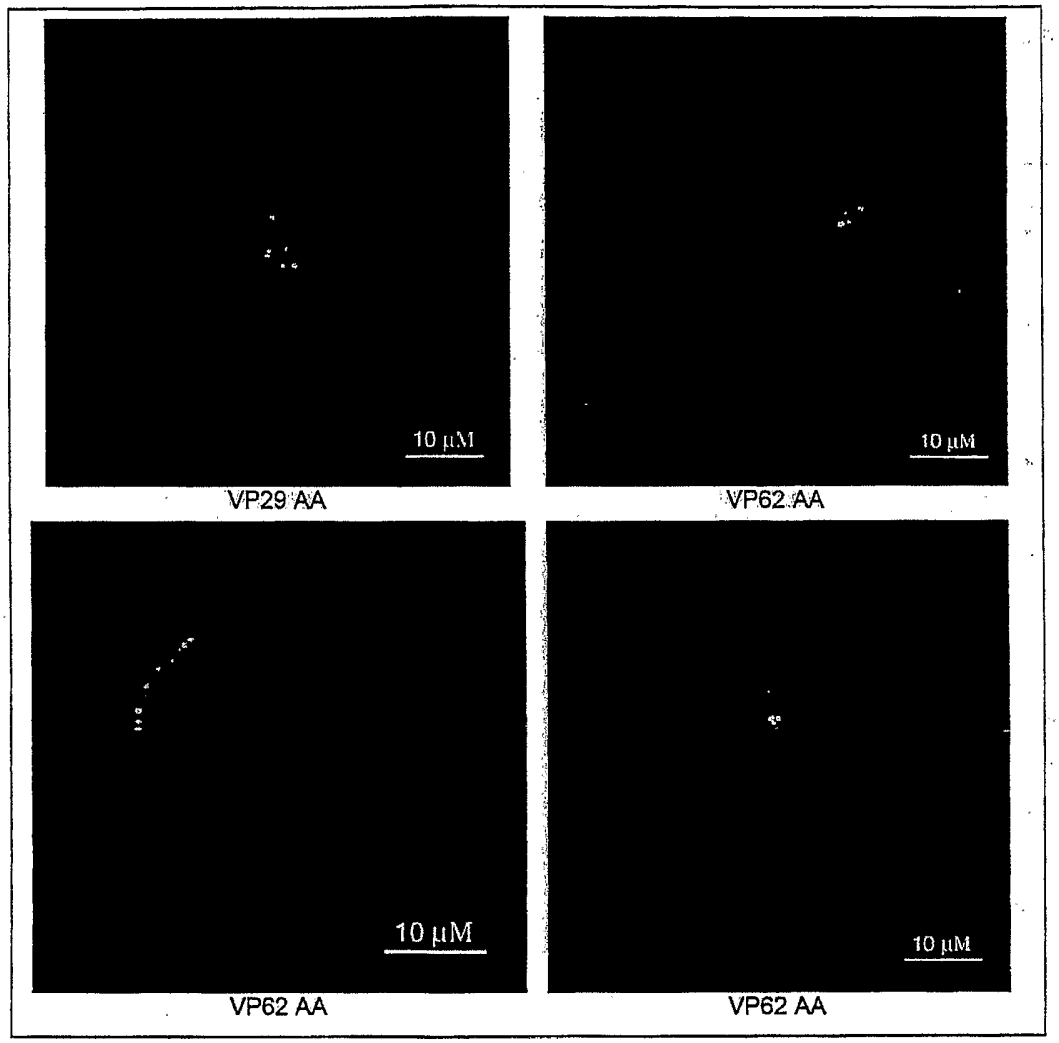


Figure 11.

HXV_{35} Gag (SEQ ID NO:3)

MGQTVTTPLS LTLQHWGDVQ RIASNQSVDV KKRRWVTF S
AEWPTFNVGW PQDGTFNLGV ISQVKSRVF PGPHGHPDQV
PYIVTWEALA YDPPPWVKPF VSPKPPPLPT APVLPPGPSA
QPPSRSALYP ALTLSIKSKP PKPQVLPDSG GPLIDLLTED
PPPYGVQPSS SARENNEEEA ATTSEVSPPS P VSRLRGRR
DPPAADSTTS QAFPLR GGD GQLQYWPFSS SDLYNWKNNN
PSFSEDPGKL TALIESVLIT HQPTWDD QQ LLGTLLTGEE
KQRVLLEAGK AVRGNDGRPT QLPNEVNAAF PLERPDWDYT
TTEGRNHLVL YRQLLLAGLQ NAGRSPTNLA KVKGITQGPN
ESPSAFLERL KEAYRRYTPY DPEDPGQETN VS SFIWQSA
PDIGRKLERL EDLKSKTLGD LVREAEKIFN KRETPEEREE
RIRREIEEKE ERRRAEDEQR ERERDRRRHR E SKLLATVV
IGQRQDRQGG ERRRPQLDKD Q AY KEKGH WAKD PKKPR
GPRGPRPQTS LLTLGD

Antigenic Candidates:

DVKKRRWVTFCSAE (SEQ ID NO:7)

EAGKAVRGNDGRPTQL (SEQ ID NO:8)

KDCPKKPRGPRGPR (SEQ ID NO:9)

Hydropathy Plot

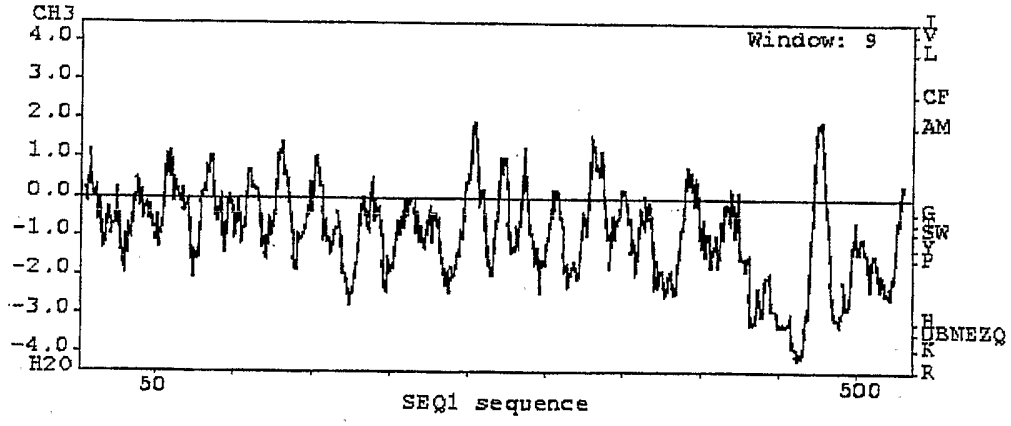
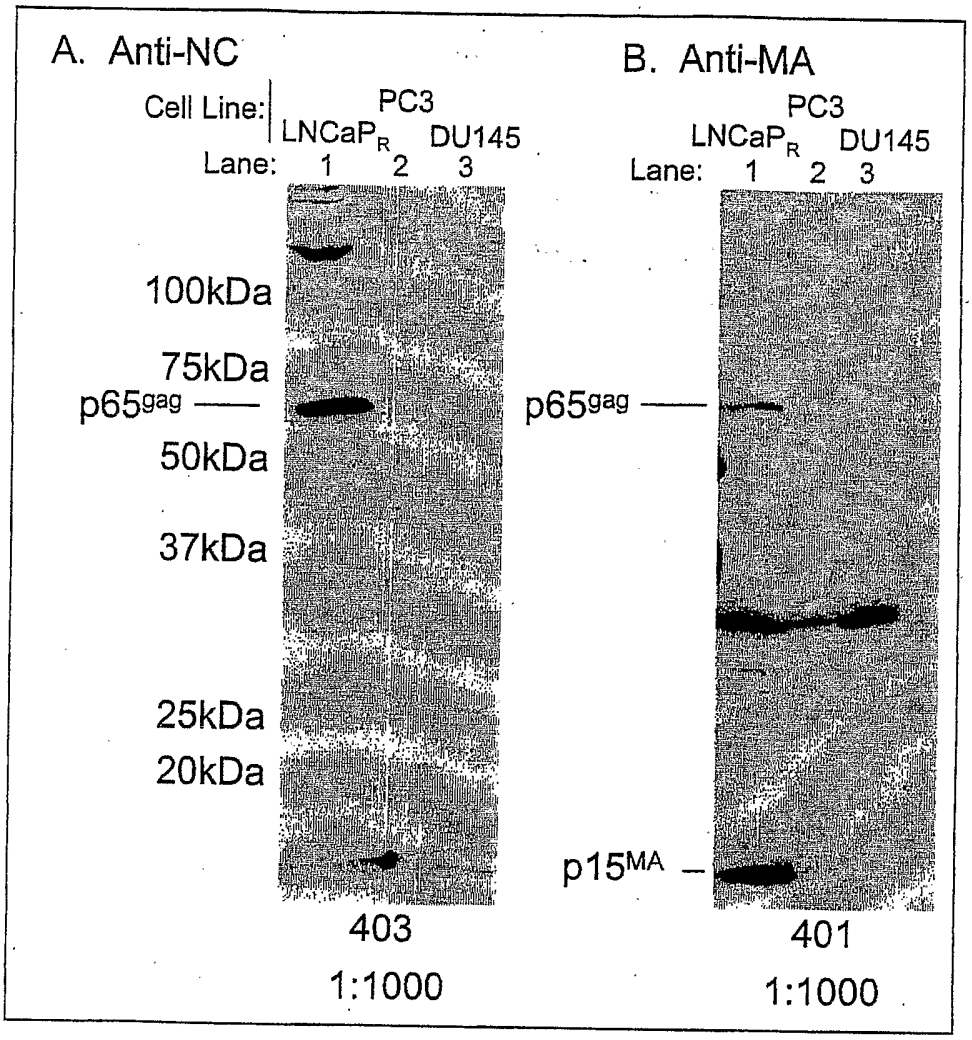


Figure 12.


A. Anti-NC
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PC3
LNCaP$_R$
DU145
Lane: 1 2 3
100kDa
75kDa
p65^{gag}
50kDa
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25kDa
20kDa
403
1:1000
B. Anti-MA
PC3
LNCaP$_R$
DU145
Lane: 1 2 3
p65^{gag}
p15^{MA}
401
1:1000

FIG. 13 Amino acid sequence of HXV_{35} (SEQ ID NO: 2)

MGQTVTTPLSLTLQHWGDVQRIASNQSVDVKKRRWVTFCSAEWPTFNVGW
PQDGTFNLGVISQVKSRVFCPGPHGHPDQVPYIVTWEALAYDPPPWVKPFVSP
KPPPLPTAPVLPPGPSAQPPSRSALYPALTLSIKSKPPKPQVLPDSGGPLIDLLTE
DPPPYGVQPSSSARENNEEEAATTSEVSPPSPMVSRLRGRRDPPAADSTTSQAF
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DDCQQLLGTLLTGEEKQRVLLEAGKAVRGNDGRPTQLPNEVNAAFPLERPD
WDYTTTEGRNHLVLYRQLLLAGLQNAGRSPTNLAKVKGITQGPNESPSAFLE
RLKEAYRRYTPYDPEDPGQETNVSMSFIWQSAPDIGRKLERLEDLKSKTLGDL
VREAEKIFNKRETPEEREERIRREIEEKEERRRAEDEQRERERDRRRHREMSKL
LATVVIGQRQDRQGGERRRPQLDKDQCAYCKEKGHWAKDCPKKPRGPRGP
RPQTSLLTLGDGGQGQEPPPEPRITLKVGGQPVTFLVDTGAQHSVLTQNPGPL
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CLEILAETHGTRPDLTDQPIPDADYTWYTDGSSFLQEGQRRAGAAVTTETEVI
WARALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHVHGEIY
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ADQAARAAAMKAVLETSTLLIEDSTPYTPPHFHYTETDLKRLRELGATYNQT
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LEPRWKGPYTVLLTTPTALKVDGISAWIHAAHVKAATTPPAGTAWKVQRSQ
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TSPDKTQECWLCLVSGPPYYEGVAVLGTYSNHTSAPANCSVTSQHKLTLSEV
TGQGLCIGAVPKTHQALCNTTQKTSDGSYYLASPAGTIWACSTGLTPCLSTTV
LNLTTDYCVLVELWPKVTYHSPNYVYGQFGKKTKYKREPVSLTLALLLGGL
TMGGIAAGVGTGTTALVATKQFEQLQAAIHTDLGALEKSVSALEKSLTSLSE
VVLQNRRGLDLLFLKEGGLCAALKKECCFYADHTGVVRDSMAKLRERLNQR
QKLFESGQGWFEGLFNRSPWFTTLISTIMGPLIVLLLILLFGPCILNRLVQFVKD
RISVVQALVLTQQYHQLKSIDPEEVESRE

FIG. 14 Amino Acid sequence of HXV_{35} GAG (SEQ ID NO: 3):

MGQTVTTPLSLTLQHWGDVQRIASNQSVDVKKRRWVTFCSAEWPTFNVGW
PQDGTFNLGVISQVKSRVFCPGPHGHPDQVPYIVTWEALAYDPPPWVKPFVSP
KPPPLPTAPVLPPGPSAQPPSRSALYPALTLSIKSKPPKPQVLPDSGGPLIDLLTE
DPPPYGVQPSSSARENNEEEAATTSEVSPPSPMVSRLRGRRDPPAADSTTSQAF
PLRMGGDGQLQYWPFSSSDLYNWKNNNPSFSEDPGKLTALIESVLITHQPTW
DDCQQLLGTLLTGEEKQRVLLEAGKAVRGNDGRPTQLPNEVNAAFPLERPD
WDYTTTEGRNHLVLYRQLLLAGLQNAGRSPTNLAKVKGITQGPNESPSAFLE
RLKEAYRRYTPYDPEDPGQETNVSMSFIWQSAPDIGRKLERLEDLKSKTLGDL
VREAEKIFNKRETPEEREERIRREIEEKEERRRAEDEQRERERDRRRHREMSKL
LATVVIGQRQDRQGGERRRPQLDKDQCAYCKEKGHWAKDCPKKPRGPRGP
RPQTSLLTLGD

FIG. 15 Amino Acid sequence of HXV_{35} PRO-POL (SEQ ID NO: 4):

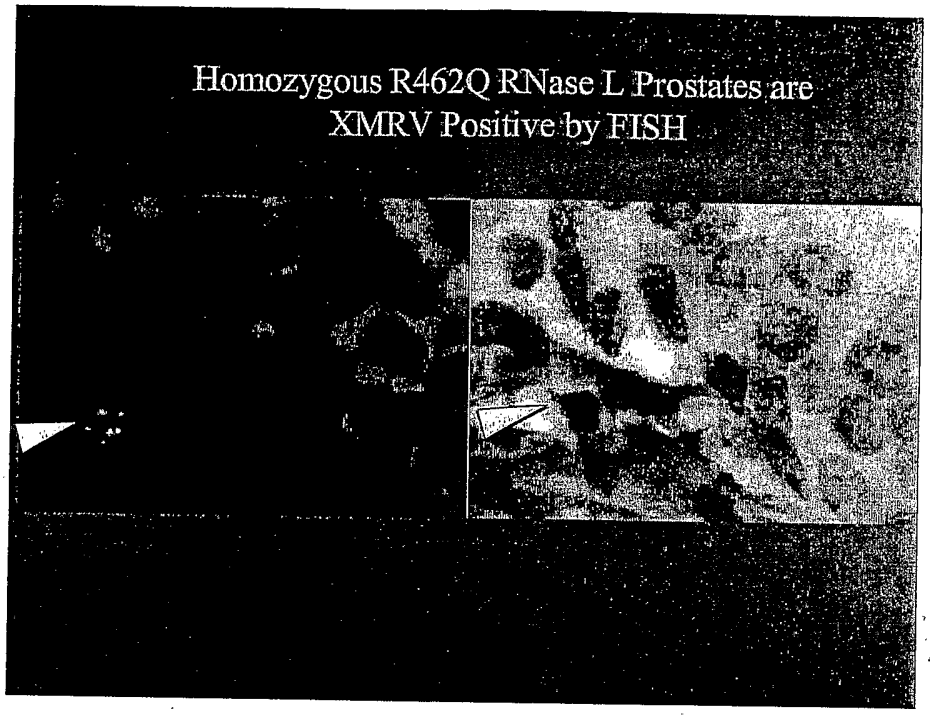
GGQGQEPPPEPRITLKVGGQPVTFLVDTGAQHSVLTQNPGPLSDKSAWVQGA
TGGKRYRWTTDRKVHLATGKVTHSFLHVPDCPYPLLGRDLLTKLKAQIHFEG
SGAQVVGPMGQPLQVLTLNIENKYRLHETSKEPDVPLGSTWLSDFPQAWAET
GGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPC
QSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSH
QWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNS
PTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSEQDCQRGTRALLQTLG
NLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQ
LREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQAL
LTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVA
AGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSN
ARMTHYQAMLLDTDRVQFGPVVALNPATLLPLPEKEAPHDCLEILAETHGTR
PDLTDQPIPDADYTWYTDGSSFLQEGQRRAGAAVTTETEVIWARALPAGTSA
QRAELIALTQALKMAEGKKLNVYTDSRYAFATAHVHGEIYRRRGLLTSEGRE
IKNKNEILALLKALFLPKRLSIIHCPGHQKGNSAEARGNRMADQAAREAAMK
AVLETSTLLIEDSTPYTPPHFHYTETDLKRLRELGATYNQTKGYWVLQGKPV
MPDQSVFELLDSLHRLTHPSPQKMKALLDREESPYYMLNRDRTIQYVTETCT
ACAQVNASKAKIGAGVRVRGHRPGTHWEVDFTEVKPGLYGYKYLLVFVDTF
SGWVEAFPTKRETAKVVSKKLLEDIFPRFEMPQVLGSDNGPAFASQVSQSVA
DLLGIDWKLHCAYKPQSSGQVERINKTIKETLTKLTLASGTKDWVLLLPLALY
RARNTPGPHGLTPYEILYGAPPPLVNFHNPEMSKLTNSPSLQAHLQALQAVQQ
EVWKPLAAAYQDQLDQPVIPHPFRVGDAVWVRRHQTKNLEPRWKGPYTVL
LTTPTALKVDGISAWIHAAHVKAATTPPAGTAWKVQRSQNPLKIRLTRGAP

FIG. 16 Amino acid sequence of HXV_{35} ENV (SEQ ID NO: 5):

MESPAFSKPLKDKINPWGPLIIMGILVRAGASVQRDSPHQVFNVTWKITNLMT
GQTANATSLLGTMTDTFPKLYFDLCDLVGDNWDDPEPDIGDGCRSPGGRKRT
RLYDFYVCPGHTVLTGCGGPREGYCGKWGCETTGQAYWKPSSSWDLISLKR
GNTPKGQGPCFDSSVGSGSIQGATPGGRCNPLVLEFTDAGKRASWDAPKTWG
LRLYRSTGADPVTLFSLTRQVLNVGPRVPIGPNPVITEQLPPSQPVQIMLPRPPR
PPPSGAASMVPGAPPPSQQPGTGDRLLNLVEGAYQALNLTSPDKTQECWLCL
VSGPPYYEGVAVLGTYSNHTSAPANCSVTSQHKLTLSEVTGQGLCIGAVPKT
HQALCNTTQKTSDGSYYLASPAGTIWACSTGLTPCLSTTVLNLTTDYCVLVEL
WPKVTYHSPNYVYGQFGKKTKYKREPVSLTLALLLGGLTMGGIAAGVGTGT
TALVATKQFEQLQAAIHTDLGALEKSVSALEKSLTSLSEVVLQNRRGLDLLFL
KEGGLCAALKKECCFYADHTGVVRDSMAKLRERLNQRQKLFESGQGWFEG
LFNRSPWFTTLISTIMGPLIVLLLILLFGPCILNRLVQFVKDRISVVQALVLTQQ
YHQLKSIDPEEVESRE

FIG. 17 Sequence from LNCaP RNA RTPCR product. (7084-7750bp) (SEQ ID NO: 6)

aaaagagagcccggtgtcattaactctggccctgtctgttgggaggacttactatgggcggcatagctccaggagttggaa
cagggactacagccctagtggccaccaaacaattcgagcagctccaggcagccatacatacagaccttggggccttagaa
aaaatcagtcagtgccctagaaaagtctctgacctcgttgtctgaggtggtcctacagaaccggaggggattagatctactgt
tcctaaaagaaggaggattatgtgctgccctaaaagaagaatgctgtttttacgcggaccacactggcgtagtaagagatag
catggcaaagctaagagaaaggttaaaccagagacaaaaattgttcgaatcaggacaagggtggtttgagggactgtttaa
caggtccccatggttcacgaccctgatatccaccaccattatgggccctctgatagtacttttattaatcctacttttcggaccct
gtattctcaaccgcttggtccagtttgtaaaagacacagaatttcggtagtgcaggccctggttctgacccagcagtatcacc
aactcaaatcaatagatccagaagaagtggaatcacgtgaataaaagattttattcagtttccagaaagagggggggaatgaa
agacccccccataaggc



Analysis of prostate from patient VP62. Left panel: Immunohistochemistry (IHC)(red) with a mouse anti-cytokeratin AE1/AE3 (20:1 mixture of AE1 to AE3) monoclonal Ab cocktail from Roche. The anti-keratin AE1 Ab recognizes the 56.5, 50, 50', 48 and 40 kDa keratins of the acidic subfamily. The anti-keratin AE3 Ab recognizes all 6 members of the basic subfamily. The IHC (red) labels prostate epithelial cells. The green label is FISH for HXV35 env probe as described in the legend to Fig. 9. Blue is DAPI staining of nuclei. Right panel. Hematoxylin and eosin staining.

Figure 18A

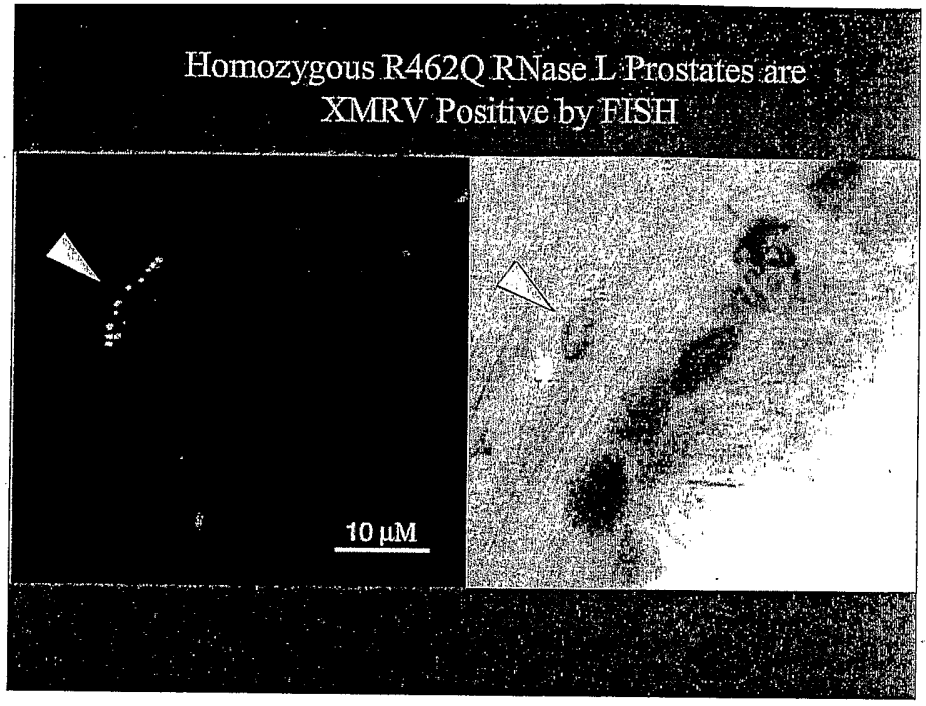

Homozygous R462Q RNase L Prostates are
XMRV Positive by FISH
10 µM

Figure 18B

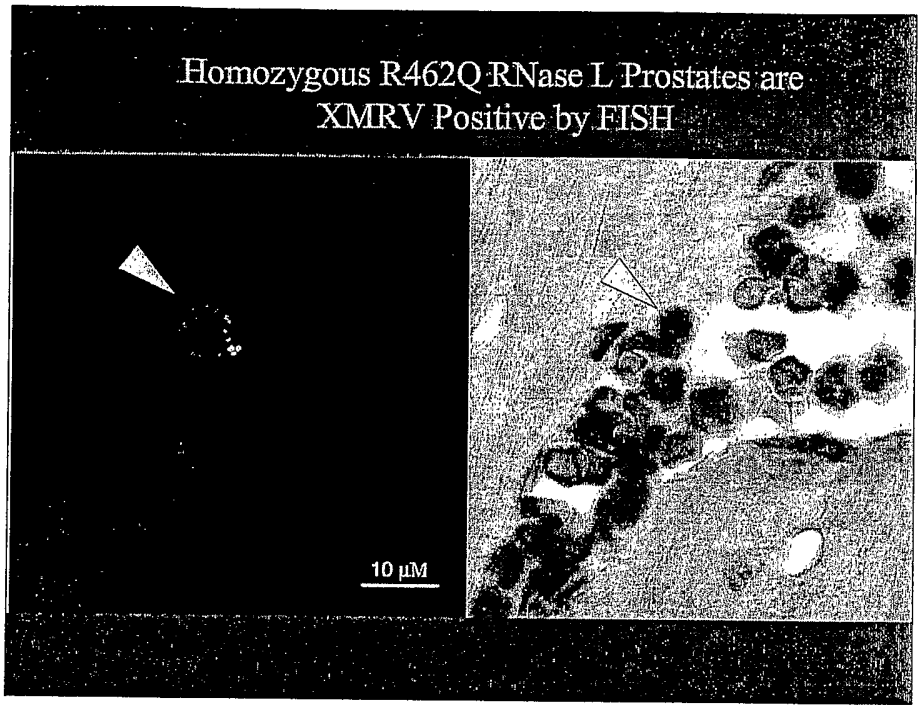

Homozygous R462Q RNase L Prostates are
XMRV Positive by FISH
10 µM

Figure 18C

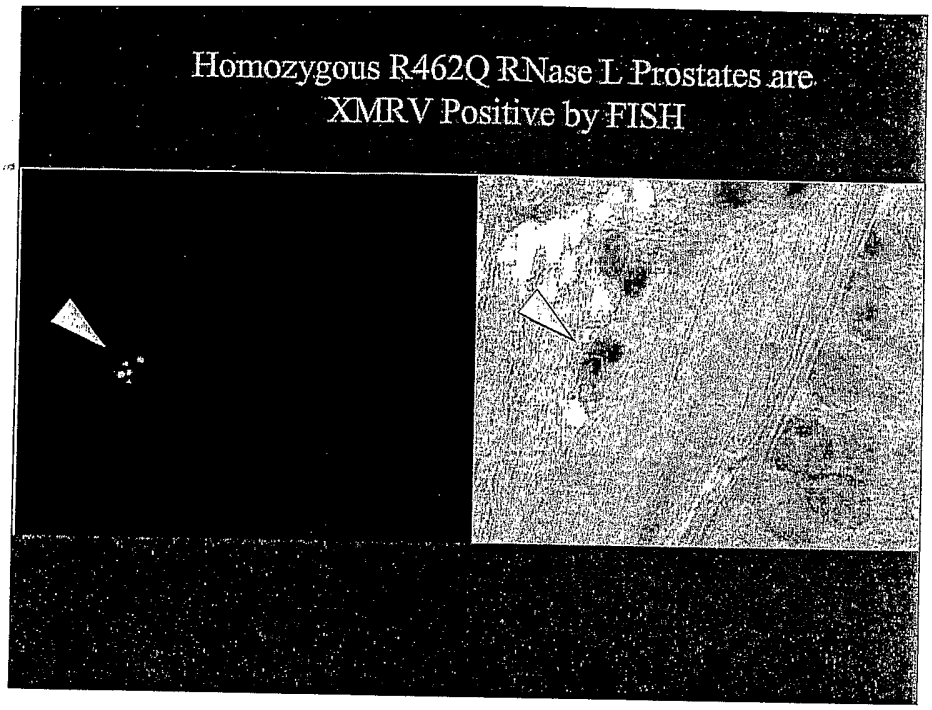
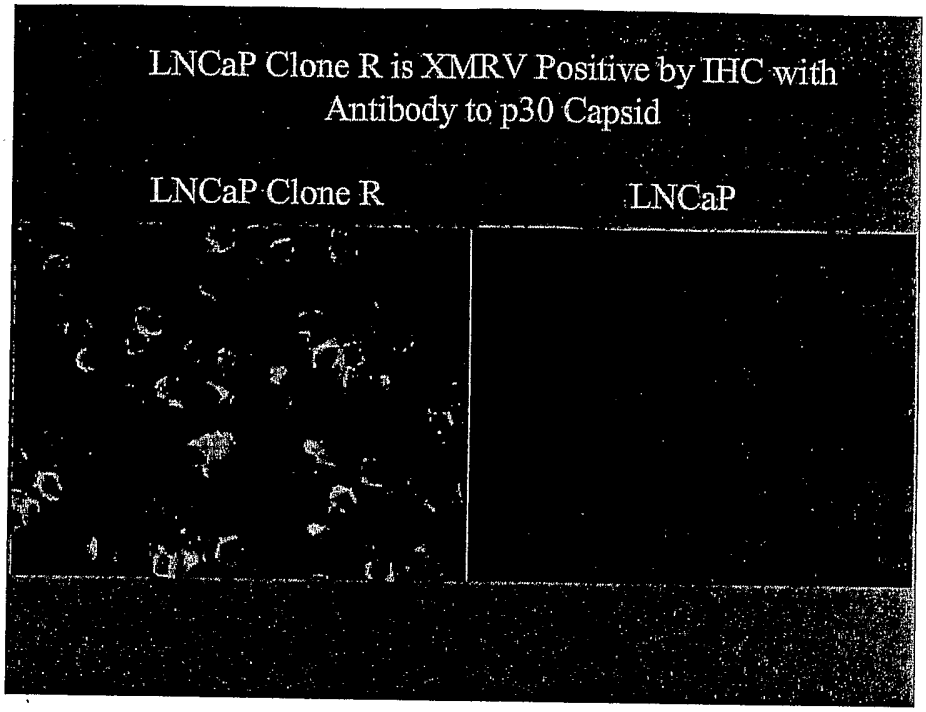
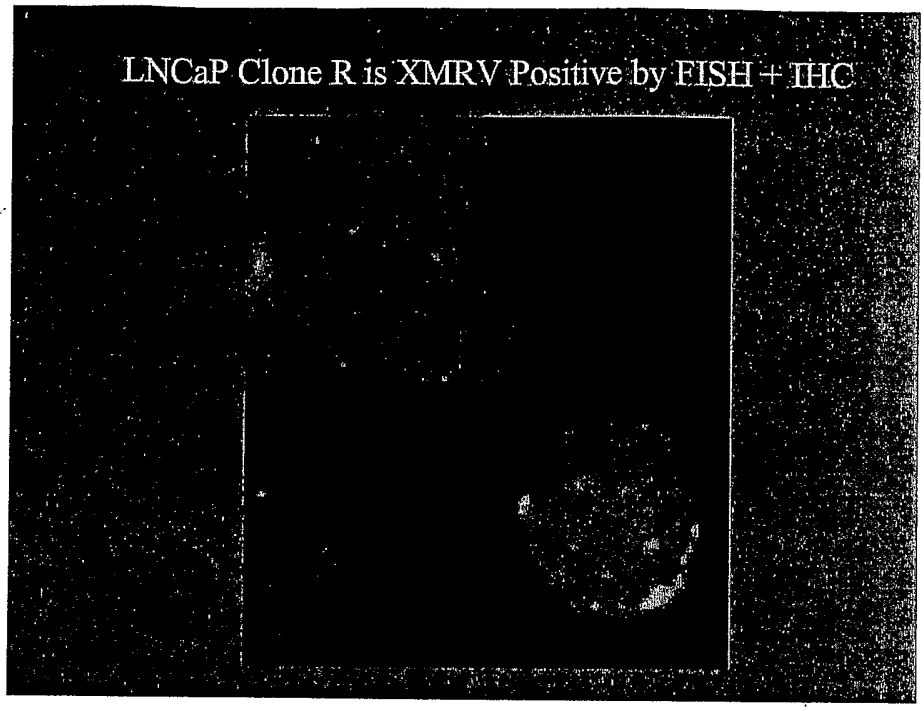

Homozygous R462Q RNase L Prostates are
XMRV Positive by FISH

Figure 18D



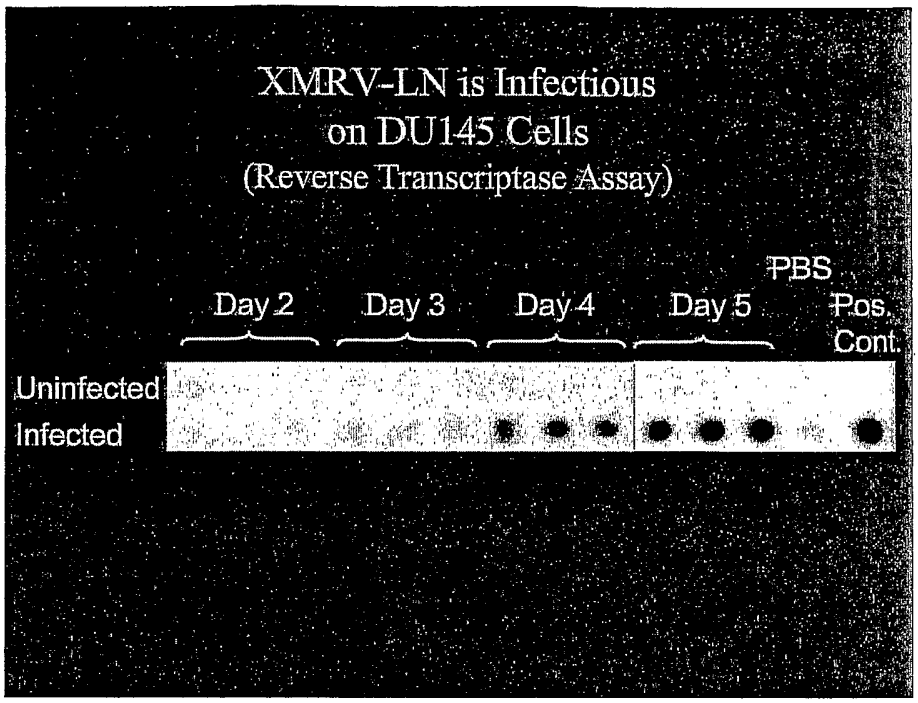
Analysis of LNCaP, clone R. Right panel: immunohistochemistry with specific antiserum prepared in goats to Rauscher mouse leukemia virus p30 protein (ATCC, catalog no. VR-1564AS-Gt) showing labeling of HXV gag protein plus DAPI (blue) staining of nuclei. Right panel: DAPI staining of nuclei.

Figure 19



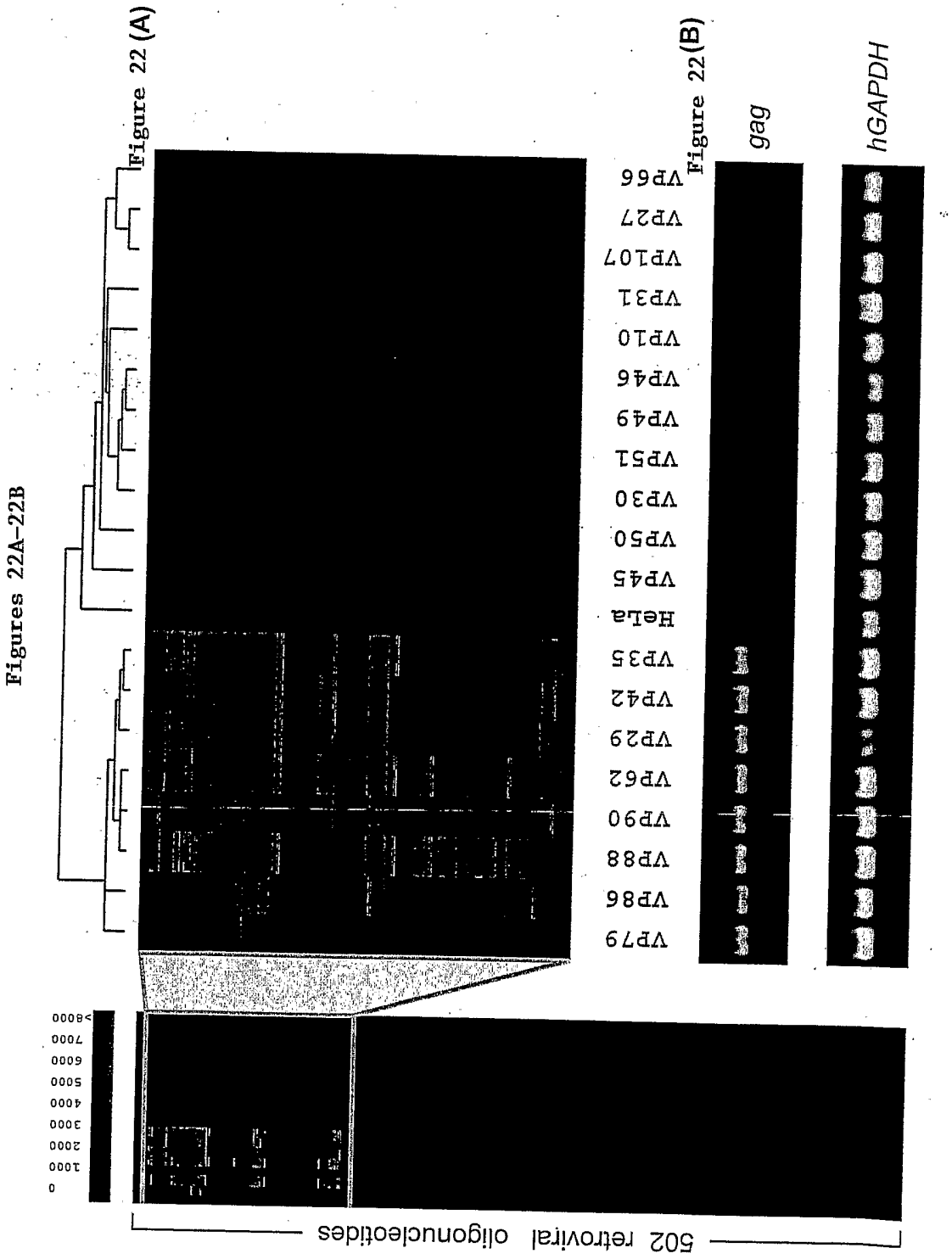
Doubling labeling by IHC and FISH of HXV in LNCaP infected cells. Immunohistochemistry with specific antiserum prepared in goats to Rauscher mouse leukemia virus p30 protein (ATCC, catalog no. VR-1564AS-Gt) showing labeling of HXV gag protein plus DAPI (blue) staining of nuclei plus FISH labeling(green) of virus nucleic acid.

Figure 20



The virus (XMRV-LN) produced by LNCaP cells is infectious when used to infect DU145 human prostate cancer cells. DU145 cellswere infected with 500 uls of LNCaP infected supernatant for 3 hours in the presence of 8ug/ml polybrene, -FBS. Virus was monitored by reverse transcriptase assay.

Figure 21


Figures 22A-22B
Figure 22 (A)
Figure 22(B)
gag
hGAPDH
VP79
VP86
VP88
VP90
VP62
VP29
VP42
VP35
HeLa
VP45
VP50
VP30
VP51
VP49
VP46
VP10
VP31
VP107
VP27
VP66
502 retroviral oligonucleotides
0
1000
2000
3000
4000
5000
6000
7000
>8000

Figures 23A–23C

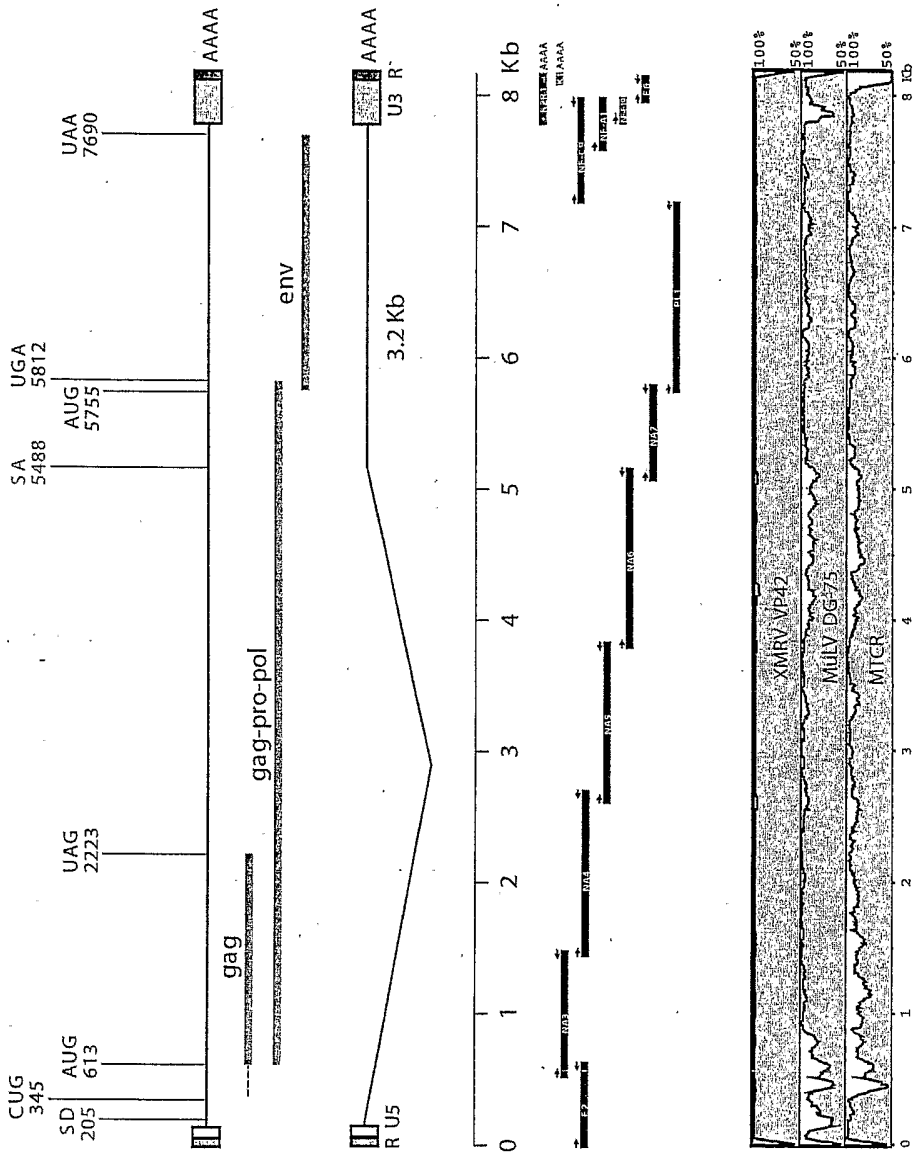
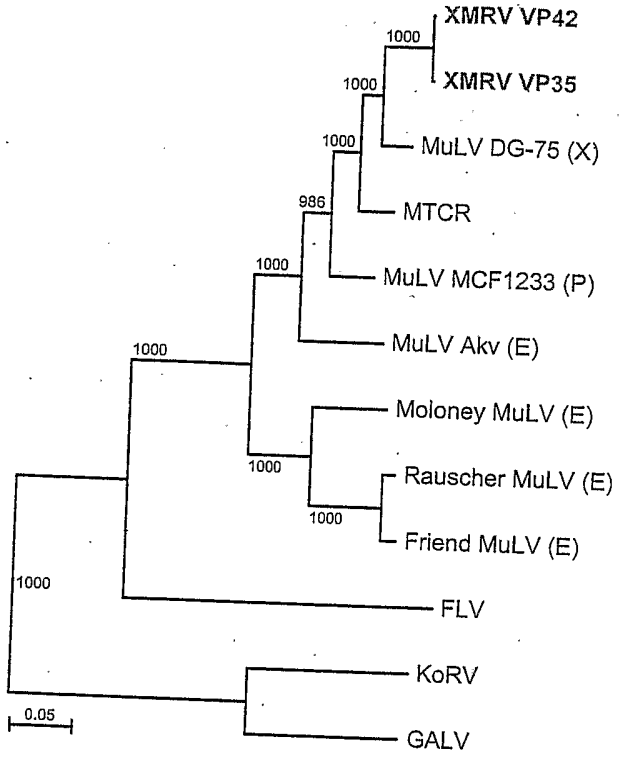


Figure 23 A

Figure 23 B

Figure 23 C

Figure 24



Complete genomes

Figure 25

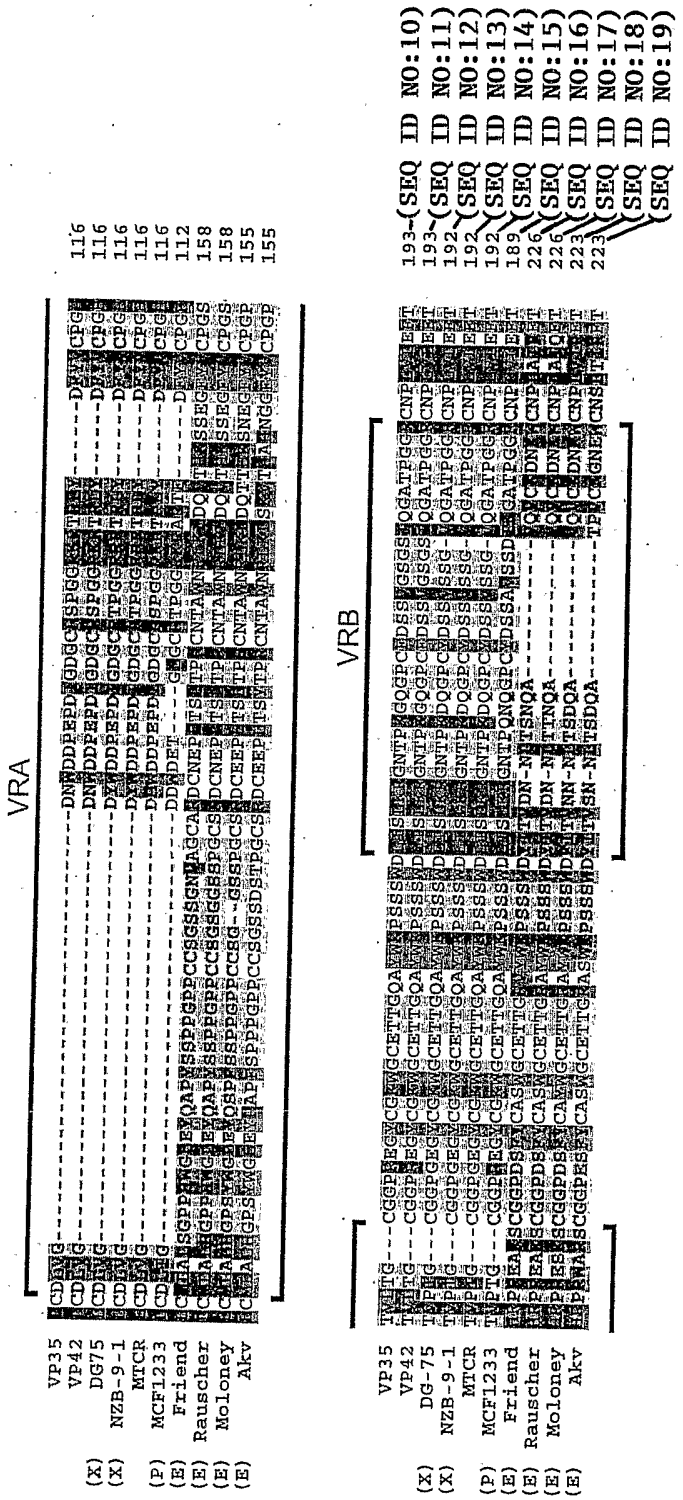

VRA
VRB
VP35
VP42
DG75
NZB-9-1
MTCR
MCF1233
Friend
Rauscher
Moloney
Akv
(X)
(P)
(E)
193-(SEQ ID NO:10)
193-(SEQ ID NO:11)
192-(SEQ ID NO:12)
192-(SEQ ID NO:13)
189-(SEQ ID NO:14)
226-(SEQ ID NO:15)
226-(SEQ ID NO:16)
223-(SEQ ID NO:17)
223-(SEQ ID NO:18)
(SEQ ID NO:19)

Figure 26

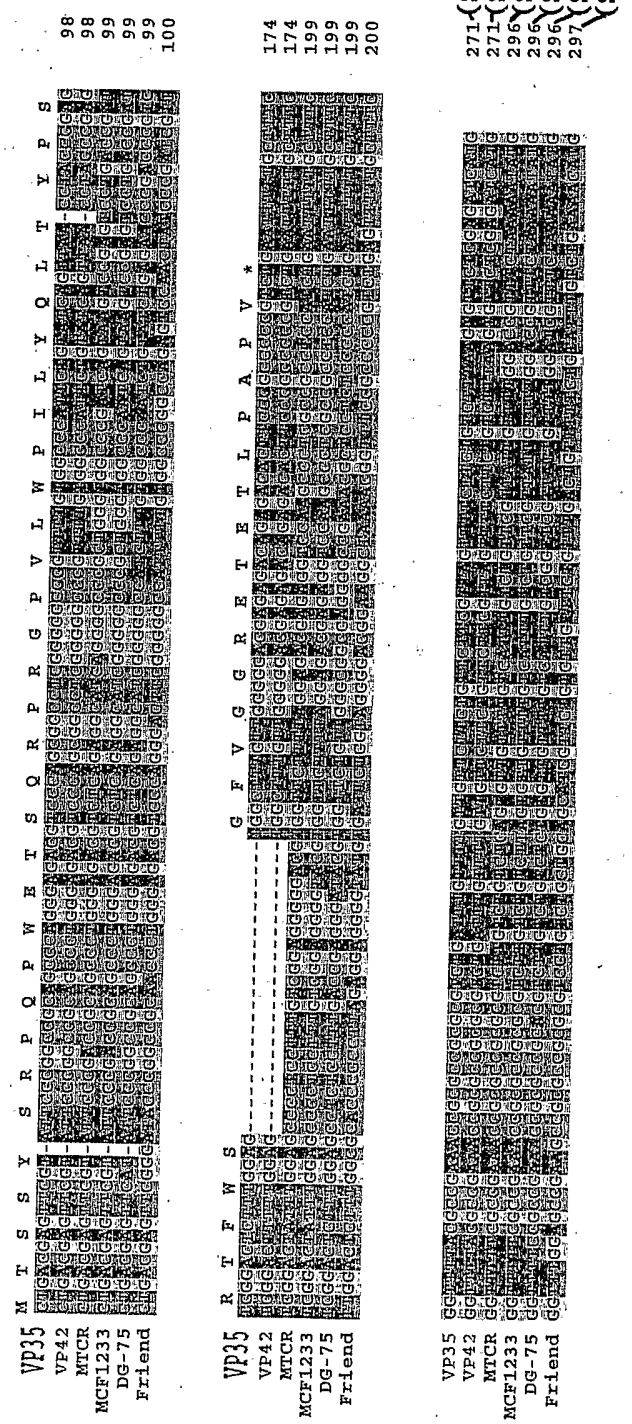

VP35 M T S S Y S R P Q P W E T S Q R P R G P V L W P I L Y Q L T Y P S
VP42
MTCR
MCF1233
DG-75
Friend
98
98
99
99
99
100
VP35 R T F W S G F V G G R E T E T L P A P V *
VP42
MTCR
MCF1233
DG-75
Friend
174
174
199
199
199
200
VP35
VP42
MTCR
MCF1233
DG-75
Friend
271 (SEQ ID NO:20)
271 (SEQ ID NO:21)
296 (SEQ ID NO:22)
296 (SEQ ID NO:23)
296 (SEQ ID NO:24)
297 (SEQ ID NO:25)

Figure 27A–27B

Figure 27 A

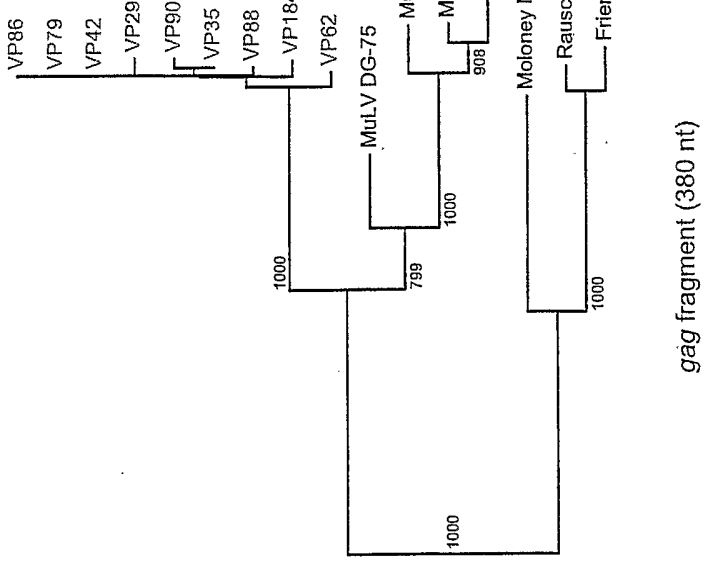


Figure 27 B

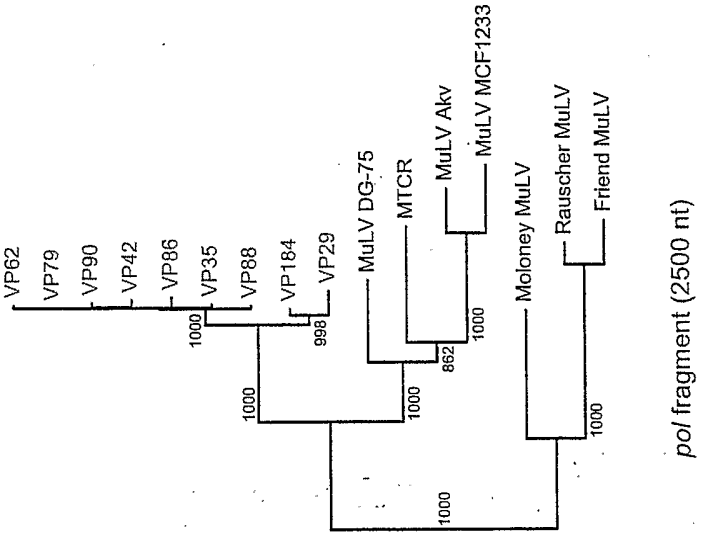


Figure 28A

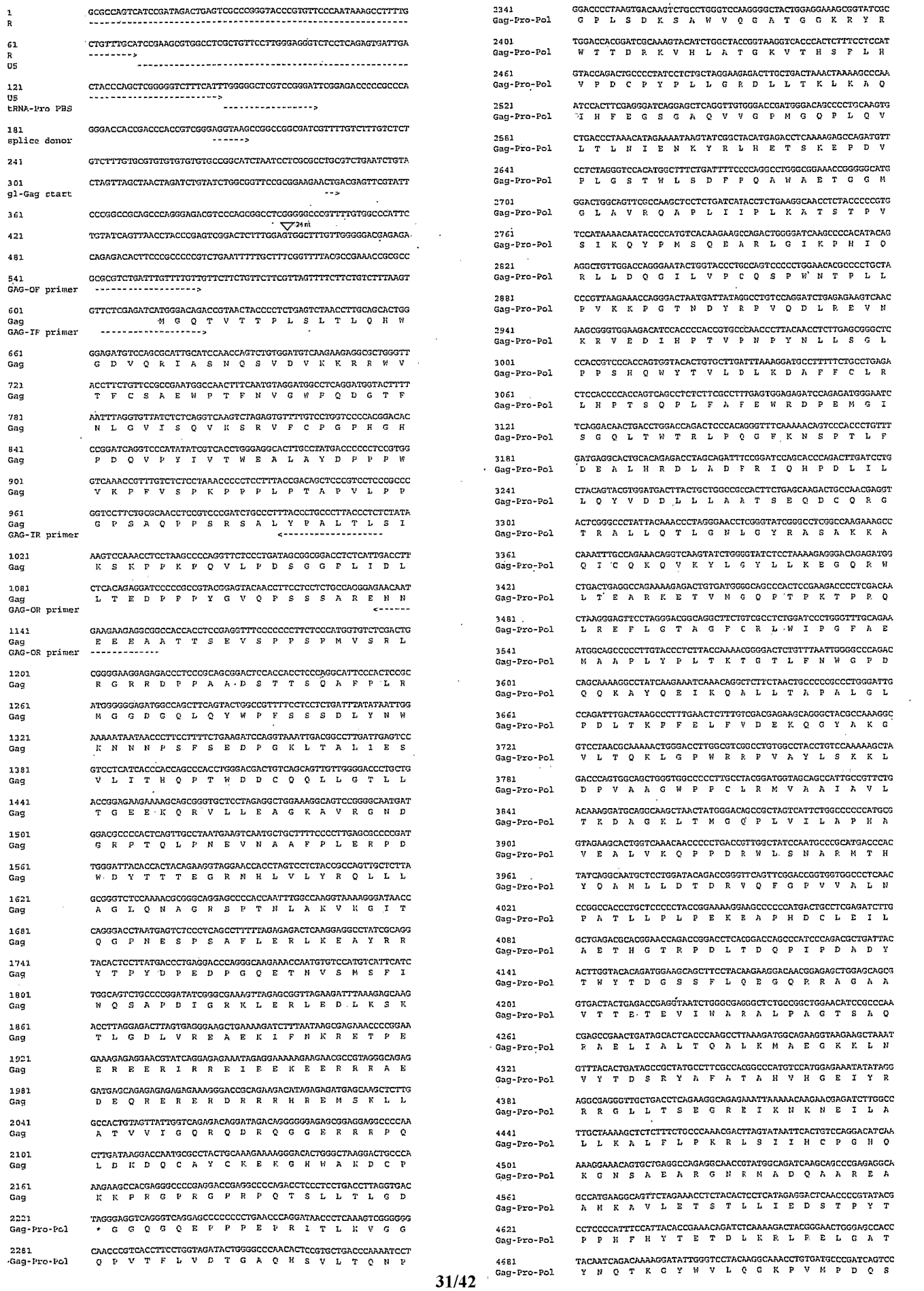
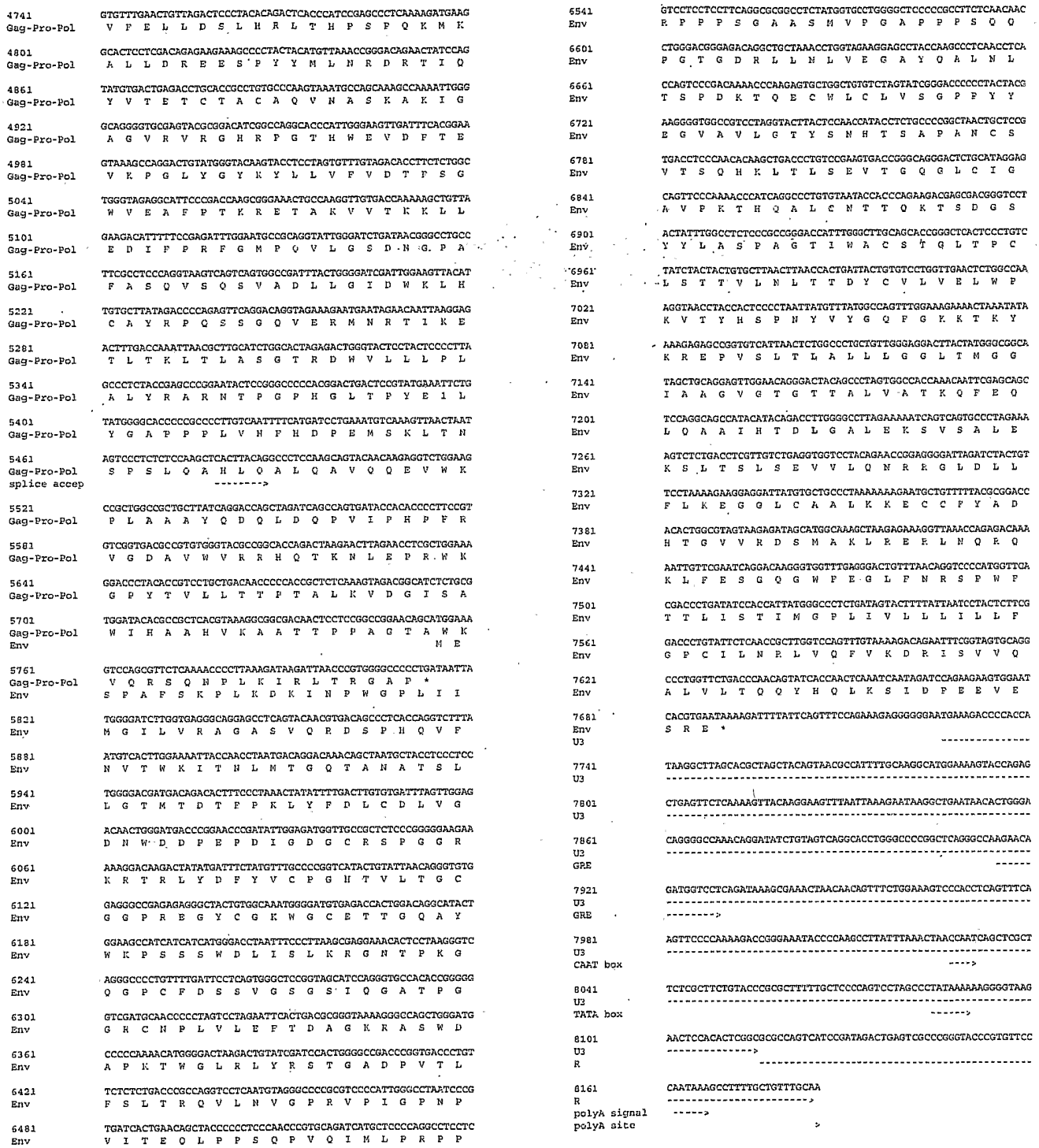

1 GCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCCCAATAAAGCCTTTTG
R
61 CTGTTTGCATCCGAAGCGTGGCCTCGCTGTTCCTTGGGAGGGTCTCCTCAGAGTGATTGA
R
U5
121 CTACCCAGCTCGGGGGTCTTTCATTTGGGGGCTCGTCCGGGATTCGGAGACCCCGCCCA
U5
tRNA-Pro PBS
181 GGGACCACCGACCCACCGTCGGGAGGTAAGCGGCCGGCGATCGTTTTGTCTTTGTCTCT
splice donor
241 GTCTTTGTGCGTGTGTGTGTGCCGGCATCTAATCCTCGCGCCTGCGTCTGAATCTGTA
301 CTAGTTAGCTAACTAGATCTGTATCTGGCGGTTCCGCGGAAGAACTGACGAGTTCGTATT
gl-Gag start
361 CCCGGCCGCAGCCCAGGGAGACGTCCCAGCGGCCTCGGGGGCCCGTTTTGTGGCCCATTC
421 TGTATCAGTTAACCTACCCGAGTCGGACTCTTTGGAGTGGCTTTGTTGGGGGACGAGAGA
481 CAGAGACACTTCCCGCCCCCGTCTGAATTTTTGCTTTCGGTTTTACGCCGAAACCGCGCC
541 GCGCGTCTGATTTGTTTTGTTGTTCTTCTGTTCTTCGTTAGTTTTCTTCTGTCTTTAAGT
GAG-OF primer
601 GTTCTCGAGATCATGGGACAGACCGTAACTACCCCTCTGAGTCTAACCTTGCAGCACTGG
Gag M G Q T V T T P L S L T L Q H W
GAG-IF primer
661 GGAGATGTCCAGCGCATTGCATCCAACCAGTCTGTGGATGTCAAGAAGAGGCGCTGGGTT
Gag G D V Q R I A S N Q S V D V K K R R W V
721 ACCTTCTGTTCCGCCGAATGGCCAACTTTCAATGTAGGATGGCCTCAGGATGGTACTTTT
Gag T F C S A E W P T F N V G W P Q D G T F
781 AATTTAGGTGTTATCTCTCAGGTCAAGTCTAGAGTGTTTTGTCCTGGTCCCCACGGACAC
Gag N L G V I S Q V K S R V F C P G P H G H
841 CCGGATCAGGTCCCATATATCGTCACCTGGGAGGCACTTGCCTATGACCCCCTCCGTGG
Gag P D Q V P Y I V T W E A L A Y D P P P W
901 GTCAAACCGTTTGTCTCTCCTAAACCCCCTCCTTTACCGACAGCTCCCGTCCTCCCGCCC
Gag V K P F V S P K P P P L P T A P V L P P
961 GGTCCTTCTGCGCAACCTCCGTCCCGATCTGCCCTTTACCCTGCCCTTACCCTCTCTATA
Gag G P S A Q P P S R S A L Y P A L T L S I
GAG-IR primer
1021 AAGTCCAAACCTCCTAAGCCCCAGGTTCTCCCTGATAGCGGCGGACCTCTCATTGACCTT
Gag K S K P P K P Q V L P D S G G P L I D L
1081 CTCACAGAGGATCCCCCGCCGTACGGAGTACAACCTTCCTCCTCTGCCAGGGAGAACAAT
Gag L T E D P P P Y G V Q P S S S A R E N N
GAG-OR primer
1141 GAAGAAGAGGCGGCCACCACCTCCGAGGTTTCCCCCCCTTCTCCCATGGTGTCTCGACTG
Gag E E E A A T T S E V S P P S P M V S R L
GAG-OR primer
1201 CGGGGAAGGAGAGACCCTCCCGCAGCGGACTCCACCACCTCCCAGGCATTCCCACTCCGC
Gag R G R R D P P A A D S T T S Q A F P L R
1261 ATGGGGGGAGATGGCCAGCTTCAGTACTGGCCGTTTTCCTCCTCTGATTTATATAATTGG
Gag M G G D G Q L Q Y W P F S S S D L Y N W
1321 AAAAATAATAACCCTTCCTTTTCTGAAGATCCAGGTAAATTGACGGCCTTGATTGAGTCC
Gag K N N N P S F S E D P G K L T A L I E S
1381 GTCCTCATCACCCACCAGCCCACCTGGGACGACTGTCAGCAGTTGTTGGGGACCCTGCTG
Gag V L I T H Q P T W D D C Q Q L L G T L L
1441 ACCGGAGAAGAAAAGCAGCGGGTGCTCCTAGAGGCTGGAAAGGCAGTCCGGGGCAATGAT
Gag T G E E K Q R V L L E A G K A V R G N D
1501 GGACGCCCCACTCAGTTGCCTAATGAAGTCAATGCTGCTTTTCCCCTTGAGCGCCCCGAT
Gag G R P T Q L P N E V N A A F P L E R P D
1561 TGGGATTACACCACTACAGAAGGTAGGAACCACCTAGTCCTCTACCGCCAGTTGCTCTTA
Gag W D Y T T T E G R N H L V L Y R Q L L L
1621 GCGGGTCTCCAAAACGCGGGCAGGAGCCCCACCAATTTGGCCAAGGTAAAAGGGATAACC
Gag A G L Q N A G R S P T N L A K V K G I T
1681 CAGGGACCTAATGAGTCTCCCTCAGCCTTTTTAGAGAGACTCAAGGAGGCCTATCGCAGG
Gag Q G P N E S P S A F L E R L K E A Y R R
1741 TACACTCCTTATGACCCTGAGGACCCAGGGCAAGAAACCAATGTGTCCATGTCATTCATC
Gag Y T P Y D P E D P G Q E T N V S M S F I
1801 TGGCAGTCTGCCCCGGATATCGGGCGAAAGTTAGAGCGGTTAGAAGATTTAAAGAGCAAG
Gag W Q S A P D I G R K L E R L E D L K S K
1861 ACCTTAGGAGACTTAGTGAGGGAAGCTGAAAAGATCTTTAATAAGCGAGAAACCCCGGAA
Gag T L G D L V R E A E K I F N K R E T P E
1921 GAAAGAGAGGAACGTATCAGGAGAGAAATAGAGGAAAAAGAAGAACGCCGTAGGGCAGAG
Gag E R E E R I R R E I E E K E E R R R A E
1981 GATGAGCAGAGAGAGAGAGAAAGGACCGCAGAAGACATAGAGAGATGAGCAAGCTCTTG
Gag D E Q R E R E R D R R R H R E M S K L L
2041 GCCACTGTAGTTATTGGTCAGAGACAGGATAGACAGGGGGGAGAGCGGAGGAGGCCCCAA
Gag A T V V I G Q R Q D R Q G G E R R R P Q
2101 CTTGATAAGGACCAATGCGCCTACTGCAAAGAAAAGGGACACTGGGCTAAGGACTGCCCA
Gag L D K D Q C A Y C K E K G H W A K D C P
2161 AAGAAGCCACGAGGGCCCCGAGGACCGAGGCCCCAGACCTCCCTCCTGACCTTAGGTGAC
Gag K K P R G P R G P R P Q T S L L T L G D
2221 TAGGGAGGTCAGGGTCAGGAGCCCCCCCCTGAACCCAGGATAACCCTCAAAGTCGGGGGG
Gag-Pro-Pol * G G Q G Q E P P P E P R I T L K V G G
2281 CAACCCGTCACCTTCCTGGTAGATACTGGGGCCCAACACTCCGTGCTGACCCAAAATCCT
Gag-Pro-Pol Q P V T F L V D T G A Q H S V L T Q N P
2341 GGACCCCTAAGTGACAAGTCTGCCTGGGTCCAAGGGGCTACTGGAGGAAAGCGGTATCGC
Gag-Pro-Pol G P L S D K S A W V Q G A T G G K R Y R
2401 TGGACCACGGATCGCAAAGTACATCTGGCTACCGGTAAGGTCACCCACTCTTTCCTCCAT
Gag-Pro-Pol W T T D R K V H L A T G K V T H S F L H
2461 GTACCAGACTGCCCCTATCCTCTGCTAGGAAGAGACTTGCTGACTAAACTAAAAGCCCAA
Gag-Pro-Pol V P D C P Y P L L G R D L L T K L K A Q
2521 ATCCACTTCGAGGGATCAGGAGCTCAGGTTGTGGGACCGATGGGACAGCCCCTGCAAGTG
Gag-Pro-Pol I H F E G S G A Q V V G P M G Q P L Q V
2581 CTGACCCTAAACATAGAAATAAGTATCGGCTACATGAGACCTCAAAAGAGCCAGATGTT
Gag-Pro-Pol L T L N I E N K Y R L H E T S K E P D V
2641 CCTCTAGGGTCCACATGGCTTTCTGATTTTCCCCAGGCCTGGGCGGAAACCGGGGGCATG
Gag-Pro-Pol P L G S T W L S D F P Q A W A E T G G M
2701 GGACTGGCAGTTCGCCAAGCTCCTCTGATCATACCTCTGAAGGCAACCTCTACCCCCGTG
Gag-Pro-Pol G L A V R Q A P L I I P L K A T S T P V
2761 TCCATAAAACAATACCCCATGTCACAAGAAGCCAGACTGGGGATCAAGCCCCACATACAG
Gag-Pro-Pol S I K Q Y P M S Q E A R L G I K P H I Q
2821 AGGCTGTTGGACCAGGGAATACTGGTACCCTGCCAGTCCCCCTGGAACACGCCCCTGCTA
Gag-Pro-Pol R L L D Q G I L V P C Q S P W N T P L L
2881 CCCGTTAAGAAACCAGGGACTAATGATTATAGGCCTGTCCAGGATCTGAGAGAAGTCAAC
Gag-Pro-Pol P V K K P G T N D Y R P V Q D L R E V N
2941 AAGCGGGTGGAAGACATCCACCCCACCGTGCCCAACCCTTACAACCTCTTGAGCGGGCTC
Gag-Pro-Pol K R V E D I H P T V P N P Y N L L S G L
3001 CCACCGTCCCACCAGTGGTACACTGTGCTTGATTTAAAGGATGCCTTTTTCTGCCTGAGA
Gag-Pro-Pol P P S H Q W Y T V L D L K D A F F C L R
3061 CTCCACCCCACCAGTCAGCCTCTCTTCGCCTTTGAGTGGAGAGATCCAGAGATGGGAATC
Gag-Pro-Pol L H P T S Q P L F A F E W R D P E M G I
3121 TCAGGACAACTGACCTGGACCAGACTCCCACAGGGTTTCAAAAACAGTCCCACCCTGTTT
Gag-Pro-Pol S G Q L T W T R L P Q G F K N S P T L F
3181 GATGAGGCACTGCACAGAGACCTAGCAGATTTCCGGATCCAGCACCCAGACTTGATCCTG
Gag-Pro-Pol D E A L H R D L A D F R I Q H P D L I L
3241 CTACAGTACGTGGATGACTTACTGCTGGCCGCCACTTCTGAGCAAGACTGCCAACGAGGT
Gag-Pro-Pol L Q Y V D D L L L A A T S E Q D C Q R G
3301 ACTCGGGCCCTATTACAAACCCTAGGGAACCTCGGGTATCGGGCCTCGGCCAAGAAAGCC
Gag-Pro-Pol T R A L L Q T L G N L G Y R A S A K K A
3361 CAAATTTGCCAGAAACAGGTCAAGTATCTGGGGTATCTCCTAAAAGAGGGACAGAGATGG
Gag-Pro-Pol Q I C Q K Q V K Y L G Y L L K E G Q R W
3421 CTGACTGAGGCCAGAAAAGAGACTGTGATGGGGCAGCCCACTCCGAAGACCCCTCGACAA
Gag-Pro-Pol L T E A R K E T V M G Q P T P K T P R Q
3481 CTAAGGGAGTTCCTAGGGACGGCAGGCTTCTGTCGCCTCTGGATCCCTGGGTTTGCAGAA
Gag-Pro-Pol L R E F L G T A G F C R L W I P G F A E
3541 ATGGCAGCCCCCTTGTACCCTCTTACCAAAACGGGGACTCTGTTTAATTGGGGCCCAGAC
Gag-Pro-Pol M A A P L Y P L T K T G T L F N W G P D
3601 CAGCAAAAGGCCTATCAAGAAATCAAACAGGCTCTTCTAACTGCCCCGCCCTGGGATTG
Gag-Pro-Pol Q Q K A Y Q E I K Q A L L T A P A L G L
3661 CCAGATTTGACTAAGCCCTTTGAACTCTTTGTCGACGAGAAGCAGGGCTACGCCAAAGGC
Gag-Pro-Pol P D L T K P F E L F V D E K Q G Y A K G
3721 GTCCTAACGCAAAAACTGGGACCTTGGCGTCGGCCTGTGGCCTACCTGTCCAAAAAGCTA
Gag-Pro-Pol V L T Q K L G P W R K P V A Y L S K K L
3781 GACCCAGTGGCAGCTGGGTGGCCCCCTTGCCTACGGATGGTAGCAGCCATTGCCGTTCTG
Gag-Pro-Pol D P V A A G W P P C L R M V A A I A V L
3841 ACAAAGGATGCAGGCAAGCTAACTATGGGACAGCCGCTAGTCATTCTGGCCCCCCATGCG
Gag-Pro-Pol T K D A G K L T M G Q P L V I L A P H A
3901 GTAGAAGCACTGGTCAAACAACCCCCTGACCGTTGGCTATCCAATGCCCGCATGACCCAC
Gag-Pro-Pol V E A L V K Q P P D R W L S N A R M T H
3961 TATCAGGCAATGCTCCTGGATACAGACCGGGTTCAGTTCGGACCGGTGGTGGCCCTCAAC
Gag-Pro-Pol Y Q A M L L D T D R V Q F G P V V A L N
4021 CCGGCCACCCTGCTCCCCCTACCGGAAAAGGAAGCCCCCCATGACTGCCTCGAGATCTTG
Gag-Pro-Pol P A T L L P L P E K E A P H D C L E I L
4081 GCTGAGACGCACGGAACCAGACCGGACCTCACGGACCAGCCCATCCCAGACGCTGATTAC
Gag-Pro-Pol A E T H G T R P D L T D Q P I P D A D Y
4141 ACTTGGTACACAGATGGAAGCAGCTTCCTACAAGAAGGACAACGGAGAGCTGGAGCAGCG
Gag-Pro-Pol T W Y T D G S S F L Q E G Q Q R A G A A
4201 GTGACTACTGAGACCGAGGTAATCTGGGCGAGGGCTCTGCCGGCTGGAACATCCGCCCAA
Gag-Pro-Pol V T T E T E V I W A R A L P A G T S A Q
4261 CGAGCCGAACTGATAGCACTCACCCAAGCCTTAAAGATGGCAGAAGGTAAGAAGCTAAAT
Gag-Pro-Pol R A E L I A L T Q A L K M A E G K K L N
4321 GTTTACACTGATAGCCGCTATGCCTTCGCCACGGCCCATGTCCATGGAGAAATATATAGG
Gag-Pro-Pol V Y T D S R Y A F A T A H V H G E I Y R
4381 AGGCGAGGGTTGCTGACCTCAGAAGGCAGAGAAATTAAAAACAAGAACGAGATCTTGCC
Gag-Pro-Pol R R G L L T S E G R E I K N K N E I L A
4441 TTGCTAAAAGCTCTCTTTCTGCCCAAACGACTTAGTATAATTCACTGTCCAGGACATCAA
Gag-Pro-Pol L L K A L F L P K R L S I I H C P G H Q
4501 AAAGGAAACAGTGCTGAGGCCAGAGGCAACCGTATGGCAGATCAAGCAGCCCGAGAGGCA
Gag-Pro-Pol K G N S A E A R G N R M A D Q A A R E A
4561 GCCATGAAGGCAGTTCTAGAAACCTCTACACTCCTCATAGAGGACTCAACCCCGTATACG
Gag-Pro-Pol A M K A V L E T S T L L I E D S T P Y T
4621 CCTCCCCATTTCCATTACACCGAAACAGATCTCAAAAGACTACGGGAACTGGGAGCCACC
Gag-Pro-Pol P P H F H Y T E T D L K E L R E L G A T
4681 TACAATCAGACAAAAGGATATTGGGTCCTACAAGGCAAACCTGTGATGCCCGATCAGTCC
Gag-Pro-Pol Y N Q T K G Y W V L Q G K P V M P D Q S

Figure 28B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4801 GCACTCCTCGACAGAGAAGAAAGCCCCTACTACATGTTAAACCGGGACAGAACTATCCAG
Gag-Pro-Pol A L L D R E E S P Y Y M L N R D R T I Q
4861 TATGTGACTGAGACCTGCACCGCCTGTGCCCAAGTAAATGCCAGCAAAGCCAAAATTGGG
Gag-Pro-Pol Y V T E T C T A C A Q V N A S K A K I G
4921 GCAGGGGTGCGAGTACGCGGACATCGGCCAGGCACCCATTGGGAAGTTGATTTCACGGAA
Gag-Pro-Pol A G V R V R G H R P G T H W E V D F T E
4981 GTAAAGCCAGGACTGTATGGGTACAAGTACCTCCTAGTGTTTGTAGACACCTTCTCTGGC
Gag-Pro-Pol V K P G L Y G Y K Y L L V F V D T F S G
5041 TGGGTAGAGGCATTCCCGACCAAGCGGGAAACTGCCAAGGTTGTGACCAAAAAGCTGTTA
Gag-Pro-Pol W V E A F P T K R E T A K V V T K K L L
5101 GAAGACATTTTTCCGAGATTTGGAATGCCGCAGGTATTGGGATCTGATAACGGGCCTGCC
Gag-Pro-Pol E D I F P R F G M P Q V L G S D N G P A
5161 TTCGCCTCCCAGGTAAGTCAGTCAGTGGCCGATTTACTGGGGATCGATTGGAAGTTACAT
Gag-Pro-Pol F A S Q V S Q S V A D L L G I D W K L H
5221 TGTGCTTATAGACCCCAGAGTTCAGGACAGGTAGAAAGAATGAATAGAACAATTAAGGAG
Gag-Pro-Pol C A Y R P Q S S G Q V E R M N R T I K E
5281 ACTTTGACCAAATTAACGCTTGCATCTGGCACTAGAGACTGGGTACTCCTACTCCCCTTA
Gag-Pro-Pol T L T K L T L A S G T R D W V L L L P L
5341 GCCCTCTACCGAGCCCGGAATACTCCGGGCCCCCACGGACTGACTCCGTATGAAATTCTG
Gag-Pro-Pol A L Y R A R N T P G P H G L T P Y E I L
5401 TATGGGGCACCCCCGCCCCTTGTCAATTTTCATGATCCTGAAATGTCAAAGTTAACTAAT
Gag-Pro-Pol Y G A P P P L V N F H D P E M S K L T N
5461 AGTCCCTCTCTCCAAGCTCACTTACAGGCCCTCCAAGCAGTACAACAAGAGGTCTGGAAG
Gag-Pro-Pol S P S L Q A H L Q A L Q A V Q Q E V W K
splice accep -------->
5521 CCGCTGGCCGCTGCTTATCAGGACCAGCTAGATCAGCCAGTGATACCACACCCCTTCCGT
Gag-Pro-Pol P L A A A Y Q D Q L D Q P V I P H P F R
5581 GTCGGTGACGCCGTGTGGGTACGCCGGCACCAGACTAAGAACTTAGAACCTCGCTGGAAA
Gag-Pro-Pol V G D A V W V R R H Q T K N L E P R W K
5641 GGACCCTACACCGTCCTGCTGACAACCCCCACCGCTCTCAAAGTAGACGGCATCTCTGCG
Gag-Pro-Pol G P Y T V L L T T P T A L K V D G I S A
5701 TGGATACACGCCGCTCACGTAAAGGCGGCGACAACTCCTCCGGCCGGAACAGCATGGAAA
Gag-Pro-Pol W I H A A H V K A A T T P P A G T A W K
Env M E
5761 GTCCAGCGTTCTCAAAACCCCTTAAAGATAAGATTAACCCGTGGGGCCCCCTGATAATTA
Gag-Pro-Pol V Q R S Q N P L K I R L T R G A P *
Env S P A F S K P L K D K I N P W G P L I I
5821 TGGGGATCTTGGTGAGGGCAGGAGCCTCAGTACAACGTGACAGCCCTCACCAGGTCTTTA
Env M G I L V R A G A S V Q R D S P H Q V F
5881 ATGTCACTTGGAAAATTACCAACCTAATGACAGGACAAACAGCTAATGCTACCTCCCTCC
Env N V T W K I T N L M T G Q T A N A T S L
5941 TGGGGACGATGACAGACACTTTCCCTAAACTATATTTTGACTTGTGTGATTTAGTTGGAG
Env L G T M T D T F P K L Y F D L C D L V G
6001 ACAACTGGGATGACCCGGAACCCGATATTGGAGATGGTTGCCGCTCTCCCGGGGGAAGAA
Env D N W D D P E P D I G D G C R S P G G R
6061 AAAGGACAAGACTATATGATTTCTATGTTTGCCCCGGTCATACTGTATTAACAGGGTGTG
Env K R T R L Y D F Y V C P G H T V L T G C
6121 GAGGGCCGAGAGAGGGCTACTGTGGCAAATGGGGATGTGAGACCACTGGACAGGCATACT
Env G G P R E G Y C G K W G C E T T G Q A Y
6181 GGAAGCCATCATCATCATGGGACCTAATTTCCCTTAAGCGAGGAAACACTCCTAAGGGTC
Env W K P S S S W D L I S L K R G N T P K G
6241 AGGGCCCCTGTTTTGATTCCTCAGTGGGCTCCGGTAGCATCCAGGGTGCCACACCGGGGG
Env Q G P C F D S S V G S G S I Q G A T P G
6301 GTCGATGCAACCCCCTAGTCCTAGAATTCACTGACGCGGGTAAAAGGGCCAGCTGGGATG
Env G R C N P L V L E F T D A G K R A S W D
6361 CCCCCAAAACATGGGGACTAAGACTGTATCGATCCACTGGGGCCGACCCGGTGACCCTGT
Env A P K T W G L R L Y R S T G A D P V T L
6421 TCTCTCTGACCCGCCAGGTCCTCAATGTAGGGCCCCGCGTCCCCATTGGGCCTAATCCCG
Env F S L T R Q V L N V G P R V P I G P N P
6481 TGATCACTGAACAGCTACCCCCCTCCCAACCCGTGCAGATCATGCTCCCCAGGCCTCCTC
Env V I T E Q L P P S Q P V Q I M L P R P P
6541 GTCCTCCTCCTTCAGGCGCGGCCTCTATGGTGCCTGGGGCTCCCCCGCCTTCTCAACAAC
Env E P P P S G A A S M V P G A P P P S Q Q
6601 CTGGGACGGGAGACAGGCTGCTAAACCTGGTAGAAGGAGCCTACCAAGCCCTCAACCTCA
Env P G T G D R L L N L V E G A Y Q A L N L
6661 CCAGTCCCGACAAAACCCAAGAGTGCTGGCTGTGTCTAGTATCGGGACCCCCCTACTACG
Env T S P D K T Q E C W L C L V S G P P Y Y
6721 AAGGGGTGGCCGTCCTAGGTACTTACTCCAACCATACCTCTGCCCCGGCTAACTGCTCCG
Env E G V A V L G T Y S N H T S A P A N C S
6781 TGACCTCCCAACACAAGCTGACCCTGTCCGAAGTGACCGGGCAGGGACTCTGCATAGGAG
Env V T S Q H K L T L S E V T G Q G L C I G
6841 CAGTTCCCAAAACCCATCAGGCCCTGTGTAATACCACCCAGAAGACGAGCGACGGGTCCT
Env A V P K T H Q A L C N T T Q K T S D G S
6901 ACTATTTGGCCTCTCCCGCCGGGACCATTTGGGCTTGCAGCACCGGGCTCACTCCCTGTC
Env Y Y L A S P A G T I W A C S T G L T P C
6961 TATCTACTACTGTGCTTAACTTAACCACTGATTACTGTGTCCTGGTTGAACTCTGGCCAA
Env L S T T V L N L T T D Y C V L V E L W P
7021 AGGTAACCTACCACTCCCCTAATTATGTTTATGGCCAGTTTGGAAAGAAACTAAATATA
Env K V T Y H S P N Y V Y G Q F G K K T K Y
7081 AAAGAGAGCCGGTGTCATTAACTCTGGCCCTGCTGTTGGGAGGACTTACTATGGGCGGCA
Env K R E P V S L T L A L L L G G L T M G G
7141 TAGCTGCAGGAGTTGGAACAGGGACTACAGCCCTAGTGGCCACCAAACAATTCGAGCAGC
Env I A A G V G T G T T A L V A T K Q F E Q
7201 TCCAGGCAGCCATACATACAGACCTTGGGGCCTTAGAAAAATCAGTCAGTGCCCTAGAAA
Env L Q A A I H T D L G A L E K S V S A L E
7261 AGTCTCTGACCTCGTTGTCTGAGGTGGTCCTACAGAACCGGAGGGGATTAGATCTACTGT
Env K S L T S L S E V V L Q N R E G L D L L
7321 TCCTAAAAGAAGGAGGATTATGTGCTGCCCTAAAAAAAGAATGCTGTTTTACGCGGACC
Env F L K E G G L C A A L K K E C C F Y A D
7381 ACACTGGCGTAGTAAGAGATAGCATGGCAAAGCTAAGAGAAAGGTTAAACCAGAGACAAA
Env H T G V V R D S M A K L R E E L N Q R Q
7441 AATTGTTCGAATCAGGACAAGGGTGGTTTGAGGGACTGTTTAACAGGTCCCCATGGTTCA
Env K L F E S G Q G W F E G L F N R S P W F
7501 CGACCCTGATATCCACCATTATGGGCCCTCTGATAGTACTTTTATTAATCCTACTCTTCG
Env T T L I S T I M G P L I V L L L I L L F
7561 GACCCTGTATTCTCAACCGCTTGGTCCAGTTTGTAAAAGACAGAATTTCGGTAGTGCAGG
Env G P C I L N R L V Q F V K D R I S V V Q
7621 CCCTGGTTCTGACCCAACAGTATCACCAACTCAAATCAATAGATCCAGAAGAAGTGGAAT
Env A L V L T Q Q Y H Q L K S I D P E E V E
7681 CACGTGAATAAAAGATTTTATTCAGTTTCCAGAAAGAGGGGGGAATGAAAGACCCCACCA
Env S R E *
U3 ----------
7741 TAAGGCTTAGCACGCTAGCTACAGTAACGCCATTTTGCAAGGCATGGAAAAGTACCAGAG
U3 ----------
7801 CTGAGTTCTCAAAAGTTACAAGGAAGTTTAATTAAAGAATAAGGCTGAATAACACTGGGA
U3 ----------
7861 CAGGGGCCAAACAGGATATCTGTAGTCAGGCACCTGGGCCCCGGCTCAGGGCCAAGAACA
U3 ----------
GRE ------
7921 GATGGTCCTCAGATAAAGCGAAACTAACAACAGTTTCTGGAAAGTCCCACCTCAGTTTCA
U3 ----------
GRE -------->
7981 AGTTCCCCAAAAGACCGGGAAATACCCCAAGCCTTATTTAAACTAACCAATCAGCTCGCT
U3 ----------
CAAT box ---->
8041 TCTCGCTTCTGTACCCGCGCTTTTTGCTCCCCAGTCCTAGCCCTATAAAAAAGGGGTAAG
U3 ----------
TATA box ------>
8101 AACTCCACACTCGGCGCGCCAGTCATCCGATAGACTGAGTCGCCCGGGTACCCGTGTTCC
U3 ---------->
R ----------
8161 CAATAAAGCCTTTTGCTGTTTGCAA
R ---------->
polyA signal ----->
polyA site >

Figures 29A–29B

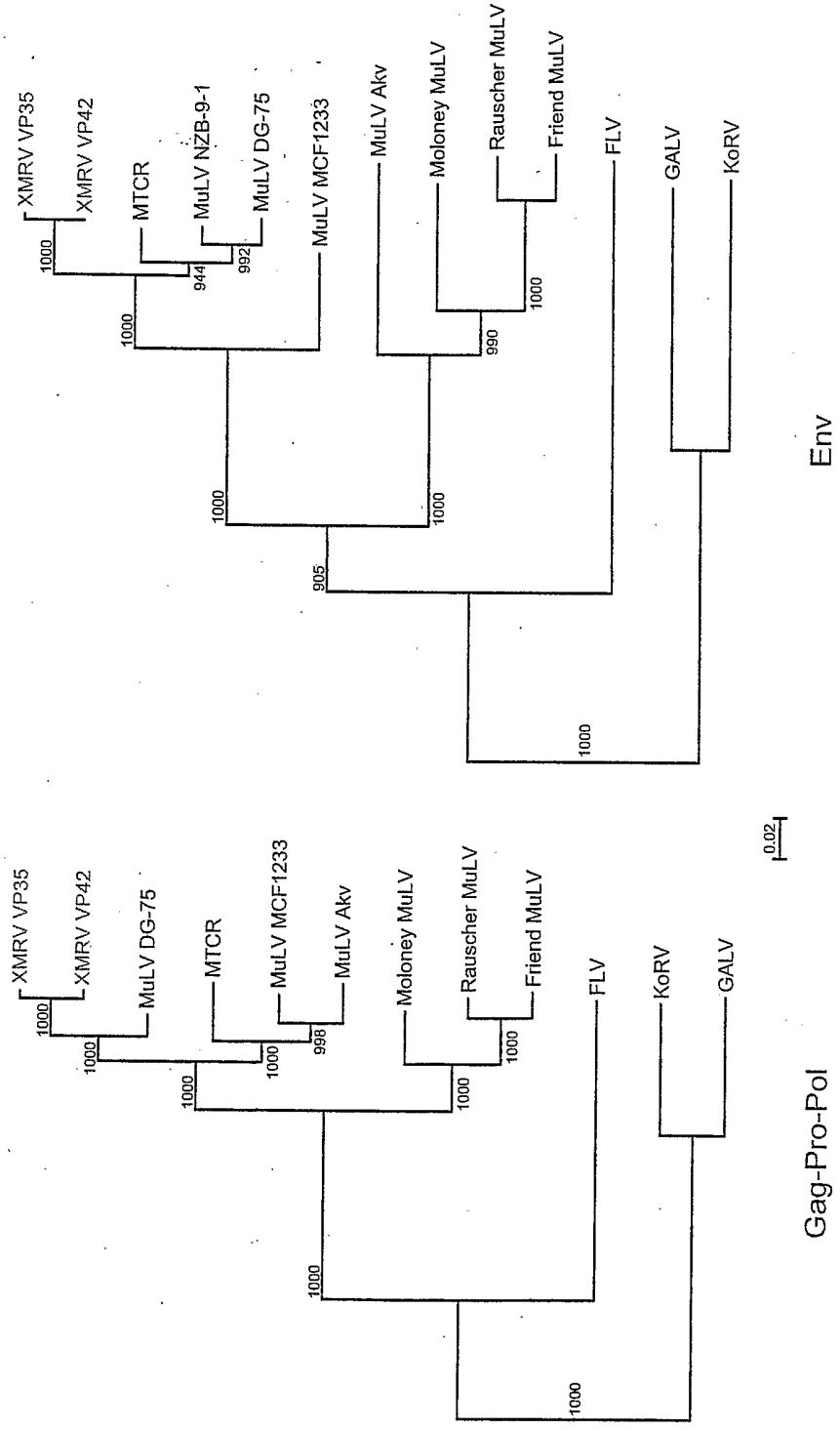
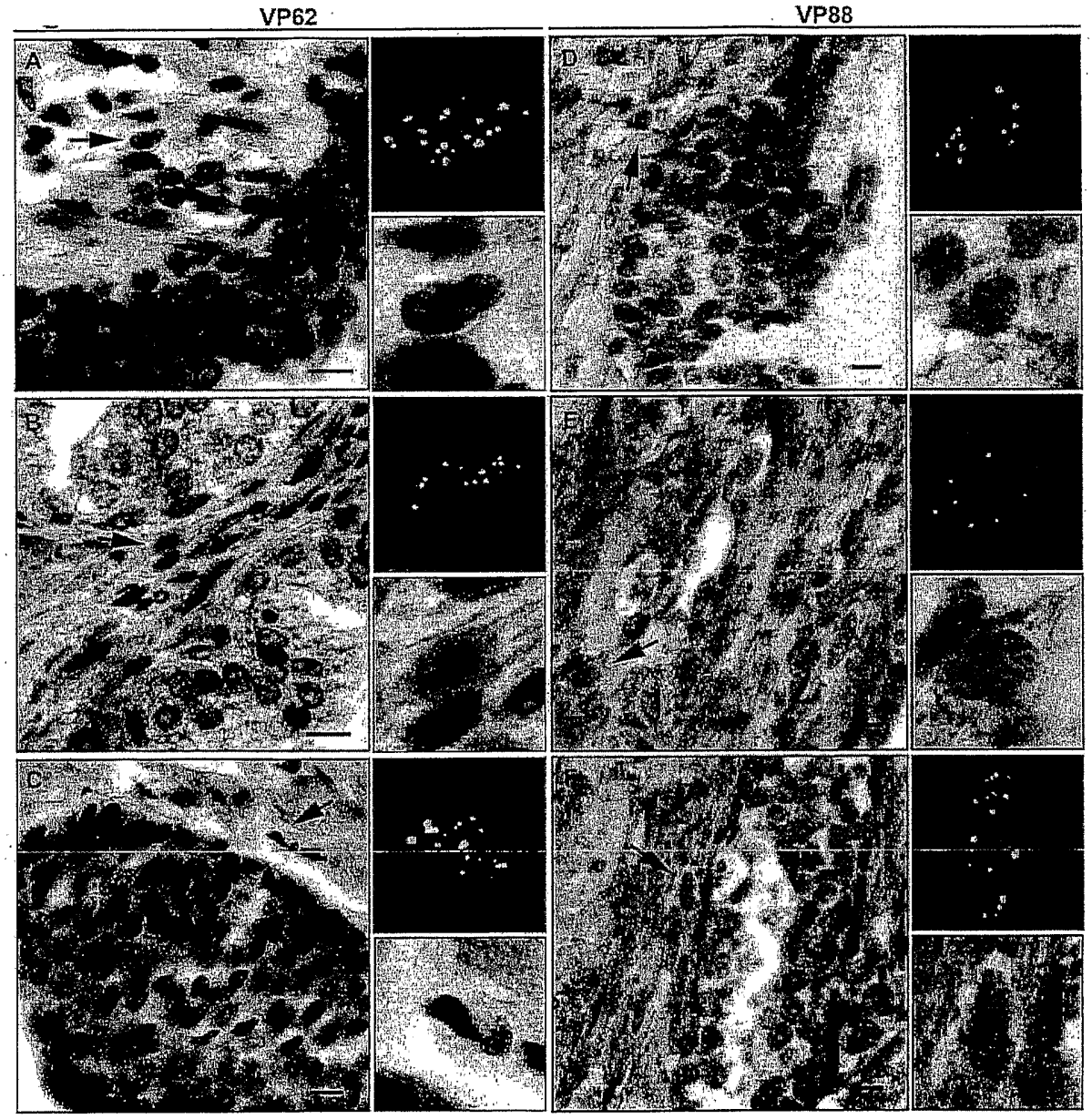
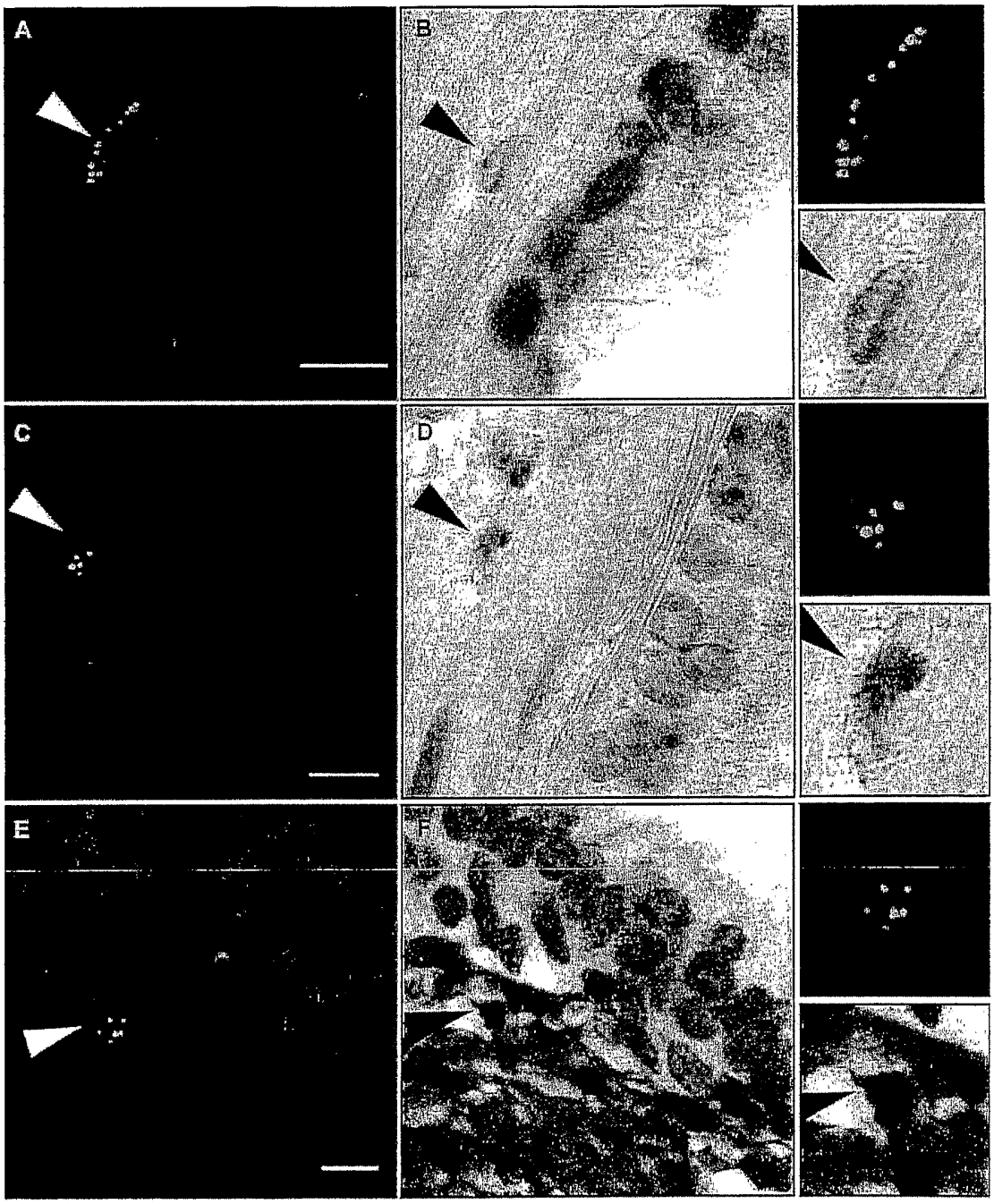


Figure 29A

Figure 29B


VP62
VP88
A
B
C
D
E
F


A
B
C
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E
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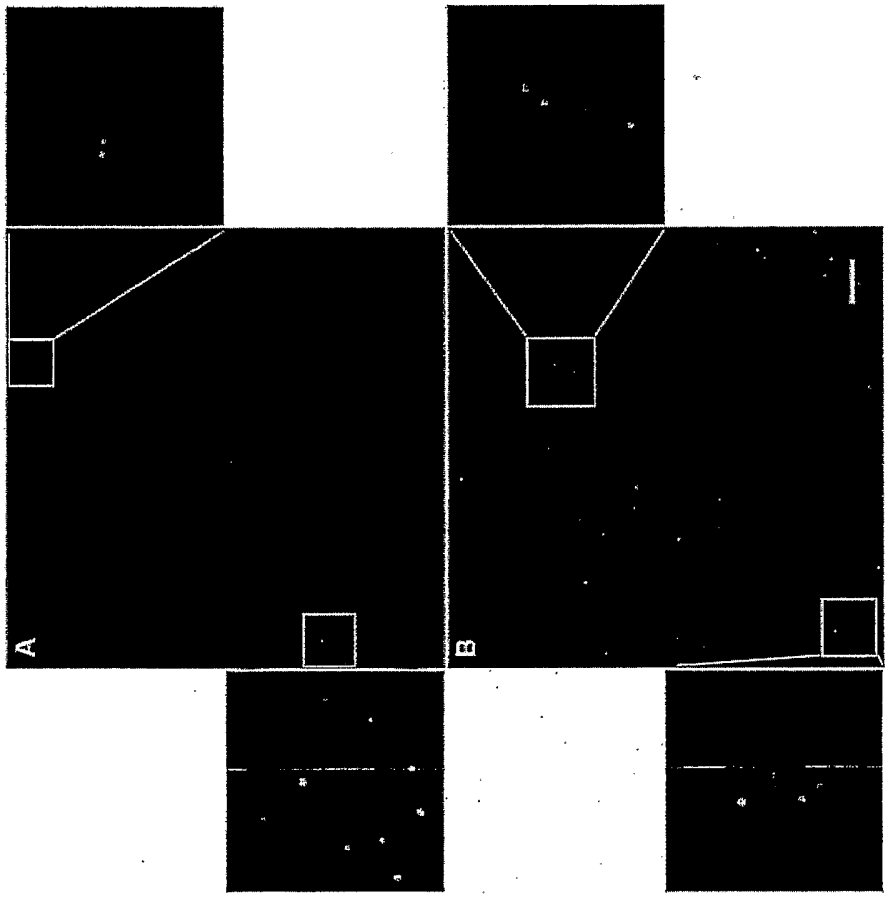
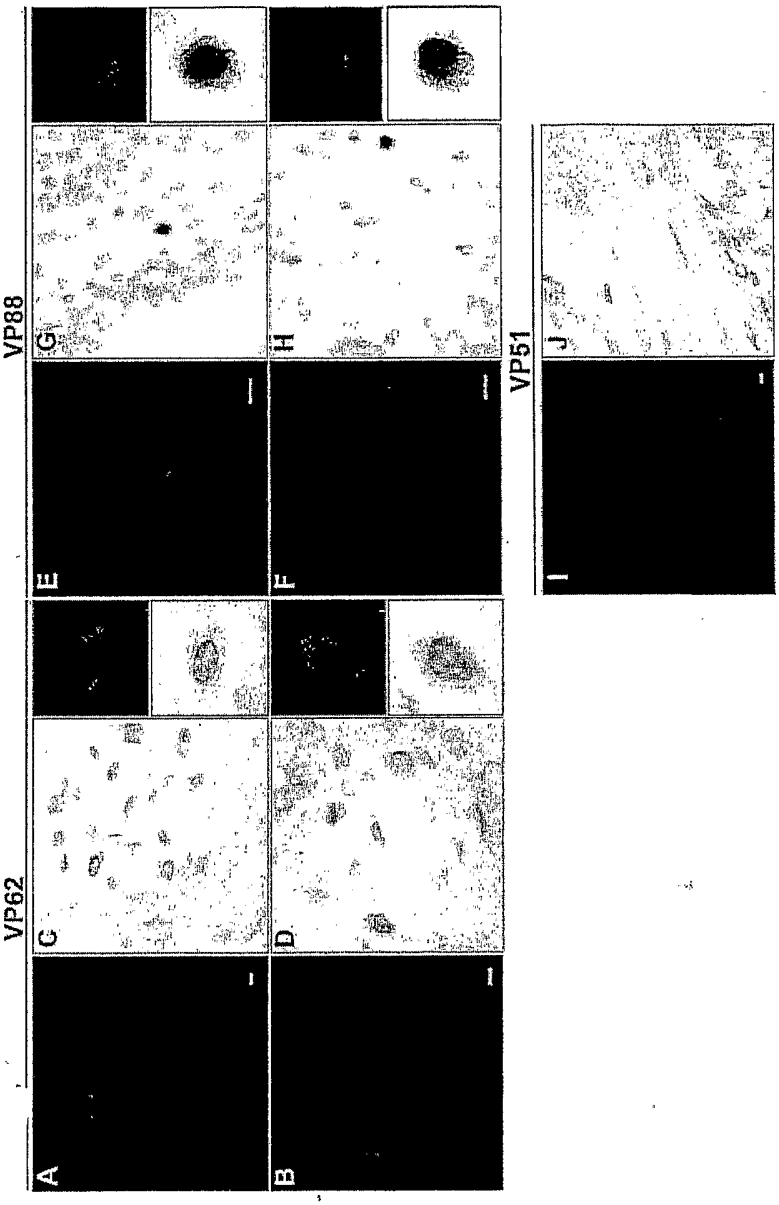
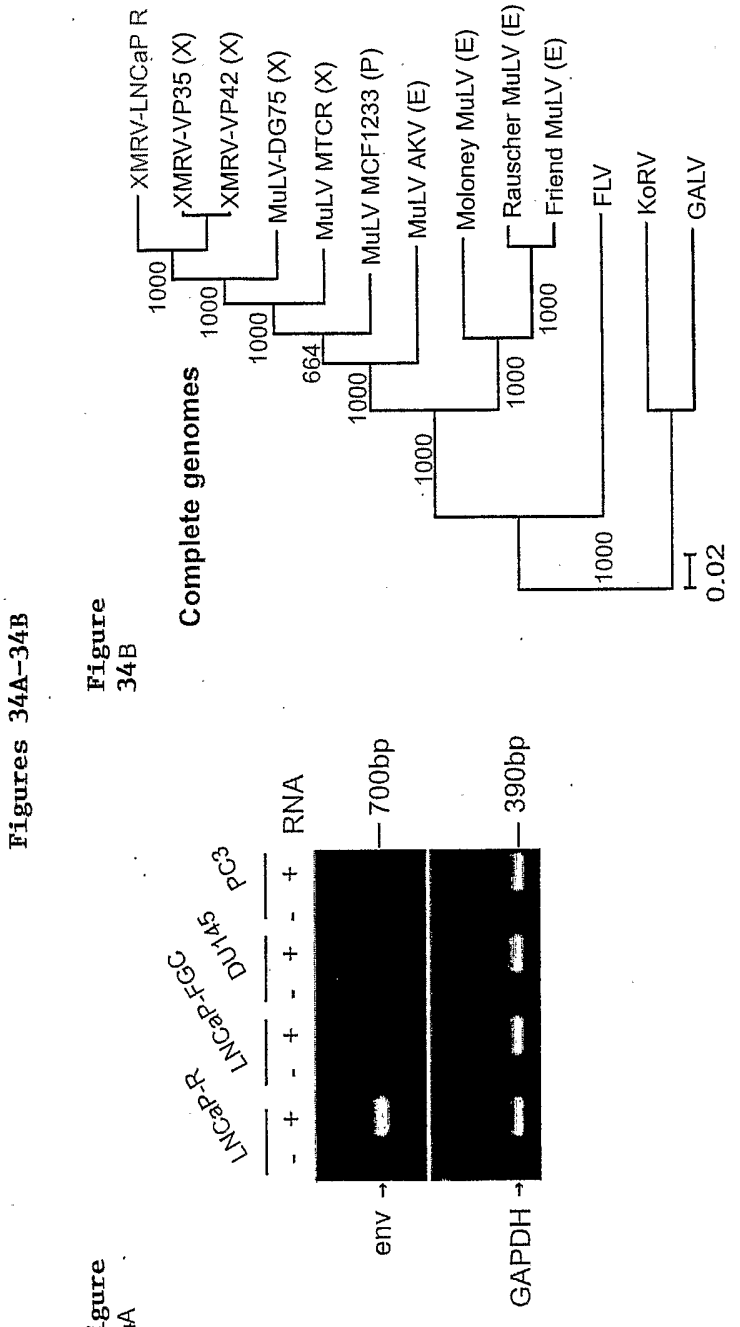


Figure 32

Figure 33




Figures 34A–34B
Figure 34A
LNCaP-R
LNCaP-FGC
DU145
PC3
RNA
- + - + - + - +
700bp
390bp
env →
GAPDH →
Figure 34B
Complete genomes
XMRV-LNCaP R
XMRV-VP35 (X)
XMRV-VP42 (X)
MuLV-DG75 (X)
MuLV MTCR (X)
MuLV MCF1233 (P)
MuLV AKV (E)
Moloney MuLV (E)
Rauscher MuLV (E)
Friend MuLV (E)
FLV
KoRV
GALV
1000
1000
1000
664
1000
1000
1000
1000
1000
0.02

Figures 34C–34D

Figure 34C

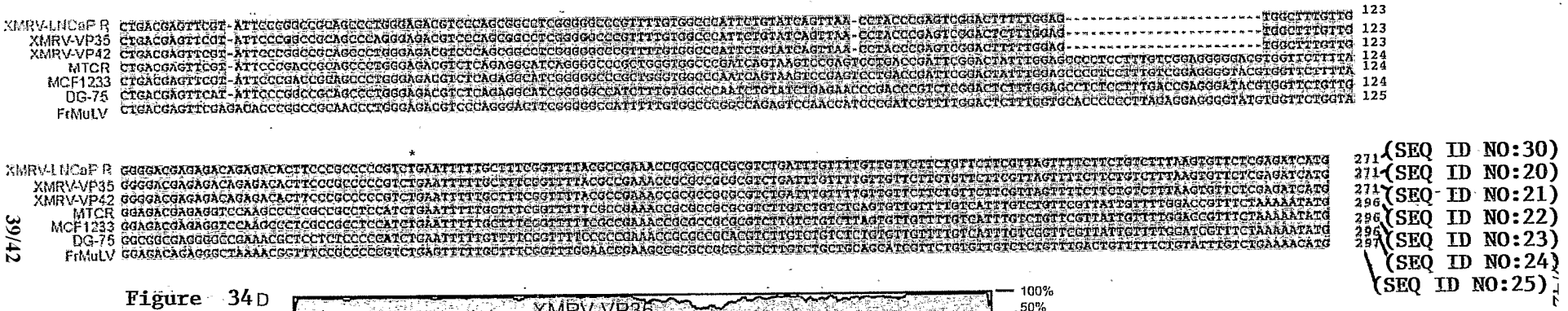
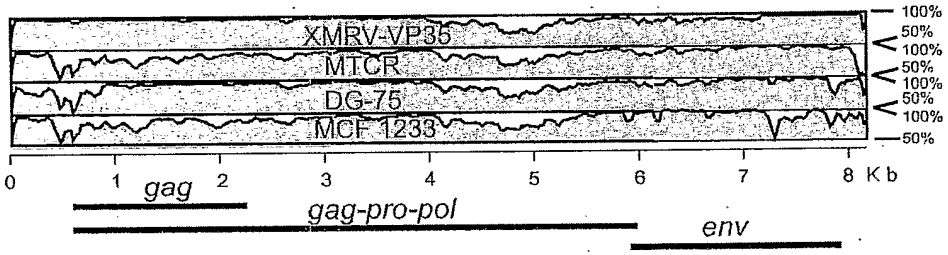


Figure 34D



Figures 35A–35C

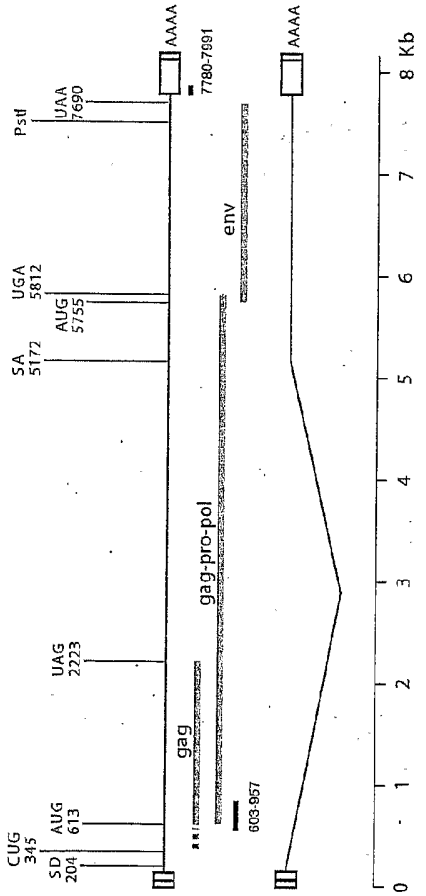


Figure 35A

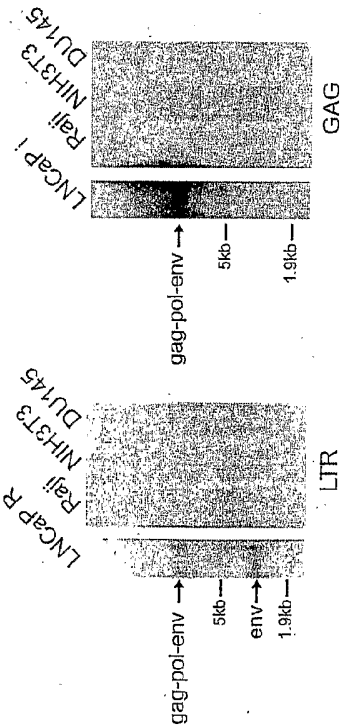


Figure 35B

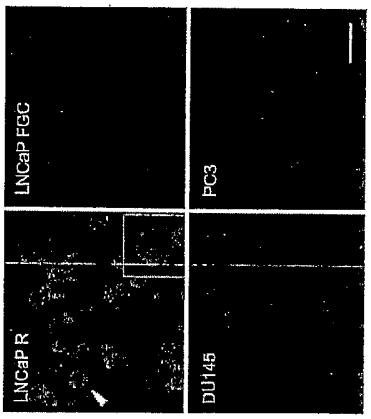
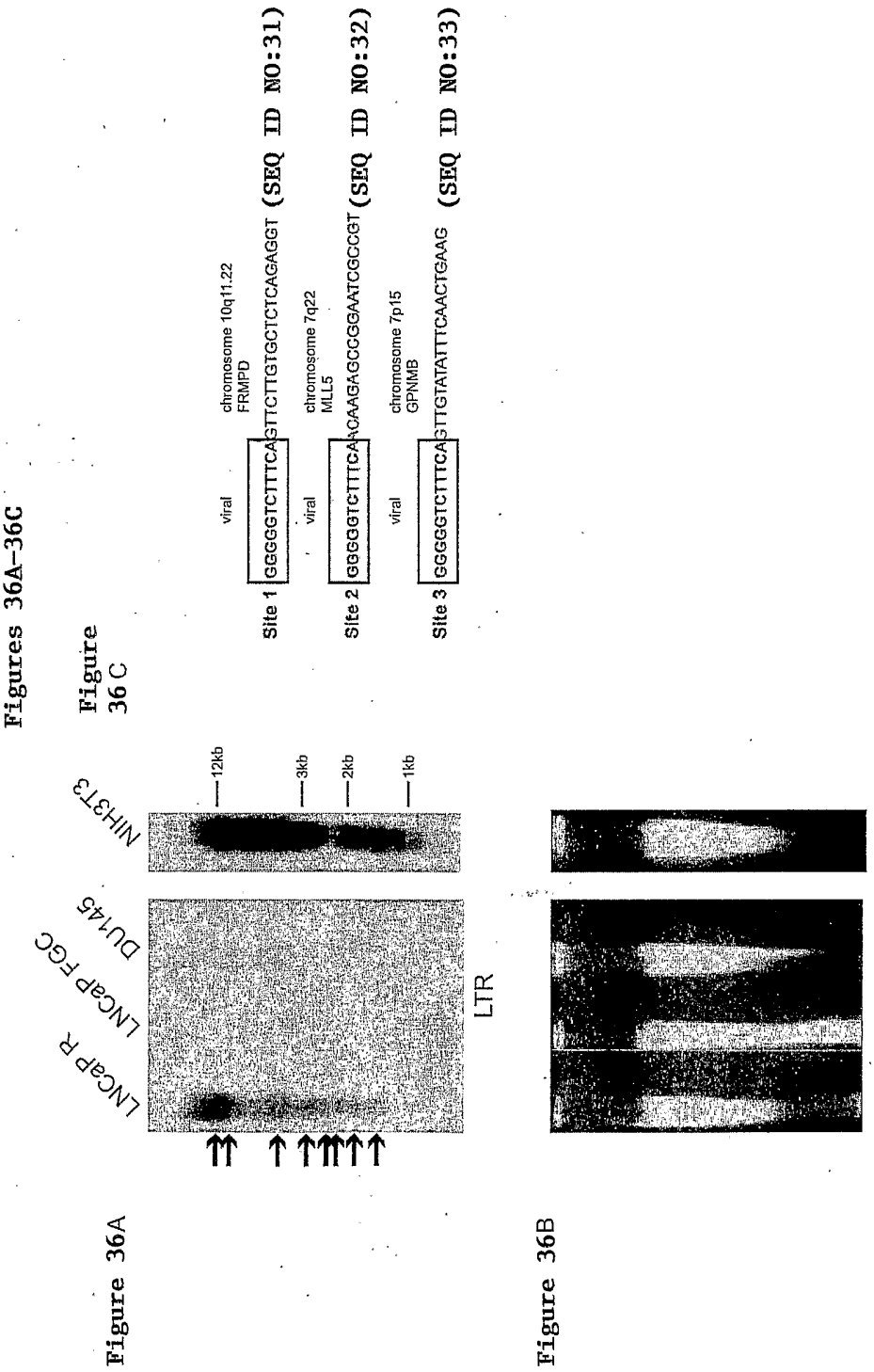


Figure 35C

Figures 36A–36C



Figures 37A-37C

Figure 37 A

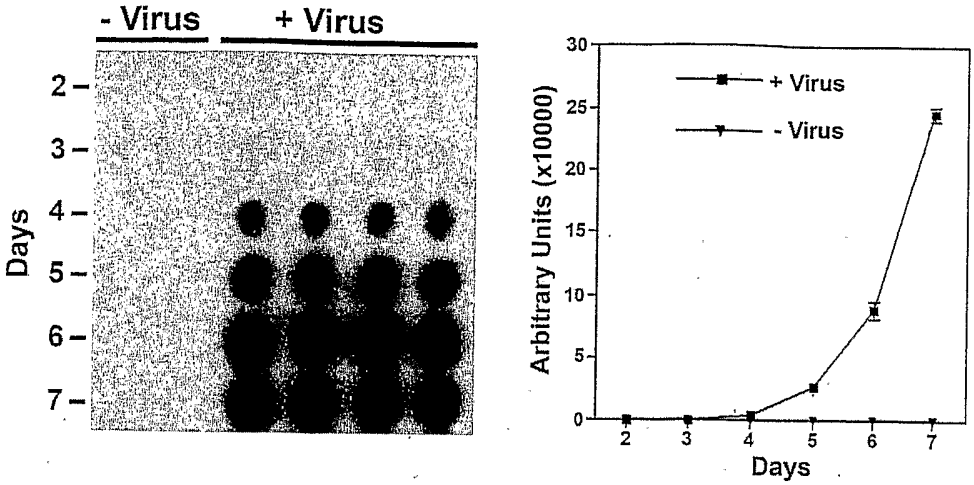


Figure 37 B

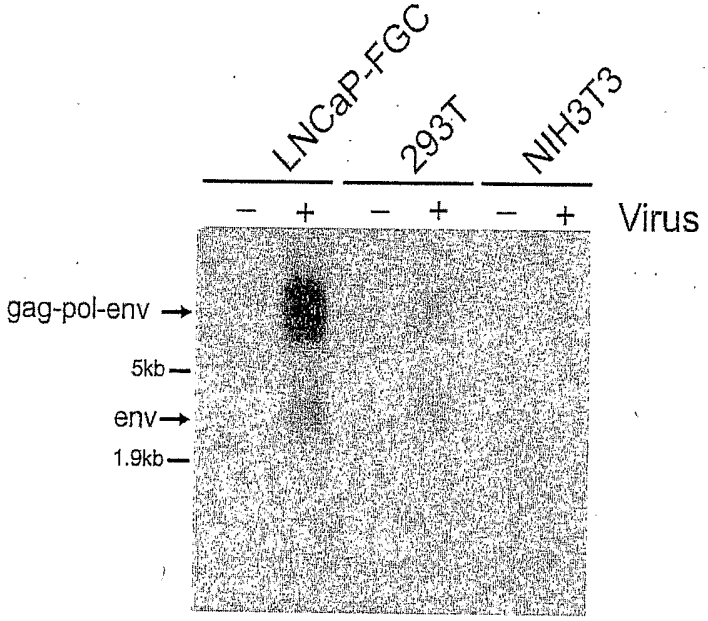


Figure 37 C