PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/31, C07K 14/22, C12Q 1/68, G01N 33/53, A61K 39/095

A2

(11) International Publication Number: WO 00/66741

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/IB00/00642

(22) International Filing Date: 28 April 2000 (28.04.00)

(30) Priority Data:

9910168.5	30 April 1999 (30.04.99)	GB
0005728.1	9 March 2000 (09.03.00)	GB

(71) Applicant *(for all designated States except US)*: CHIRON S.P.A. [IT/IT]; Via Fiorentina, 1, I–53100 Siena (IT).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: RAPPUOLI, Rino [IT/IT]; Chiron S.p.A., Via Fiorentina, 1, I–53100 Siena (IT).

(74) Agents: HALLYBONE, Huw, George et al.; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

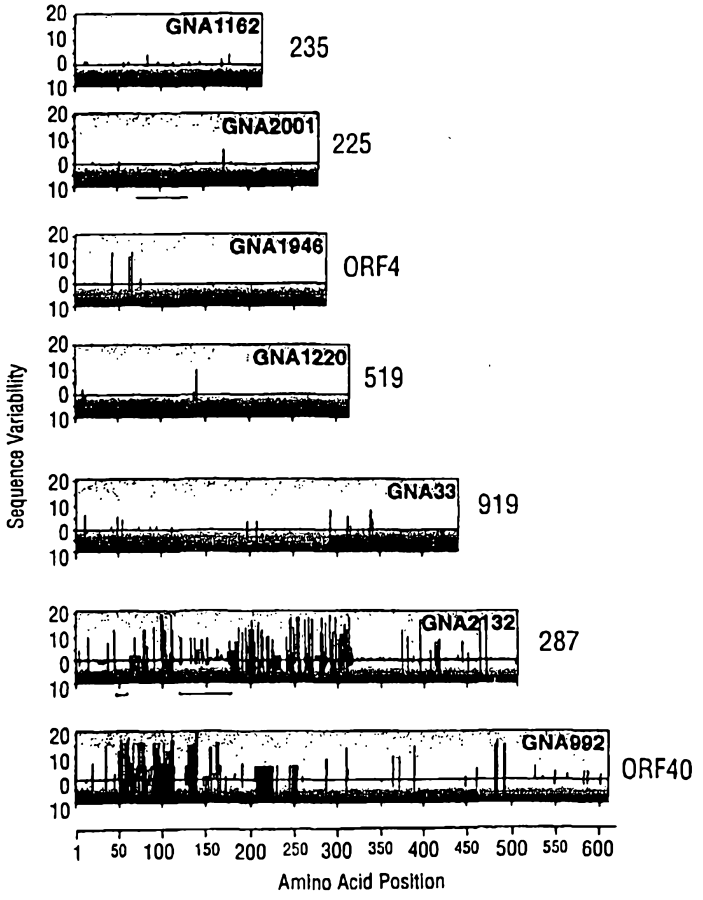
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: CONSERVED NEISSERIAL ANTIGENS

(57) Abstract

To ensure maximum cross–strain recognition and reactivity, regions of proteins that are conserved between different Neisserial species, serogroups and strains can be used. The invention provides proteins which comprise stretches of amino acid sequence that are shared across the majority of *Neisseria*, particularly *N. meningitidis* and *N.gonorrhoeae*.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

CONSERVED NEISSERIAL ANTIGENS

The contents of all documents cited herein are incorporated by reference in their entirety.

FIELD OF THE INVENTION

This invention relates to conserved antigens from the *Neisseria* bacteria.

BACKGROUND ART

Neisseria meningitidis and *Neisseria gonorrhoeae* are non-motile, gram negative diplococci that are pathogenic in humans.

Based on the organism's capsular polysaccharide, 12 serogroups of *N.meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries.

The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. This approach cannot be used for Meningococcus B, however, because the menB capsular polysaccharide is a polymer of α(2-8)-linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. One approach to a menB vaccine uses mixtures of outer membrane proteins (OMPs) To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed [*eg.* Poolman (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28]. Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability [*eg.* Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53].

A large number of Neisserial protein and nucleotide sequences are disclosed in WO99/24578, WO99/36544, WO99/57280 and WO00/22430. The contents of these four applications are incorporated herein by reference. Comprehensive sequence data from strain MC58 is disclosed in Tettelin *et al.* [*Science* (2000) 287:1809-1815], the contents of which are also incorporated herein by reference.

DESCRIPTION OF THE INVENTION

To ensure maximum cross-strain recognition and reactivity, regions of proteins that are conserved between different Neisserial species, serogroups and strains can be used. The invention therefore provides proteins which comprise stretches of amino acid sequence that are shared across the majority of *Neisseria*, particularly *N.meningitidis* and *N.gonorrhoeae*.

The invention provides a protein comprising a fragment of a Neisserial protein, wherein said fragment consists of *n* consecutive conserved amino acids, with the proviso that the invention does not include within its scope full-length Neisserial proteins. Depending on the particular protein, *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20 or more). The fragment preferably comprises an antigenic or immunogenic region of the Neisserial protein.

A "conserved" amino acid is one that is present in a particular Neisserial protein in at least *x*% of *Neisseria*. The value of *x* may be 50% or more *eg.* 66%, 75%, 80%, 90%, 95% or even 100% (*ie.* the amino acid is found in the protein in question in all *Neisseria*).

In order to determine whether an amino acid is "conserved" in a particular Neisserial protein, it is necessary to compare that amino acid residue in the sequences of the protein in question from a plurality of different *Neisseria* (a "reference population"). The reference population may include a number of different *Neisseria* species (preferably *N.meningitidis* and *N.gonorrhoeae*) or may include a single species. The reference population may include a number of different serogroups of a particular species (such as the A, B, C, W135, X, Y, Z and 29E serogroups of *N.meningitidis*) or a single serogroup. The reference population may also include a number of different strains from a particular serogroup (such as the NG6/88, BZ198, NG3/88, 297-0, BZ147, BZ169, 528, BZ133, NGE31, NGH38, NGH15, BZ232, BZ83, and 44/76 strains of *N.meningitidis* B). A preferred reference population consists of the 5 most common strains of *N. meningitidis* and/or the 5 most common strains of *N.gonorrhoeae*.

The reference population preferably comprises *k* strains taken from *k* different branches of a suitable phylogenetic tree, such as those disclosed in (a) Ni *et al.* (1992) *Epidemiol Infect* 109:227-239 (b) Wolff *et al.* (1992) *Nucleic Acids Res* 20:4657 (c) Bygraves & Maiden (1992) *J. Gen. Microbiol.* 138:523-531 (d) Caugant *et al.* (1987) *J. Bacteriol.* 69:2781-2792. Another phylogenetic tree that can be used is shown in Figure 8 herein, and another in Figure 9b.

It will be appreciated that a particular species, serogroup or strain should only be included in the reference population if it encodes the protein in which the amino acid in question is located. In

the case of amino acids within ORF40 described below, for instance, the reference population should not include *N.gonorrhoeae* because this species does not contain ORF40.

For proteins found in both *N.meningitidis* and *N.gonorrhoeae*, therefore, a preferred reference population comprises:

- *N.meningitidis* A, strain Z2491
- *N.meningitidis* B, strains NG6/88
- *N.meningitidis* W, strains A22
- *N.gonorrhoeae*, strain Ng F62

These are described in (a) Seiler A. *et al.* (1996) *Mol. Microbiol.* 19(4):841-856 (b) Maiden *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95:3140-3145 (c) Virji *et al.* (1992) *Mol. Microbiol.* 6:1271-1279 (d) Dempsey *et al.* (1991) *J. Bacteriol.* 173:5476-5486.

For proteins found only in *N.meningitidis*, however, a preferred reference population comprises:

- *N.meningitidis* A, strain Z2491
- *N.meningitidis* B, strains NG6/88
- *N.meningitidis* W, strains A22

Amino acid sequences of different *Neissieriae* can easily be compared using computers. This will typically involve the alignment of a number of sequences using an algorithm such as CLUSTAL [Thompson *et al.* (1994) *Nucleic Acids Res* 22:4673-4680; *Trends Biochem Sci* (1998) 23:403-405] or, preferably, PILEUP [part of the GCG Wisconsin package, preferably version 9.0].

Conserved amino acids are readily apparent in a multiple sequence alignment – at the amino acid position in question a majority of the aligned sequences will contain a particular amino acid. Conserved amino acids can be made more visually apparent by using a program such as BOXSHADE [available, for instance, at the NIH on-line], PRETTYBOX [GCG Wisconsin, version 10] or JALVIEW [available on-line at EBI].

The protein preferably comprises a fragment of one of the proteins disclosed in WO99/24578, WO99/36544, WO99/57280 or WO00/22430, or of one of the 2158 ORFs disclosed in Tettelin *et al.* [*Science* (2000) 287:1809-1815]. More particularly, it preferably comprises a fragment of one or more of ORF4, ORF40, ORF46, protein 225, protein 235, protein 287, protein 519, protein 726, protein 919 and protein 953 disclosed therein (see examples herein). Typically, the

protein of the invention will not comprise a protein sequence explicitly disclosed in WO99/24578, WO99/36544, WO99/57280, WO00/22430, or Tettelin *et al.*

The invention also provides a protein comprising one of the sequences shown in the Figures.

The proteins of the invention can, of course, be prepared by various means (*eg.* recombinant expression, native expression, purification from cell culture, chemical synthesis *etc.*) and in various forms (*eg.* native, fusions *etc.*). They are preferably prepared in substantially pure form (*ie.* substantially free from other Neisserial or host cell proteins)

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means.

According to a further aspect, the invention provides nucleic acid encoding the proteins of the invention. It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to these (*eg.* for antisense or probing purposes).

Furthermore, the invention provides nucleic acid which can hybridise to the *N.meningitidis* nucleic acid disclosed in the examples, preferably under "high stringency" conditions (*eg.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Nucleic acid according to the invention can, of course, be prepared in many ways (*eg.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*eg.* single stranded, double stranded, vectors, probes *etc.*).

In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (*eg.* expression vectors) and host cells transformed with them.

According to a further aspect, the invention provides compositions comprising protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (*eg.* as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of: (i) a medicament for treating or preventing infection due to Neisserial bacteria; (ii) a diagnostic reagent for detecting

the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria; and/or (iii) a reagent which can raise antibodies against Neisserial bacteria. The use is preferably applicable to all species of *Neisseria*.

Where a Neisserial protein contains more than *q%* conserved amino acids, the invention provides the use of the Neisserial protein, or a fragment thereof, as a non-strain-specific protein that exhibits cross-reactivity between many species, serogroups and strains. The value of *q* may be 50%, 60%, 75%, 80%, 90%, 95% or even 100%.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression.

A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

A summary of standard techniques and procedures which may be employed in order to perform the invention (*eg.* to utilise the disclosed sequences for vaccination or diagnostic purposes) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

General

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature *eg.* Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide*

Synthesis (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

All publications, patents, and patent applications cited herein are incorporated in full by reference. In particular, the contents of international patent applications WO99/24578, WO99/36544, WO99/57280 and WO00/22430 are incorporated herein.

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "comprising" means "including" as well as "consisting" *eg.* a composition "comprising" X may consist exclusively of X or may include something additional to X, such as X+Y.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been assembled in a single protein in an arrangement not found in nature.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate

proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A "mutant" sequence is defined as DNA, RNA or amino acid sequence differing from but having sequence identity with the native or disclosed sequence. Depending on the particular sequence, the degree of sequence identity between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more, calculated using the Smith-Waterman algorithm as described above). As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs essentially at the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions (*eg.* see US patent 5,753,235).

Expression systems

The Neisserial nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual, 2nd ed.]*.

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallotheionein gene, also provide useful promoter sequences. Expression may be either

constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormone-responsive cells.

The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) *Science 236:*1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.]. Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer [Dijkema et al (1985) *EMBO J. 4*:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al. (1982b) *Proc. Natl. Acad. Sci. 79*:6777] and from human cytomegalovirus [Boshart et al. (1985) *Cell 41*:521]. Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) *Trends Genet. 2*:215; Maniatis et al. (1987) *Science* 236:1237].

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus triparite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) *Cell 41*:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci. 14*:105]. These sequences direct the

transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminater/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*].

Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 [Gluzman (1981) *Cell 23*:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replicaton systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) *Mol. Cell. Biol. 9*:946] and pHEBO [Shimizu et al. (1986) *Mol. Cell. Biol. 6*:1074].

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*eg.* Hep G2), and a number of other cell lines.

ii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous

recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) *17*:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, *42*:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A

baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol. 69*:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, *73*:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human α-interferon, Maeda et al., (1985), *Nature 315*:592; human gastrin-releasing peptide, Lebacq-Verheyden et al., (1988), *Molec. Cell. Biol. 8*:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, *82*:8404; mouse IL-3, (Miyajima et al., (1987) *Gene 58*:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, *7*:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome.

Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) *3*:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays 4*:91.The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μm in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of recombinant virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti* , *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol. 56*:153; Wright (1986) *Nature 321*:718; Smith et al., (1983) *Mol. Cell. Biol. 3*:2156; and see generally, Fraser, *et al.* (1989) *In Vitro Cell. Dev. Biol. 25*:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. *See, eg.* Summers and Smith *supra.*

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively,

where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, eg. HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, eg. proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iii. Plant Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., Nucleic Acids Research 15:2515-2535 (1987); Wirsel et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*,. Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a

prokaryote selectable marker; and, for Agrobacterium transformations, T DNA sequences for Agrobacterium-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Reptr*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be

conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersion*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and

alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in Escherichia coli (E. coli) [Raibaud *et al.* (1984) *Annu. Rev. Genet. 18*:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature 198*:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.* (1980) *Nuc. Acids Res. 8*:4057; Yelverton *et al.* (1981) *Nucl. Acids Res. 9*:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The g-laotamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes."

In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature 292*:128] and T5 [US patent 4,689,406] promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene 25*:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci. 80*:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol. 189*:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci. 82*:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature 254*:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' and of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in Escherichia coli." In *Molecular Cloning: A Laboratory Manual*].

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* on *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid

sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai *et al.* (1984) *Nature 309*:810]. Fusion proteins can also be made with sequences from the *lacZ* [Jia *et al.* (1987) *Gene 60*:197], *trpE* [Allen et al. (1987) J. Biotechnol. 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol. 135*:11], and *Chey* [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated [Miller *et al.* (1989) *Bio/Technology 7*:698].

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [US patent 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) [Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghrayeb *et al.* (1984) *EMBO J. 3*:2437] and the *E. coli* alkaline phosphatase signal sequence (*phoA*) [Oka *et al.* (1985) *Proc. Natl. Acad. Sci. 82*:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various Bacillus strains can be used to secrete heterologous proteins from *B. subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 244 042].

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various Bacillus strains integrate into the Bacillus chromosome (EP-A- 0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol. 32*:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: Bacillus subtilis [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], Escherichia coli [Shimatake *et al.* (1981) *Nature 292*:128; Amann *et al.* (1985) *Gene 40*:183; Studier *et al.* (1986) *J. Mol. Biol. 189*:113; EP-A-0 036 776,EP-A-0 136 829 and EP-A-0 136 907], Streptococcus cremoris [Powell *et al.* (1988) *Appl.*

Environ. Microbiol. 54:655]; Streptococcus lividans [Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655], Streptomyces lividans [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See *eg.* [Masson *et al.* (1989) *FEMS Microbiol. Lett. 60*:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, Bacillus], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci. 85*:856; Wang *et al.* (1990) *J. Bacteriol. 172*:949, Campylobacter], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci. 69*:2110; Dower *et al.* (1988) *Nucleic Acids Res. 16*:6127; Kushner (1978) "An improved method for transformation of Escherichia coli with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol. 53*:159; Taketo (1988) *Biochim. Biophys. Acta 949*:318; Escherichia], [Chassy *et al.* (1987) *FEMS Microbiol. Lett. 44*:173 Lactobacillus]; [Fiedler *et al.* (1988) *Anal. Biochem 170*:38, Pseudomonas]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett. 66*:203, Staphylococcus], [Barany *et al.* (1980) *J. Bacteriol. 144*:698; Harlander (1987) "Transformation of Streptococcus lactis by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun. 32*:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology 1*:412, Streptococcus].

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast *PHO5* gene, encoding

acid phosphatase, also provides useful promoter sequences [Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:1].

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (US Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the *ADH2*, *GAL4*, *GAL10*, OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, [Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA 77*:1078; Henikoff *et al.* (1981) *Nature 283*:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol. 96*:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast Saccharomyces cerevisiae," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene 11*:163; Panthier *et al.* (1980) *Curr. Genet. 2*:109;].

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See *eg.* EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (*eg.* WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for

secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EP-A-0 012 873; JPO. 62,096,086) and the A-factor gene (US patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EP-A-0 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (US Patents 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (*eg.* see WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 [Botstein *et al.* (1979) *Gene 8*:17-24], pCl/1 [Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA 81*:4642-4646], and YRp17 [Stinchcomb *et al.* (1982) *J. Mol. Biol. 158*:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number

vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See *eg.* Brake *et al.*, *supra.*

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver *et al.* (1983) *Methods in Enzymol. 101*:228-245]. An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra.* One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions [Butt *et al.* (1987) *Microbiol, Rev. 51*:351].

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts:Candida albicans [Kurtz, *et al.* (1986) *Mol. Cell. Biol. 6*:142], Candida maltosa [Kunze, *et al.* (1985) *J. Basic Microbiol. 25*:141]. Hansenula polymorpha [Gleeson, *et al.* (1986) *J. Gen. Microbiol. 132*:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet. 202*:302], Kluyveromyces fragilis [Das, *et al.* (1984) *J. Bacteriol. 158*:1165], Kluyveromyces lactis [De Louvencourt *et al.* (1983) *J. Bacteriol. 154*:737; Van den Berg *et al.* (1990) *Bio/Technology 8*:135], Pichia guillerimondii [Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141], Pichia pastoris [Cregg, *et al.* (1985) *Mol. Cell. Biol. 5*:3376; US Patent

Nos. 4,837,148 and 4,929,555], Saccharomyces cerevisiae [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA 75*:1929; Ito *et al.* (1983) *J. Bacteriol. 153*:163], Schizosaccharomyces pombe [Beach and Nurse (1981) *Nature 300*:706], and Yarrowia lipolytica [Davidow, *et al.* (1985) *Curr. Genet. 10*:380471 Gaillardin, *et al.* (1985) *Curr. Genet. 10*:49].

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See *eg.* [Kurtz *et al.* (1986) *Mol. Cell. Biol. 6*:142; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141; Candida]; [Gleeson *et al.* (1986) *J. Gen. Microbiol. 132*:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet. 202*:302; Hansenula]; [Das *et al.* (1984) *J. Bacteriol. 158*:1165; De Louvencourt *et al.* (1983) *J. Bacteriol. 154*:1165; Van den Berg *et al.* (1990) *Bio/Technology 8*:135; Kluyveromyces]; [Cregg *et al.* (1985) *Mol. Cell. Biol. 5*:3376; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141; US Patent Nos. 4,837,148 and 4,929,555; Pichia]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA 75*;1929; Ito *et al.* (1983) *J. Bacteriol. 153*:163 Saccharomyces]; [Beach and Nurse (1981) *Nature 300*:706; Schizosaccharomyces]; [Davidow *et al.* (1985) *Curr. Genet. 10*:39; Gaillardin *et al.* (1985) *Curr. Genet. 10*:49; Yarrowia].

Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanised antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying Neisserial proteins.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 μg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using

Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (*eg.* 1,000*g* for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein [*Nature* (1975) 256:495-96], or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells expressing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*eg.* hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (*eg.* in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I, or with an anti-biotin MAb labeled with HRP. Other permutations

and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Pharmaceutical Compositions

Pharmaceutical compositions can comprise either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions

are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines according to the invention may either be prophylactic (*ie.* to prevent infection) or therapeutic (*ie.* to treat disease after infection).

Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, *etc.* pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer

such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (*eg.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*eg.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59™ are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The immunogenic compositions (*eg.* the immunising antigen/immunogen/polypeptide/protein/ nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (*eg.* nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating

doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

The immunogenic compositions are conventionally administered parenterally, *eg.* by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (*eg.* WO98/20734). Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed [*eg.* Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; see later herein].

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses *eg.* MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma

Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (*eg.* HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC Nol VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this

invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (*ie.* there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in US Serial No. 08/405,627, filed March 15, 1995,WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, Nature 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example

ATCC VR-925; Triniti virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in US 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell

targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in US 5,149,655; use of ionizing radiation for activating transferred gene, as described in US 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, Biochemistry, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in *eg.* WO93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A.Polypeptides

One example are polypeptides which include, without limitation: asioloorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

B.Hormones, Vitamins, *etc.*

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C.Polyalkylenes, Polysaccharides, *etc.*

Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethlylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

D.Lipids, and Liposomes

The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, *eg.* Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammelar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See *eg.* Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77); Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348); Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E.Lipoproteins

In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells

expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) Annu Rev. Biochem 54:699; Law (1986) Adv. Exp Med. Biol. 151:162; Chen (1986) J Biol Chem 261:12918; Kane (1980) Proc Natl Acad Sci USA 77:2465; and Utermann (1984) Hum Genet 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750. Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Techniologies, Inc., Stoughton, Massachusetts, USA. Further description of lipoproteins can be found in WO98/06437.

F.Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents

have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic aid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and purtrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides/polypeptides.

Immunodiagnostic Assays

Neisserial antigens of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-Neisserial antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to Neisserial proteins within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridisation

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook *et al.* [*supra*] Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated Tm of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1μg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 μg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/μg. For a single-copy mammalian gene a conservative approach would start with 10 μg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/μg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (Tm) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$Tm = 81 + 16.6(\log_{10}Ci) + 0.4[\%(G + C)] - 0.6(\%formamide) - 600/n - 1.5(\%mismatch).$$

where Ci is the salt concentration (monovalent ions) and *n* is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (*ie.* stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 to 7 show BOXSHADE-rendered alignments of (1) ORF40 (2) ORF4 (3) 225 (4) 235 (5) 287 (6) 519 (7) 919. Conserved amino acids have a solid background.

Figure 8 shows a phylogenetic tree.

Figure 9A illustrates amino acid sequence variability within *N.meningitidis* for ORF4, ORF40, 225, 235, 287, 519, and 919. These sequences were used to construct the phylogenetic tree shown in Figure 9B.

Figures 10 to 19 show BOXSHADE-rendered alignments of (10) ORF4 (11) ORF40 (12) ORF46 (13) 225 (14) 235 (15) 287 (16) 519 (17) 726 (18) 919 (19) 953.

Figure 20 shows Western blots for ORF4, 225, 235, 519 and 919.

EXAMPLES

Example 1

Example 1 of WO99/36544 discloses the cloning and expression of a Neisserial protein referred to as "ORF40". Protein and DNA sequences from serogroup A and B *N.meningitidis* are disclosed, and the complete protein sequences show 83.7% identity over 601 aa overlap.

ORF40 was sequenced for a reference population of 21 strains of *N.meningitidis*:

Identification number	Strain	Reference
Group B		
zn02_1	BZ198	Seiler *et al.*(1996)
zn03_1	NG3/88	Seiler *et al.*(1996)
zn04_1	297-0	Seiler *et al.*(1996)
zn06_1	BZ147	Seiler *et al.*(1996)
zn07_1	BZ169	Seiler *et al.*(1996)
zn08_1	528	Seiler *et al.*(1996)
zn10_1	BZ133	Seiler *et al.*(1996)
zn11_1ass	NGE31	Seiler *et al.*(1996)
zn14_1	NGH38	Seiler *et al.*(1996)
zn16_1	NGH15	Seiler *et al.*(1996)
zn18_1	BZ232	Seiler *et al.*(1996)
zn19_1	BZ83	Seiler *et al.*(1996)
zn20_1	44/76	Seiler *et al.*(1996)
zn21_1	MC58	Virji *et al.* (1992)
Group A		
zn22_1	205900	Chiron SpA
zn23_1	F6124	Chiron SpA
z2491_1	Z2491	Maiden *et al.*(1998)
Group C		
zn24_1	90/18311	Chiron SpA
zn25_1ass	93/4286	Chiron SpA
Others		
zn28_1ass	860800 (group Y)	Maiden *et al.*(1998)
zn29_1ass	E32 (group Z)	Maiden *et al.*(1998)

An alignment of these 21 sequences is shown in Figure 1. Stretches of conserved amino acids are evident. The first 17 amino acids, for instance, are conserved (MNKIYRIIWNSALNAWV), although

the serine at residue 11 is not present in 100% of *Neisseria*. This is followed by an amino acid which is not conserved, which is in turn followed by a stretch of 16 conserved amino acids (VSELTRNHTKRASATV). The C-terminal of the protein consists of 116 conserved amino acids.

The conserved regions identified in this example confirm that fragments of the full-length ORF40 protein are suitable as multi-specific vaccines or diagnostic reagents.

ORF40 was re-sequenced for 31 strains in total, and the sequences were aligned. The results are shown in Figure 11.

Conserved regions of particular interest are:

- MNKIYRIIWNSALNAWV
- VSELTRNHTKRASATV
- TAVLATLL
- TLKAGDNLKIKQ
- FTYSLKKDLTDLTSV
- TEKLSFGANG
- KVNITSDTKGLNFAKETAGTNGD
- TVHLNGIGSTLTDTL
- RAAS(V/I)KDVLNAGWNIKGVK
- NVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTGK
- KGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGT
- GTTATVSKDDQGNITV
- YDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETVNINAGNNIEITRNGKNIDIATSM
- PQFSSVSLGAGADAPTLSVD
- NKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKSMMAI GGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW.

Example 2

Example 26 of WO99/24578 discloses the cloning and expression of a Neisserial protein referred to as "ORF4". Protein and DNA sequences from serogroup A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*. The identity between the sequences at an amino acid level are:

	N.meningitidis A	*N.gonorrhoeae*
N.meningitidis B	99.7% over 287 aa	97.6% over 288 aa

ORF4 was sequenced for a reference population of 32 strains of *Neisseria*:

Identification number	Strain	Reference
Group B		
zv01_4	NG6/88	Seiler *et al.* (1996)
zv02_4	BZ198	Seiler *et al.* (1996)
zv03_4ass	NG3/88	Seiler *et al.* (1996)
zv04_4	297-0	Seiler *et al.* (1996)
zv05_4	1000	Seiler *et al.* (1996)
zv06_4	BZ147	Seiler *et al.* (1996)
zv07_4	BZ169	Seiler *et al.* (1996)
zv08_4	528	Seiler *et al.* (1996)
zv09_4	NGP165	Seiler *et al.* (1996)
zv10_4	BZ133	Seiler *et al.* (1996)
zv11_4	NGE31	Seiler *et al.* (1996)
zv12_4ass	NGF26	Seiler *et al.* (1996)
zv13_4	NGE28	Seiler *et al.* (1996)
zv15_4	SWZ107	Seiler *et al.* (1996)
zv16_4	NGH15	Seiler *et al.* (1996)
zv17_4	NGH36	Seiler *et al.* (1996)
zv18_4	BZ232	Seiler *et al.* (1996)
zv19_4	BZ83	Seiler *et al.* (1996)
zv20_4	44/76	Seiler *et al.* (1996)
zv21_4	MC58	Virji *et al.* (1992)
zv96_4	2996	Chiron SpA
Group A		
zv22_4	205900	Chiron SpA
z2491_4	Z2491	Maiden *et al.*, 1998
Group C		
zv24_4	90/18311	Chiron SpA
zv25_4	93/4286	Chiron SpA
Others		
zv26_4ass	A22 (group W)	Maiden *et al.* (1998)
zv27_4	E26 (group X)	Maiden *et al.* (1998)
zv28_4	860800(group Y)	Maiden *et al.* (1998)
zv29_4	E32 (group Z)	Maiden *et al.* (1998)
N.gonorrhoeae		
zv32_4	Ng F62	Maiden *et al.* (1998)
zv33_4	Ng SN4	R. Moxon

fa1090_4	FA1090	Dempsey *et al.* (1991)

An alignment of the sequences generated using PILEUP is shown in Figure 2. Stretches of conserved amino acids are evident. The first 34 amino acids, for instance, are conserved, although the serine at residue 26 is not present in 100% of *Neisseria*. The C-terminal of the protein consists of 228 conserved amino acids.

The conserved regions identified in this example confirm that fragments of the full-length ORF4 protein are suitable as multi-specific vaccines or diagnostic reagents.

ORF4 was re-sequenced for 35 strains in total, and the sequences were aligned. The results are shown in Figure 10.

Conserved regions of particular interest are:

- MKTFFKTLSAAALALILAACGGQKDSAPAASASAAADNGA
- KKEIVFGTTVGDFGDMVKE
- ELEKKGYTVKLVEFTDYVRPNLALAEGELDINVFQHKPYLDDFKKEHNLDITEVFQVPTAPLGLYPGK LKSLEEVKDGSTVSAPNDPSNFARVLVMLDELGWIKLKDGINPLTASKADIAENLKNIKIVELEAAQL PRSRADVDFAVVNGNYAISSGMKLTEALFQEPSFAYVNWSAVKTADKDSQWLKDVTEAYNSDAFKAYA HKRFEGYKSPAAWNEGAAK

Example 3

Example 16 of WO99/57280 discloses the cloning and expression of a Neisserial protein referred to as "225". Protein and DNA sequences from serogroup A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*.

225 has now been sequenced for a reference population of 34 strains of *Neisseria*:

Identification number	**Strain**	**Source reference**
Group B		
zo01_225	NG6/88	Seiler *et al.*, 1996
zo02_225	BZ198	Seiler *et al.*, 1996
zo03_225	NG3/88	Seiler *et al.*, 1996
zo04_225	297-0	Seiler *et al.*, 1996
zo05_225	1000	Seiler *et al.*, 1996
zo06_225	BZ147	Seiler *et al.*, 1996
zo07_225	BZ169	Seiler *et al.*, 1996
zo08_225	528	Seiler *et al.*, 1996

zo09_225	NGP165	Seiler *et al.*, 1996
zo10_225	BZ133	Seiler *et al.*, 1996
zo11_225	NGE31	Seiler *et al.*, 1996
zo12_225	NGF26	Seiler *et al.*, 1996
zo13_225	NGE28	Seiler *et al.*, 1996
zo14_225	NGH38	Seiler *et al.*, 1996
zo15_225	SWZ107	Seiler *et al.*, 1996
zo16_225	NGH15	Seiler *et al.*, 1996
zo17_225	NGH36	Seiler *et al.*, 1996
zo18_225	BZ232	Seiler *et al.*, 1996
zo19_225	BZ83	Seiler *et al.*, 1996
zo20_225	44/76	Seiler *et al.*, 1996
zo21_225	MC58	Chiron SpA
zo96_225	2996	Chiron SpA
Group A		
zo22_225	205900	Chiron SpA
zo23_225	F6124	Chiron SpA
z2491	Z2491	Maiden *et al.*, 1998
Group C		
zo24_225	90/18311	Chiron SpA
zo25_225	93/4286	Chiron SpA
Others		
zo26_225	A22 (group W)	Maiden *et al.*, 1998
zo27_225	E26 (group X)	Maiden *et al.*, 1998
zo28_225	860800(group Y)	Maiden *et al.*, 1998
zo29_225	E32 (group Z)	Maiden *et al.*, 1998
Gonococcus		
zo32_225	Ng F62	Maiden *et al.*, 1998
zo33_225	Ng SN4	Chiron SpA
fa1090	FA1090	Chiron SpA

An alignment of the sequences generated using PILEUP is shown in Figure 3. Stretches of conserved amino acids are evident. The first 74 amino acids, for instance, are conserved, although the isoleucine at residue 51 is not present in 100% of *Neisseria*. The C-terminal of the protein consists of 148 conserved amino acids. A similar alignment is shown in Figure 13.

The conserved regions identified in this example confirm that fragments of the full-length 225 protein are suitable as multi-specific vaccines or diagnostic reagents.

Example 4

Example 16 of WO99/57280 discloses the cloning and expression of a Neisserial protein referred to as "235". Protein and DNA sequences from serogroup A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*.

235 has now been sequenced for a reference population of 31 strains of *Neisseria*:

Identification number	**Strain**	**Reference**
Group B		
gnmzq01	NG6/88	Seiler *et al.*, 1996
gnmzq02	BZ198	Seiler *et al.*, 1996
gnmzq03	NG3/88	Seiler *et al.*, 1996
gnmzq04	1000	Seiler *et al.*, 1996
gnmzq05	1000	Seiler *et al.*, 1996
gnmzq07	BZ169	Seiler *et al.*, 1996
gnmzq08	528	Seiler *et al.*, 1996
gnmzq09	NGP165	Seiler *et al.*, 1996
gnmzq10	BZ133	Seiler *et al.*, 1996
gnmzq11	NGE31	Seiler *et al.*, 1996
gnmzq13	NGE28	Seiler *et al.*, 1996
gnmzq14	NGH38	Seiler *et al.*, 1996
gnmzq15	SWZ107	Seiler *et al.*, 1996
gnmzq16	NGH15	Seiler *et al.*, 1996
gnmzq17	NGH36	Seiler *et al.*, 1996
gnmzq18	BZ232	Seiler *et al.*, 1996
gnmzq19	BZ83	Seiler *et al.*, 1996
gnmzq21	MC58	Virji *et al.*, 1992
Group A		
gnmzq22	205900	Chiron SpA
gnmzq23	F6124	Chiron SpA
z2491	Z2491	Maiden *et al.*, 1998
Group C		
gnmzq24	90/18311	Chiron SpA
gnmzq25	93/4286	Chiron SpA
Others		
gnmzq26	A22 (group W)	Maiden *et al.*, 1998
gnmzq27	E26 (group X)	Maiden *et al.*, 1998

gnmzq28	860800 (group Y)	Maiden *et al.*, 1998
gnmzq29	E32 (group Z)	Maiden *et al.*, 1998
gnmzq31	*N. lactamica*	Chiron SpA
Gonococcus		
gnmzq32	Ng F62	Maiden *et al.*, 1998
gnmzq33	Ng SN4	Chiron SpA
fa1090	FA1090	Dempsey *et al.* 1991

An alignment of the sequences generated using PILEUP is shown in Figure 4. Stretches of conserved amino acids are evident. The protein is wholly conserved, although the serine at residue 168 shows some variance.

The conserved regions identified in this example confirm that fragments of the full-length 235 protein are suitable as multi-specific vaccines or diagnostic reagents.

235 was re-sequenced for 35 strains in total, and the sequences were aligned. The results are shown in Figure 14.

Example 5

Example 16 of WO99/57280 discloses the cloning and expression of a Neisserial protein referred to as "287". Protein and DNA sequences from serogroup A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*.

287 has now been sequenced for a reference population of 6 strains of *Neisseria*:

Identification number	**Strain**	**Reference**
Group B		
287_2	BZ198	Seiler *et al.*(1996)
287_9	NGP165	Seiler *et al.*(1996)
287_14	NGH38	Seiler *et al.*(1996)
287_21	MC58	Virji *et al.* (1992)
Group A		
z2491	Z2491	Maiden *et al.*(1998)
Gonococcus		
fa1090	FA1090	Dempsey *et al.* (1991)

An alignment of the sequences generated using PILEUP is shown in Figure 5. Stretches of conserved amino acids are evident. The first 42 amino acids, for instance, are well conservedand a long conserved region can be seen at the C-terminus.

The conserved regions identified in this example confirm that fragments of the full-length 287 protein are suitable as multi-specific vaccines or diagnostic reagents.

287 was re-sequenced for 35 strains in total (including C11, a serogroup C *N.meningitidis* strain), and the sequences were aligned. The results are shown in Figure 15.

Conserved regions of particular interest are:

- MFKRSVIAMACI
- ALSACGGGGGGSPDVKSADT
- SKPAAPVV
- QDMAAVS
- ENTGNGGAATTD
- QNDMPQ
- DGPSQNITLTHCK
- KSEFE
- RRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKL
- GGSYAL
- VQGEPAKGEMLAGTAVYNGEVLHFH
- GRFAAKVDFGSKSVDGIIDSGDDLHMG
- QKFKAAIDGNGFKGTWTENGGGDVSG(R/K)FYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKDRD

Example 6

Example 16 of WO99/57280 discloses the cloning and expression of a Neisserial protein referred to as "519". Protein and DNA sequences from serogroup A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*.

519 has now been sequenced for a reference population of 22 strains of *Neisseria*:

Identification number	Strain	Source
Group B		
zv01_519	NG6/88	Seiler *et al.*, 1996
zv02_519	BZ198	Seiler *et al.*, 1996
zv03_519ass	NG3/88	Seiler *et al.*, 1996
zv04_519	297-0	Seiler *et al.*, 1996
zv05_519	1000	Seiler *et al.*, 1996

zv06_519ass	BZ147	Seiler *et al.*, 1996
zv07_519	BZ169	Seiler *et al.*, 1996
zv11_519	NGE31	Seiler *et al.*, 1996
zv12_519	NGF26	Seiler *et al.*, 1996
zv18_519	BZ232	Seiler *et al.*, 1996
zv19_519	BZ83	Seiler *et al.*, 1996
zv20_519ass	44/76	Seiler *et al.*, 1996
zv21_519ass	MC58	Chiron SpA
zv96_519	2996	Chiron SpA
Group A		
zv22_519ass	205900	Chiron SpA
z2491_519	Z2491	Maiden *et al.*, 1998
Others		
zv26_519	A22 (group W)	Maiden *et al.*, 1998
zv27_519	E26 (group X)	Maiden *et al.*, 1998
zv28_519	860800 (group Y)	Maiden *et al.*, 1998
zv29_519ass	E32 (group Z)	Maiden *et al.*, 1998
Gonococcus		
zv32_519	Ng F62	Maiden *et al.*, 1998
fa1090_519	FA1090	Chiron SpA

An alignment of the sequences generated using PILEUP is shown in Figure 6. Stretches of conserved amino acids are evident, and the protein shows conservation along its complete length.

The conserved regions identified in this example confirm that fragments of the full-length 519 protein are suitable as multi-specific vaccines or diagnostic reagents.

519 was re-sequenced for 33 strains in total, and the sequences were aligned. The results are shown in Figure 16.

Conserved regions of particular interest are:

- MEFFIILL
- AVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSLKEIPLDVPSQVCITRDN TQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVV
- ALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQ QSEGEAQAAVNASNAEKIARINRAKGEAESLRLVAEANAEANRQIAAALQTQSGADAVNLKIAGQYVT AFKNLAKEDNTRIKPAKVAEIGNPNFRRHEKFSPEAKTAK

Example 7

Example 16 of WO99/57280 discloses the cloning and expression of a Neisserial protein referred to as "919". Protein and DNA sequences from serogroup A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*.

919 has now been sequenced for a reference population of 35 strains of *Neisseria*:

Identification number	Strains	Source
Group B		
zm01	NG6/88	Seiler *et al.*, 1996
zm02	BZ198	Seiler *et al.*, 1996
zm03	NG3/88	Seiler *et al.*, 1996
zm04	297-0	Seiler *et al.*, 1996
zm05	1000	Seiler *et al.*, 1996
zm06	BZ147	Seiler *et al.*, 1996
zm07	BZ169	Seiler *et al.*, 1996
zm08n	528	Seiler *et al.*, 1996
zm09	NGP165	Seiler *et al.*, 1996
zm10	BZ133	Seiler *et al.*, 1996
zm11asbc	NGE31	Seiler *et al.*, 1996
zm12	NGF26	Seiler *et al.*, 1996
zm13	NGE28	Seiler *et al.*, 1996
zm14	NGH38	Seiler *et al.*, 1996
zm15	SWZ107	Seiler *et al.*, 1996
zm16	NGH15	Seiler *et al.*, 1996
zm17	NGH36	Seiler *et al.*, 1996
zm18	BZ232	Seiler *et al.*, 1996
zm19	BZ83	Seiler *et al.*, 1996
zm20	44/76	Seiler *et al.*, 1996
zm21	MC58	Chiron SpA
zm96	2996	Chiron SpA
Group A		
zm22	205900	Chiron SpA
zm23asbc	F6124	Chiron SpA
z2491	Z2491	Maiden *et al.*, 1998
Group C		
zm24	90/18311	Chiron SpA
zm25	93/4286	Chiron SpA

Others		
zm26	A22 (group W)	Maiden *et al.*, 1998
zm27bc	E26 (group X)	Maiden *et al.*, 1998
zm28	860800 (group Y)	Maiden *et al.*, 1998
zm29asbc	E32 (group Z)	Maiden *et al.*, 1998
zm31asbc	*N. lactamica*	Chiron SpA
Gonococcus		
zm32asbc	Ng F62	Maiden *et al.*, 1998
zm33asbc	Ng SN4	Chiron SpA
fa1090	FA1090	Chiron SpA

An alignment of the sequences generated using PILEUP is shown in Figure 7. Another alignment is shown in Figure 18. Stretches of conserved amino acids are evident. The protein shows almost complete conservation.

The conserved regions identified in this example confirm that fragments of the full-length 919 protein are suitable as multi-specific vaccines or diagnostic reagents.

Conserved regions of particular interest are:

- MKKYLFRAAL
- GIAAAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTV(G/A)GGGAVYTVVPHLSLPHWAAQ DFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFERYFTPWQVAGNGSLAGTVTGYYEP VLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKALVRIRQTGKNSGTIDN
- GGTHTADLS
- FPITARTTAIKGRFEGSRFLPYHTRNQINGGALDGKAPILGYAEDPVELFFMHIQGSGRLKTPSGKYI RIGYADKNEHPYVSIG(R/K)YMADKGYLKLGQTSMQGIK
- YMRQNPQRLAEVLGQNPSYIFFREL
- NDGPVGALGTPLMGEYAGAVDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDYFWG YGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP

Example 8

Example 55 of WO99/23578 discloses the cloning and expression of a Neisserial protein referred to as "ORF46". Protein and DNA sequences from serogroups A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*.

Full-length ORF46 has been sequenced for a reference population of 6 strains of serogroup B. An alignment of these sequences is shown in Figure 12, from which stretches of conserved amino acids are evident.

Conserved regions of particular interest are:

- RKISLILSILAVCLPMHAHASDLANDSFIRQVLDRQHFEPDGKYHLFGSRGELAERSGHIGLG
- IQSHQLGNLMIQQAAIKGNIGYIVRFSDHGHEVHSPFDNHASHSDSDEAGSPVDGFSLYRIHWDGYEH HPADGYDGPQGGGYPAPKGARDIYSYDIKGVAQNIRLNLTDNRSTGQRLADRFHNAG
- MLTQGVGDGFKRATRYSPELDRSGNAAEAFNGTADIVKNIIGAAGEIVGAGDAVQGISEGSNIAVMHG LGLLSTENKMARINDLADMAQLKDYAAAAIRDWAVQNPNAAQGIEAVSNIF
- IPIKGIGAVRGKYGLGGITAHP(V/I)KRSQMGEIALPKGKSAVS
- NFADAAYAKYPSPYHSRNIRSNLEQRYGKENITSSTVPPSNGKNVKLANKRHPKTKVPFDGKGFPNFE KDVKY

The conserved regions in ORF46 confirm that fragments of this protein are suitable as multi-specific vaccines or diagnostic reagents.

Example 9

WO99/57280 discloses the cloning and expression of a Neisserial protein referred to as "726". Protein and DNA sequences from serogroups A and B *N.meningitidis* are disclosed.

726 has been sequenced for a reference population of 7 *N.meningitidis* strains in serogroups A, B and C. An alignment of these sequences is shown in Figure 17, from which stretches of conserved amino acids are evident.

Conserved regions of particular interest are:

- IYFKNGFYDDTLG
- IPEGAVAVRAEEYAALLAGQAQGGQIAADSDGRPVLTPPRPS(D/E)YHEWDGKKW
- AAAAARFAEQKTATAFRLA
- KADELKNSLLAGYPQVEIDSFYRQEKEALARQADNNAPTPMLAQIAAARGVELDVLIEKV(I/V)EKS ARLAVAAGAIIGKRQQLEDKLN
- IETAPGLDALEKEIEEWT

The conserved regions in 726 confirm that fragments of this protein are suitable as multi-specific vaccines or diagnostic reagents.

Example 10

WO99/57280 discloses the cloning and expression of a Neisserial protein referred to as "953". Protein and DNA sequences from serogroups A and B *N.meningitidis* are disclosed, along with sequences from *N.gonorrhoeae*.

953 has been sequenced for a reference population of 8 strains of *N.meningitidis* serogroups A, B and C. An alignment of these sequences is shown in Figure 19, from which stretches of conserved amino acids are evident. The protein is well-conserved.

Conserved regions of particular interest are:

- MKKIIFAALAAAAVGTASAATYKVDEYHANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDIT IP(I/V)ANLQSGSQHFTDHLKSADIFDAAQYPDIRFVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLK AEKFNCYQSPM
- ATYKVDEYHANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIP(I/V)ANLQSGSQHFTD HLKSADIFDAAQYPDIRFVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPM
- KTEVCGGDFSTTIDRTKWG(M/V)DYLVNVGMTKSVRIDIQIEAAKQ

The conserved regions in 953 confirm that fragments of this protein are suitable as multi-specific vaccines or diagnostic reagents.

Phylogenetic tree

Figure 8 is a dendrogram showing the genetic relationship among 107 *N.meningitidis* strains, based on MLST analysis of six gene fragments [adapted from Maiden *et al.* (1998) *PNAS USA* 95:3140]. The dendrogram can be used to select strains representative of meningococcus serogroup B (arrows). Five additional strains, for which genetic assignment to hypervirulent lineages has been independently determined by Wang *et al.* [*J.Infect.Dis* (1993) 167:1320], Seiler *et al.* [*Mol.Microbiol.* (1996) 19:841], and Virji *et al.* [*Mol.Microbiol.* (1992) 6:1271] are superimposed on the dendrogram and indicated by asterisks. In addition to the 22 strains of MenB, three strains of MenA, two strains of MenC, and one strain each of Men Y, X, Z and W135 were used. These are indicated by bold letters before the name. Where phylogenetic data were not available, the strains are shown outside the tree. The hypervirulent strains ET-5, ET-37 and IV-1 are indicated.

Sequence variability

Figure 9a is a schematic representation of amino acid sequence variability with *N.meningitidis* for proteins 225, 235, 287, 519, 919, ORF4 and ORF40. The horizontal axis represents the sequence of MC58. Amino acid differences within MenB strains are indicated by vertical lines above the horizontal axis; differences within serogroups A, C, Y, X, Z and W135 are indicated by lines below the axis. The height of the vertical lines represents the number of strains with amino acid differences. Peaks thus show variable regions. The bard below 225 and 287 represent sequence segments that are missing from some strains.

Figure 9b is a dendrogram of *N.meningitidis* strains obtained using the same 7 proteins. The phylogenetic analysis based on these genes provided a dendrogram which clusters the hypervirulent strains in agreement with Figure 8. Bars indicate strains which cluster with 100% bootstrap support in agreement with MLST analysis. Numbers at the base of each node are bootstrap scores (only those >80% are reported). Gene sequences from different strains were aligned with the program PILEUP from the GCG package. The phylogenetic analysis was performed using the neighbour-joining algorithm [Saitou & Nei (1987) *Mol.Biol.Evol.* 4:406] as implemented in the NEIGHBOR program of the PHYLIP package. Pairwise distances were calculated using the Kimura-two parameter [Kimura (1980) *J.Mol.Evol.* 16:111] on the 31 *N.meningitidis* strains. The N-terminal region of ORF40, the entire 287, and the tandem repeats of 225 were excluded from the analysis. A total of 1000 bootstrap replicates were allowed to evaluate the level of support. The clustering of the hypervirulent strains was confirmed by maximum parsimony analysis.

Western blots

Antigens ORF4, 225, 235, 519 and 919 were analysed by Western blot for various strains. The results are shown in Figure 20. In the case of 225, the blot shows fragments of different sizes in the different strains, with arrows indicating the band of correct size. 225 contains regions of deletion and insertion of a defined repeat and the size of the fragments on the blots matches the gene variability data.

The strains used for Figure 20 are as follows:

<u>*N. meningitidis* serogroup B:</u>

1 = NG6/88	2 = BZ198	3 = NG3/88	4 = 297-0	5 = 1000
6 = BZ147	7 = BZ169	8 = 528	9 = NGP165	10 = BZ133
11 = NGE31	12 = NGF26	13 = NGE28	14 = NGH38	15 = SWZ107
16 = NGH15	17 = NGH36	18 = BZ232	19 = BZ83	20 = 44/76
21 = MC58	96 = 2996			

<u>*N. meningitidis* serogroup A:</u>

22 = 205900 23 = F6124

<u>*N. meningitidis* serogroup C:</u>

24 = 90/18311 25 = 93/4286

Other *N.meningitidis*

26 = A22 (serogroup W)

27 = E26 (serogroup X)

28 = 860800 (serogroup Y)

29 = E32 (serogroup Z)

Other *Neisseria*

30 = *N. cinerea*

L17 = *N.lactamica*

L19 = *N.lactamica*

31 = *N. gonorrhoeae* F62

32 = *N.gonorrhoeae* SN4

It will be appreciated that the invention has been described by means of example only, and that modifications may be made whilst remaining within the spirit and scope of the invention.

CLAIMS

1. A protein comprising a fragment of a Neisserial protein, wherein said fragment consists of 7 or more consecutive conserved amino acids, provided that said protein is not a full-length Neisserial protein.

2. The protein of claim 1, wherein said fragment consists of 20 or more consecutive conserved amino acids.

3. The protein of claim 1 or claim 2, wherein the conserved amino acids are found in at least 50% or more of a *Neisseria* reference population.

4. The protein of claim 3, wherein the reference population includes a plurality of different *Neisseria* species, preferably *N.meningitidis* and *N.gonorrhoeae*.

5. The protein of claim 4, wherein the reference population includes a plurality of different serogroups of *N.meningitidis*.

6. The protein of claim 3 or claim 4, wherein the reference population comprises: *N.meningitidis* A, strain Z2491; *N.meningitidis* B, strain NG6/88; *N.meningitidis* W, strain A22; and *N.gonorrhoeae*, strain Ng F62.

7. The protein of claim 5, wherein the reference *population* comprises: *N.meningitidis* A, strain Z2491; *N.meningitidis* B, strain NG6/88; and *N.meningitidis* W, strain A22.

8. The protein of any preceding claim, comprising a fragment of a protein disclosed in WO99/24578, WO99/36544, WO99/57280 or WO00/22430.

9. The protein of claim 8, comprising a fragment of one or more of ORF4, ORF40, ORF46, protein 225, protein 235, protein 287, protein 519, protein 726, protein 919 and protein 953.

10. The protein of any one of claims 1 to 7, comprising a fragment of a protein disclosed in Tettelin *et al.* [*Science* (2000) 287:1809-1815].

11. Nucleic acid encoding a protein according to any preceding claim.

12. A protein according to any one of claims 1 to 10, or nucleic acid according to claim 11, for use as a medicament.

13. The use a protein according to any one of claims 1 to 10, or nucleic acid according to claim 11, in the manufacture of a medicament for treating or preventing infection due to Neisserial bacteria.

14. The use a protein according to any one of claims 1 to 10, or nucleic acid according to claim 11, in the manufacture of a multi-specific diagnostic reagent.

15. A protein according to claim 1, comprising one or more of the following amino acid sequences:

MNKIYRIIWNSALNAWV;

VSELTRNHTKRASATV;

TAVLATLL;

TLKAGDNLKIKQ;

FTYSLKKDLTDLTSV;

TEKLSFGANG;

KVNITSDTKGLNFAKETAGTNGD;

TVHLNGIGSTLTDTL;

RAAS(V/I)KDVLNAGWNIKGVK;

NVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTGK;

KGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGT;

GTTATVSKDDQGNITV;

YDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSPSKGKMDETVNINAGNNIEITRNGKNIDIATSM;

PQFSSVSLGAGADAPTLSVD;

NKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGLVQAYLPGKSMMAIGGGTY
RGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQW.;

MKTFFKTLSAAALALILAACGGQKDSAPAASASAAADNGA;

KKEIVFGTTVGDFGDMVKE;

ELEKKGYTVKLVEFTDYVRPNLALAEGELDINVFQHKPYLDDFKKEHNLDITEVFQVPTAPLGLYPGKLKSLE
EVKDGSTVSAPNDPSNFARVLVMLDELGWIKLKDGINPLTASKADIAENLKNIKIVELEAAQLPRSRADVDFA
VVNGNYAISSGMKLTEALFQEPSFAYVNWSAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNE
GAAK;

MFKRSVIAMACI;

ALSACGGGGGGSPDVKSADT;

SKPAAPVV;

QDMAAVS;

ENTGNGGAATTD;

DGPSQNITLTHCK;

RRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKL;

VQGEPAKGEMLAGTAVYNGEVLHFH;

GRFAAKVDFGSKSVDGIIDSGDDLHMG;

QKFKAAIDGNGFKGTWTENGGGDVSG(R/K)FYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKDRD;

MEFFIILL;

AVAVFGFKSFVVIPQQEVHVVERLGRFHRALTAGLNILIPFIDRVAYRHSLKEIPLDVPSQVCITRDNTQLTV
DGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIGRMELDKTFEERDEINSTVV;

ALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGE
AQAAVNASNAEKIARINRAKGEAESLRLVAEANAEANRQIAAALQTQSGADAVNLKIAGQYVTAFKNLAKEDN
TRIKPAKVAEIGNPNFRRHEKFSPEAKTAK;

MKKYLFRAAL;

GIAAAILAACQSKSIQTFPQPDTSVINGPDRPVGIPDPAGTTV(G/A)GGGAVYTVVPHLSLPHWAAQDFAKS
LQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFERYFTPWQVAGNGSLAGTVTGYYEPVLKGDDRRTA
QARFPIYGIPDDFISVPLPAGLRSGKALVRIRQTGKNSGTIDN;

GGTHTADLS;

FPITARTTAIKGRFEGSRFLPYHTRNQINGGALDGKAPILGYAEDPVELFFMHIQGSGRLKTPSGKYIRIGYA
DKNEHPYVSIG(R/K)YMADKGYLKLGQTSMQGIK;

YMRQNPQRLAEVLGQNPSYIFFREL;

NDGPVGALGTPLMGEYAGAVDRHYITLGAPLFVATAHPVTRKALNRLIMAQDTGSAIKGAVRVDYFWGYGDEA
GELAGKQKTTGYVWQLLPNGMKPEYRP;

RKISLILSILAVCLPMHAHASDLANDSFIRQVLDRQHFEPDGKYHLFGSRGELAERSGHIGLG;

IQSHQLGNLMIQQAAIKGNIGYIVRFSDHGHEVHSPFDNHASHSDSDEAGSPVDGFSLYRIHWDGYEHHPADG
YDGPQGGGYPAPKGARDIYSYDIKGVAQNIRLNLTDNRSTGQRLADRFHNAG;

MLTQGVGDGFKRATRYSPELDRSGNAAEAFNGTADIVKNIIGAAGEIVGAGDAVQGISEGSNIAVMHGLGLLS
TENKMARINDLADMAQLKDYAAAAIRDWAVQNPNAAQGIEAVSNIF;

IPIKGIGAVRGKYGLGGITAHP(V/I)KRSQMGEIALPKGKSAVS;

NFADAAYAKYPSPYHSRNIRSNLEQRYGKENITSSTVPPSNGKNVKLANKRHPKTKVPFDGKGFPNFEKDVKY;

IYFKNGFYDDTLG;

IPEGAVAVRAEEYAALLAGQAQGGQIAADSDGRPVLTPPRPS(D/E)YHEWDGKKW;

AAAAARFAEQKTATAFRLA;

KADELKNSLLAGYPQVEIDSFYRQEKEALARQADNNAPTPMLAQIAAARGVELDVLIEKV(I/V)EKSARLAV
AAGAIIGKRQQLEDKLN;

IETAPGLDALEKEIEEWT;

MKKIIFAALAAAAVGTASAATYKVDEYHANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIP(I/V
)ANLQSGSQHFTDHLKSADIFDAAQYPDIRFVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPM;

ATYKVDEYHANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIP(I/V)ANLQSGSQHFTDHLKSA
DIFDAAQYPDIRFVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPM; and

KTEVCGGDFSTTIDRTKWG(M/V)DYLVNVGMTKSVRIDIQIEAAKQ.

FIG. 1A

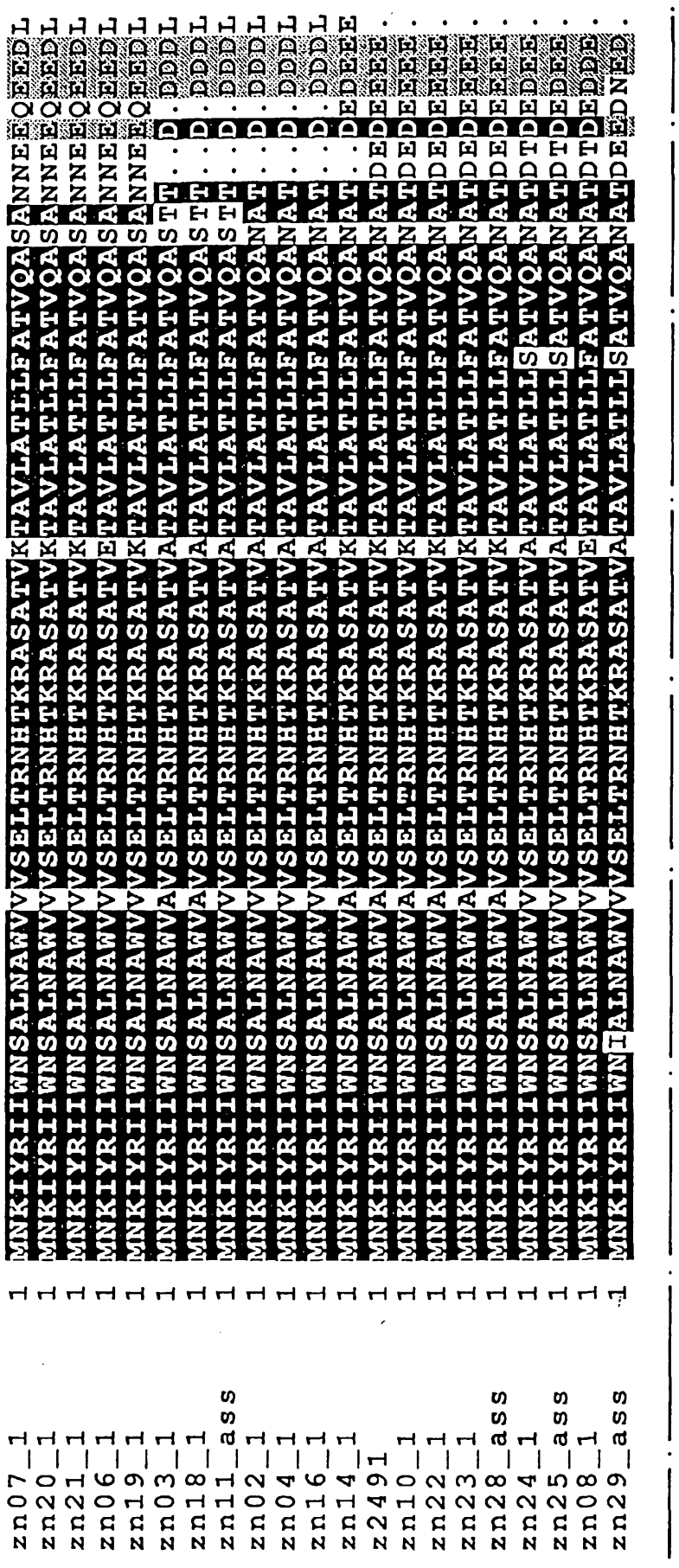


FIG. 1A(CONTD.)

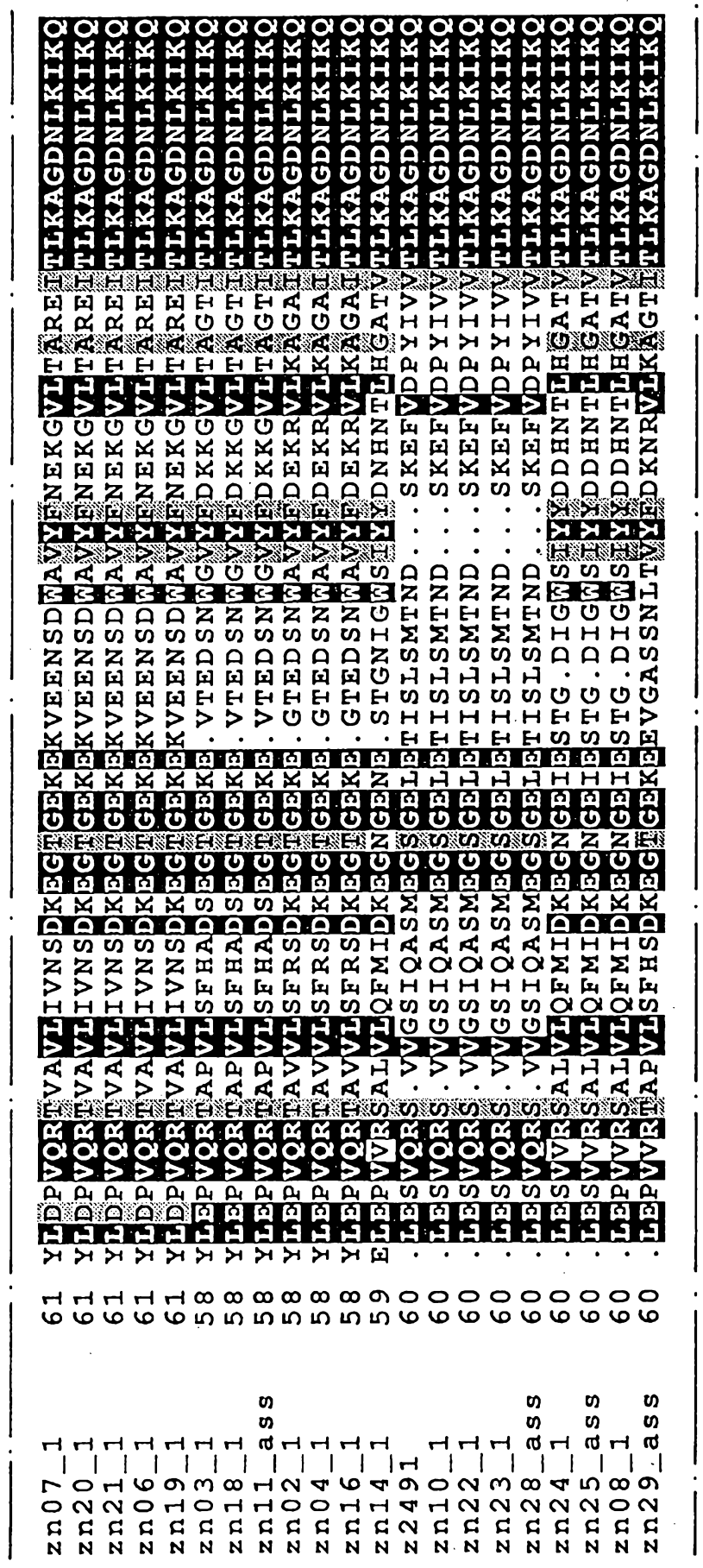


FIG. 1A(CONTD.)

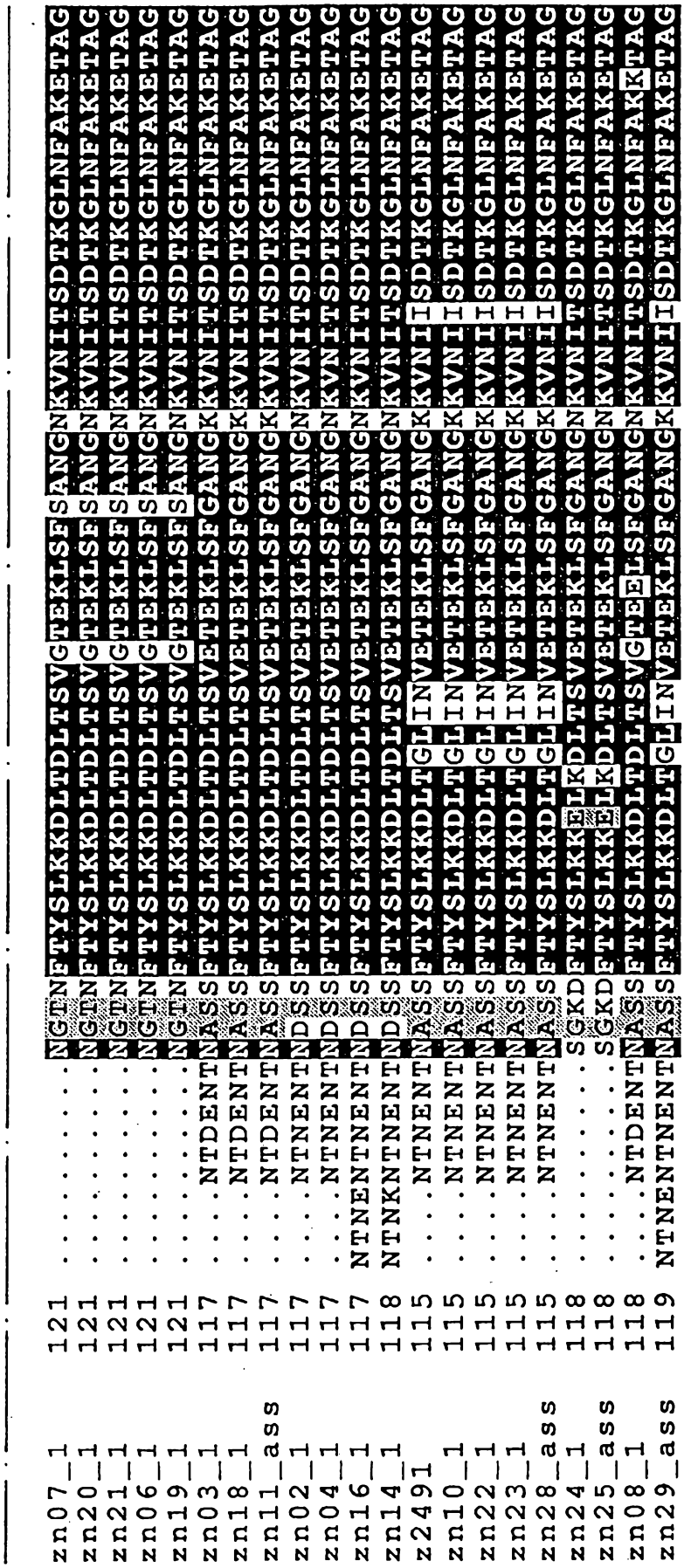


FIG. 1B

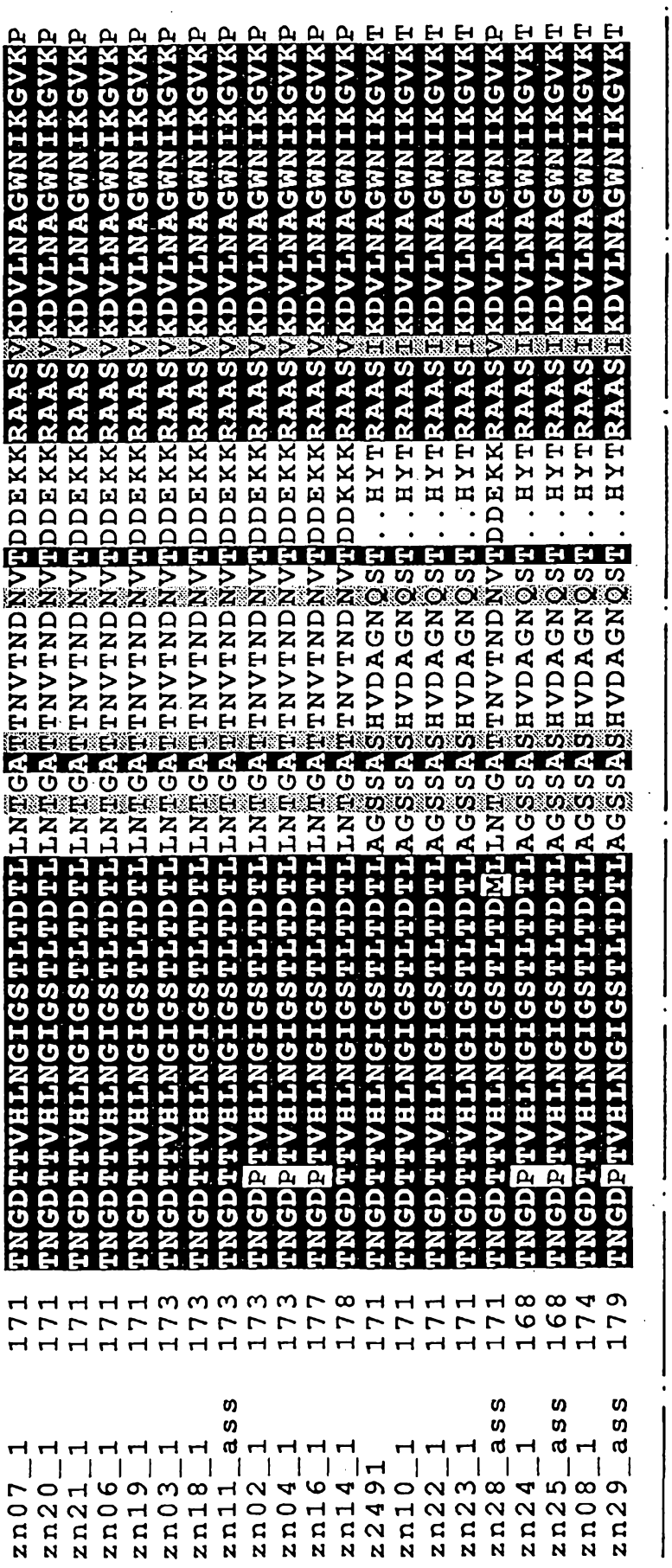


FIG. 1B(CONTD.)

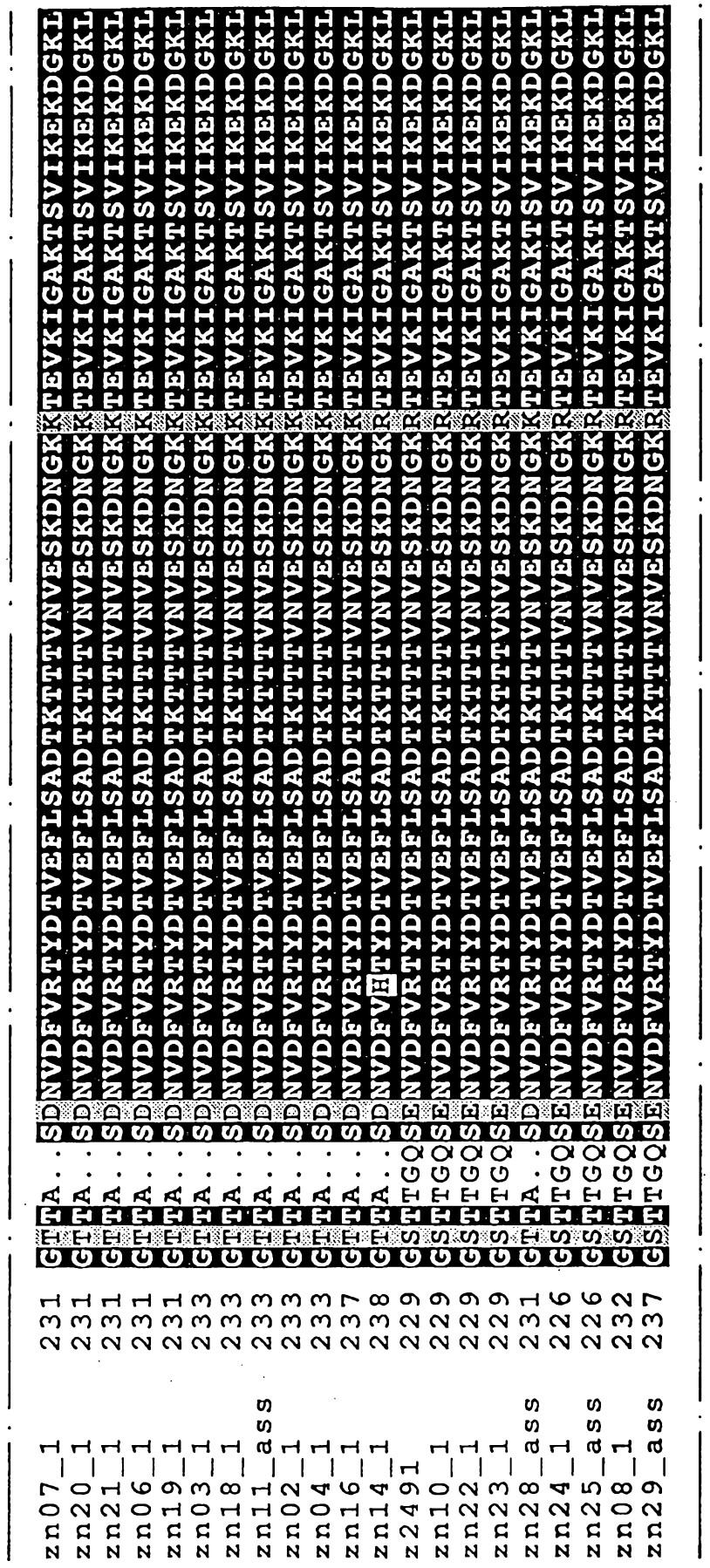

zn07_1 231 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn20_1 231 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn21_1 231 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn06_1 231 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn19_1 231 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn03_1 233 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn18_1 233 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn11_ass 233 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn02_1 233 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn04_1 233 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn16_1 237 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn14_1 238 GTTA..SDNVDFVHTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
z2491 229 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
zn10_1 229 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
zn22_1 229 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
zn23_1 229 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
zn28_ass 231 GTTA..SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKL
zn24_1 226 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
zn25_ass 226 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
zn08_1 232 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL
zn29_ass 237 GSTTGQSENVDFVRTYDTVEFLSADTKTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKL

FIG. 1B(CONTD.)

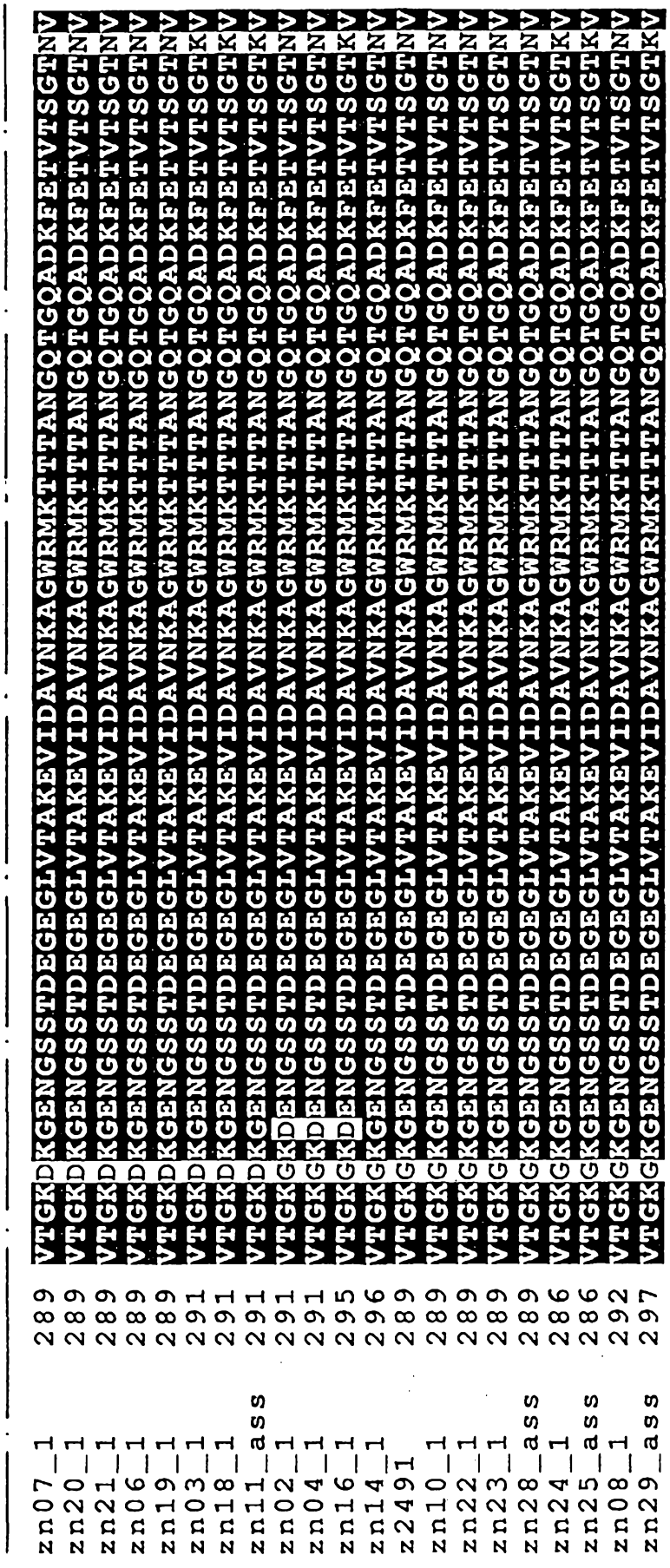

zn07_1 289 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn20_1 289 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn21_1 289 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn06_1 289 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn19_1 289 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn03_1 291 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV
zn18_1 291 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV
zn11_ass 291 VTGKDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV
zn02_1 291 VTGKGKDENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn04_1 291 VTGKGKDENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn16_1 295 VTGKGKDENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV
zn14_1 296 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
z2491 289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn10_1 289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn22_1 289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn23_1 289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn28_ass 289 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn24_1 286 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV
zn25_ass 286 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV
zn08_1 292 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNV
zn29_ass 297 VTGKGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTKV

FIG. 1C

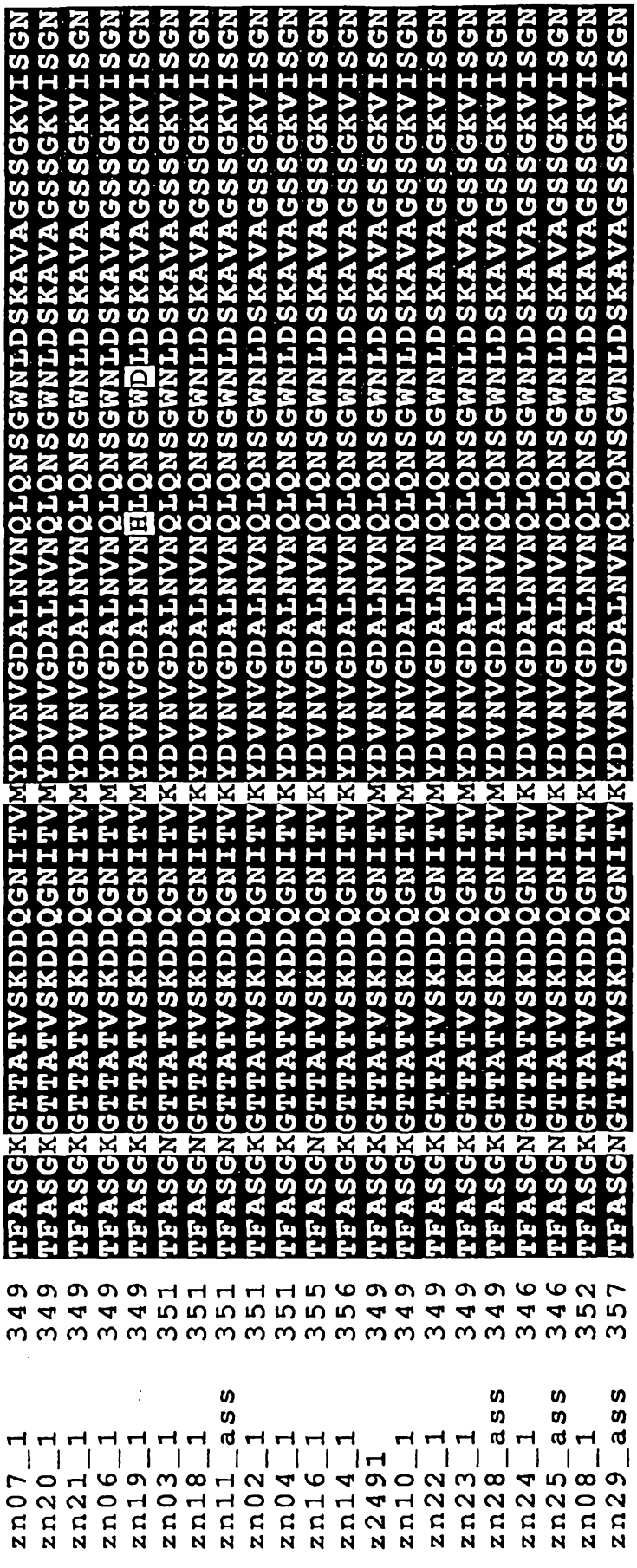

zn07_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn20_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn21_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn06_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn19_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNHLQNSGWDLDSKAVAGSSGKVISGN
zn03_1 351 TFASGNGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn18_1 351 TFASGNGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn11_ass 351 TFASGNGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn02_1 351 TFASGKGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn04_1 351 TFASGKGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn16_1 355 TFASGNGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn14_1 356 TFASGKGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
z2491 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn10_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn22_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn23_1 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn28_ass 349 TFASGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn24_1 346 TFASGNGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn25_ass 346 TFASGNGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn08_1 352 TFASGKGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN
zn29_ass 357 TFASGNGTTATVSKDDQGNITVKYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGN

FIG. 1C(CONTD.)

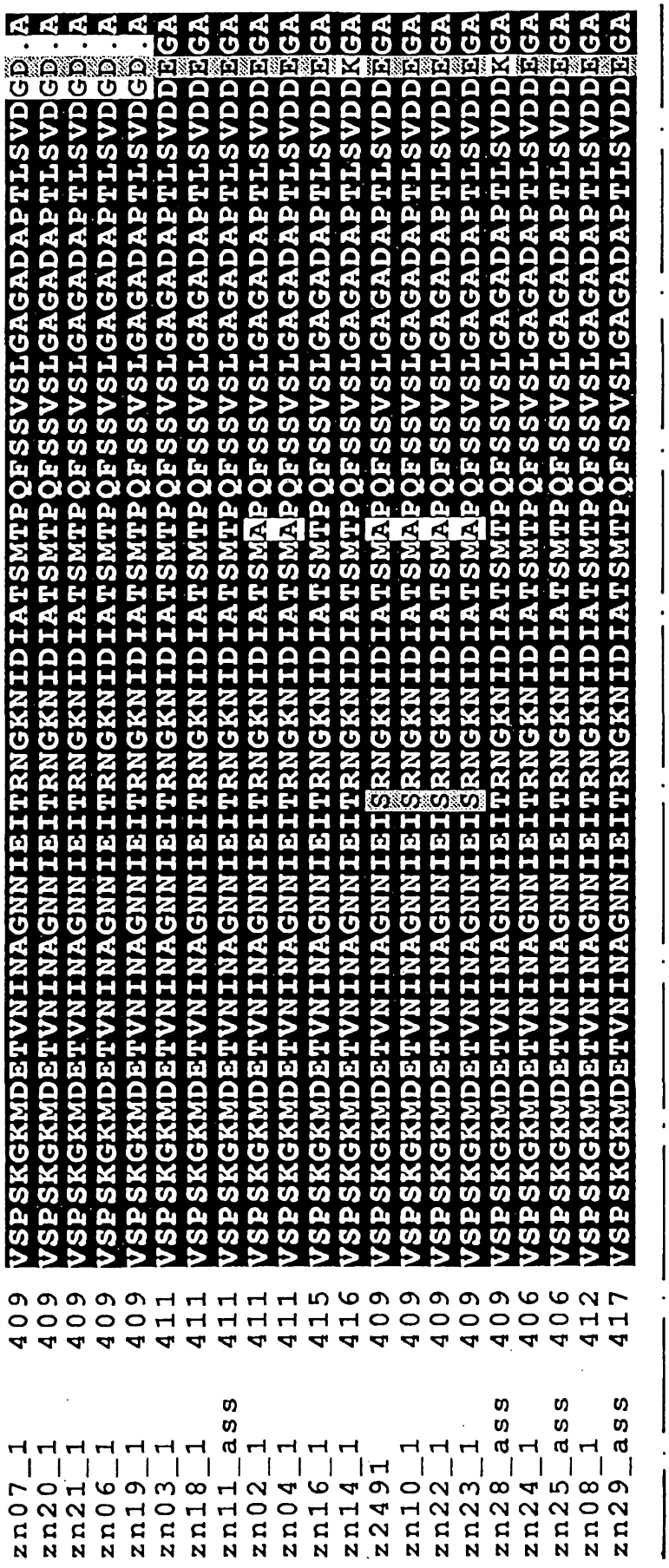

zn07_1 409 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDGD.A
zn20_1 409 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDGD.A
zn21_1 409 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDGD.A
zn06_1 409 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDGD.A
zn19_1 409 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDGD.A
zn03_1 411 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA
zn18_1 411 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA
zn11_ass 411 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA
zn02_1 411 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGA
zn04_1 411 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGA
zn16_1 415 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA
zn14_1 416 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDKGA
z2491 409 VSPSKGKMDETVNINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGA
zn10_1 409 VSPSKGKMDETVNINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGA
zn22_1 409 VSPSKGKMDETVNINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGA
zn23_1 409 VSPSKGKMDETVNINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGA
zn28_ass 409 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDKGA
zn24_1 406 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA
zn25_ass 406 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA
zn08_1 412 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA
zn29_ass 417 VSPSKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDDEGA

FIG. 1C(CONTD.)

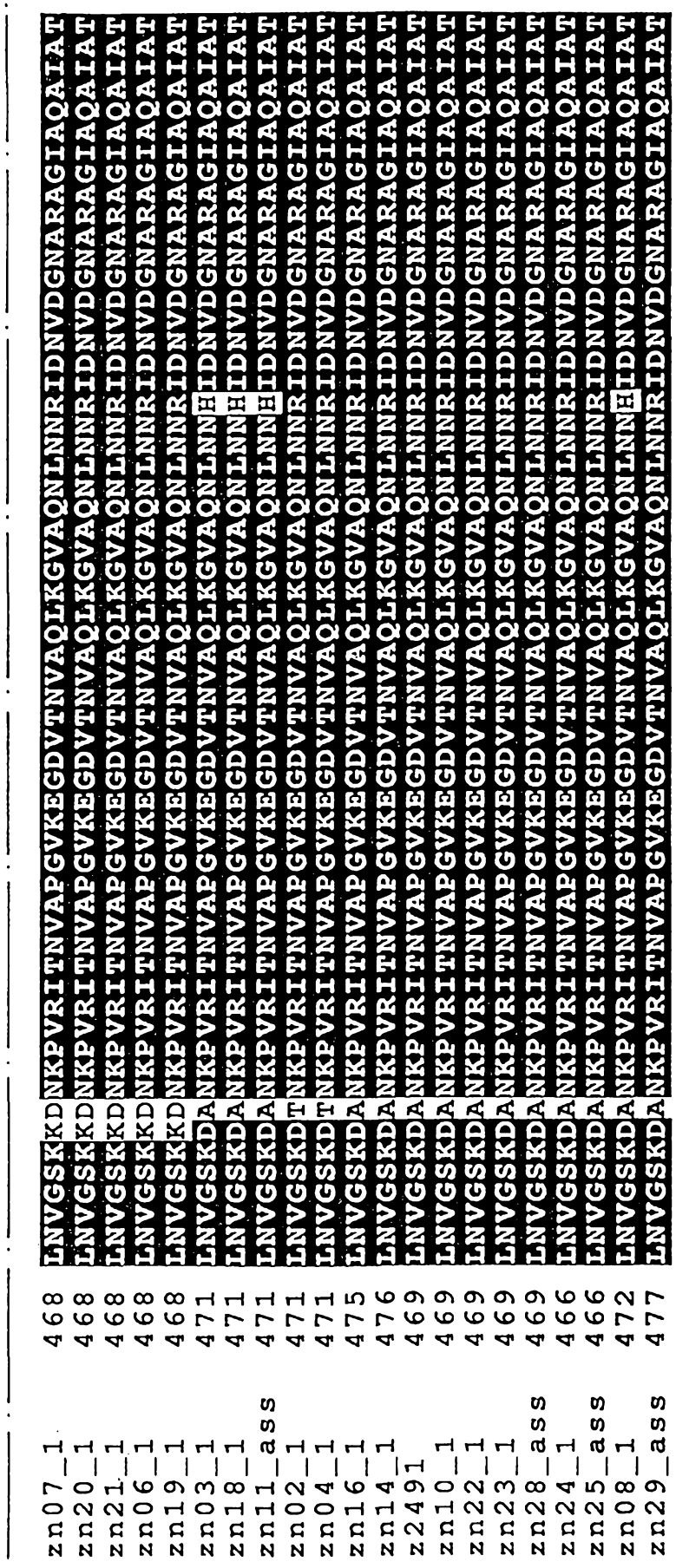

zn07_1 468 LNVGSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn20_1 468 LNVGSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn21_1 468 LNVGSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn06_1 468 LNVGSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn19_1 468 LNVGSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn03_1 471 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNHIDNVDGNARAGIAQAIAT
zn18_1 471 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNHIDNVDGNARAGIAQAIAT
zn11_ass 471 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNHIDNVDGNARAGIAQAIAT
zn02_1 471 LNVGSKDTNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn04_1 471 LNVGSKDTNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn16_1 475 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn14_1 476 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
z2491 469 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn10_1 469 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn22_1 469 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn23_1 469 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn28_ass 469 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn24_1 466 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn25_ass 466 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT
zn08_1 472 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNHIDNVDGNARAGIAQAIAT
zn29_ass 477 LNVGSKDANKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIAT

FIG. 1D

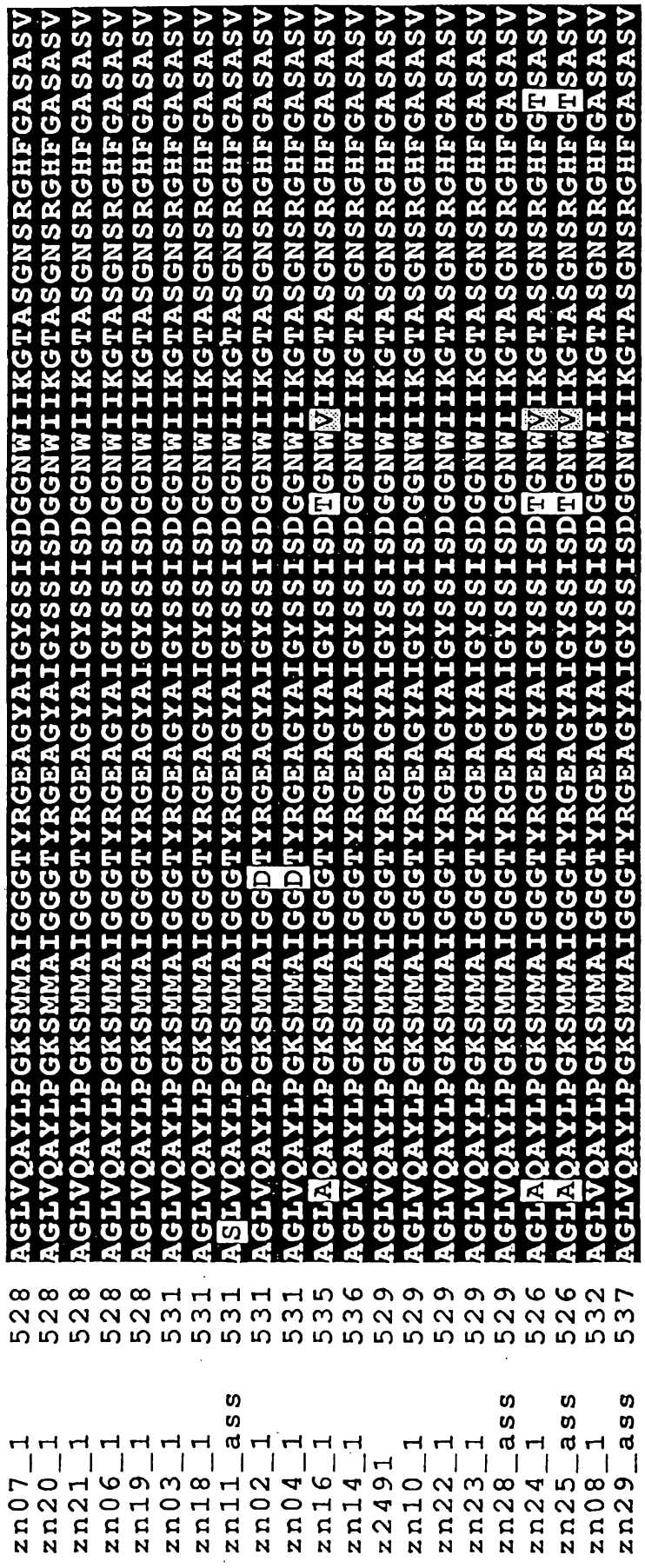
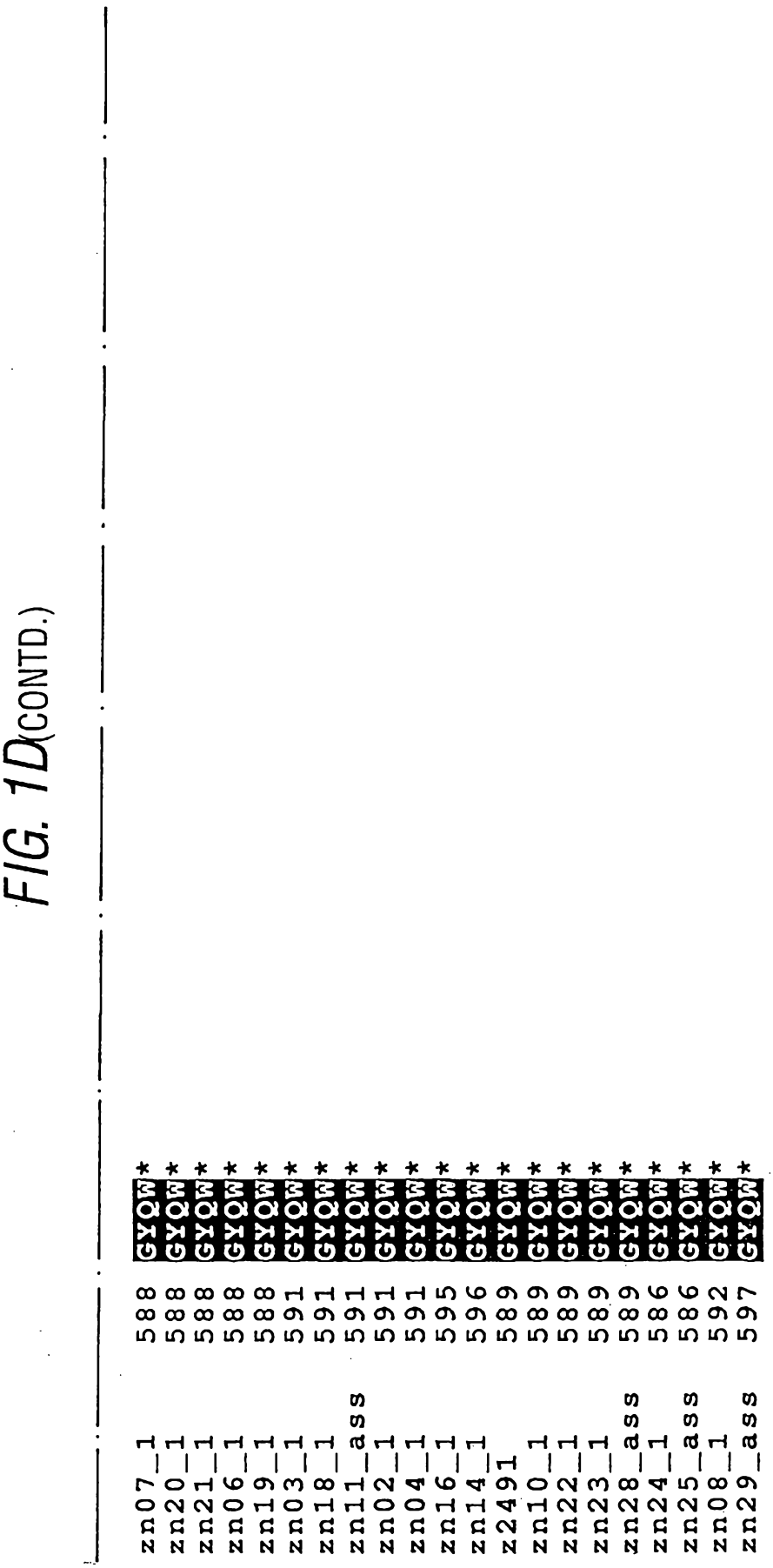
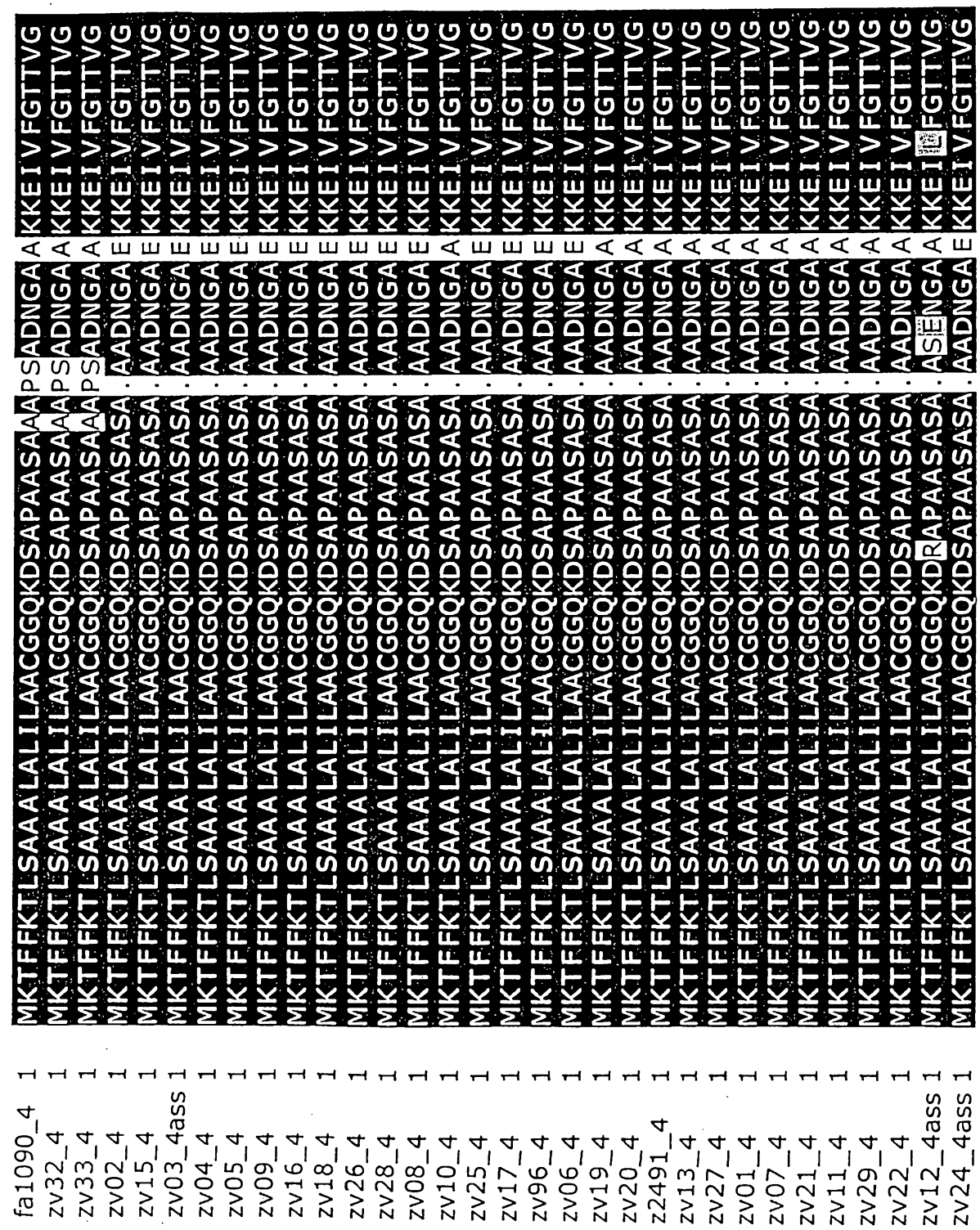

zn07_1 528 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn20_1 528 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn21_1 528 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn06_1 528 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn19_1 528 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn03_1 531 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn18_1 531 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn11_ass 531 ASLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn02_1 531 AGLVQAYLPGKSMMAIGGDTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn04_1 531 AGLVQAYLPGKSMMAIGGDTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn16_1 535 AGLAQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDTGNWVIKGTASGNSRGHFGASV
zn14_1 536 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
z2491 529 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn10_1 529 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn22_1 529 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn23_1 529 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn28_ass 529 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn24_1 526 AGLAQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDTGNWVIKGTASGNSRGHFGTSASV
zn25_ass 526 AGLAQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDTGNWVIKGTASGNSRGHFGTSASV
zn08_1 532 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV
zn29_ass 537 AGLVQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASV

FIG. 1D(CONTD.)


zn07_1 588 GYQW*
zn20_1 588 GYQW*
zn21_1 588 GYQW*
zn06_1 588 GYQW*
zn19_1 588 GYQW*
zn03_1 591 GYQW*
zn18_1 591 GYQW*
zn11_ass 591 GYQW*
zn02_1 591 GYQW*
zn04_1 591 GYQW*
zn16_1 595 GYQW*
zn14_1 596 GYQW*
z2491 589 GYQW*
zn10_1 589 GYQW*
zn22_1 589 GYQW*
zn23_1 589 GYQW*
zn28_ass 589 GYQW*
zn24_1 586 GYQW*
zn25_ass 586 GYQW*
zn08_1 592 GYQW*
zn29_ass 597 GYQW*

12/97

FIG. 2A


fa1090_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASAAAPSADNGAAKKEIVFGTTVG
zv32_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASAAAPSADNGAKKEIVFGTTVG
zv33_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASAAAPSADNGAKKEIVFGTTVG
zv02_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv15_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv03_4ass 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv04_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv05_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv09_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv16_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv18_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv26_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv28_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv08_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv10_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv25_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv17_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv96_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv06_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG
zv19_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv20_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
z2491_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv13_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv27_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv01_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv07_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv21_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv11_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv29_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv22_4 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAAKKEIVFGTTVG
zv12_4ass 1 MKTFFKTLSAAALALILAACGGQKDRAPAASASA.ASENGAAKKEILFGTTVG
zv24_4ass 1 MKTFFKTLSAAALALILAACGGQKDSAPAASASA.AADNGAEKKEIVFGTTVG

13/97

FIG. 2A(CONTD.)

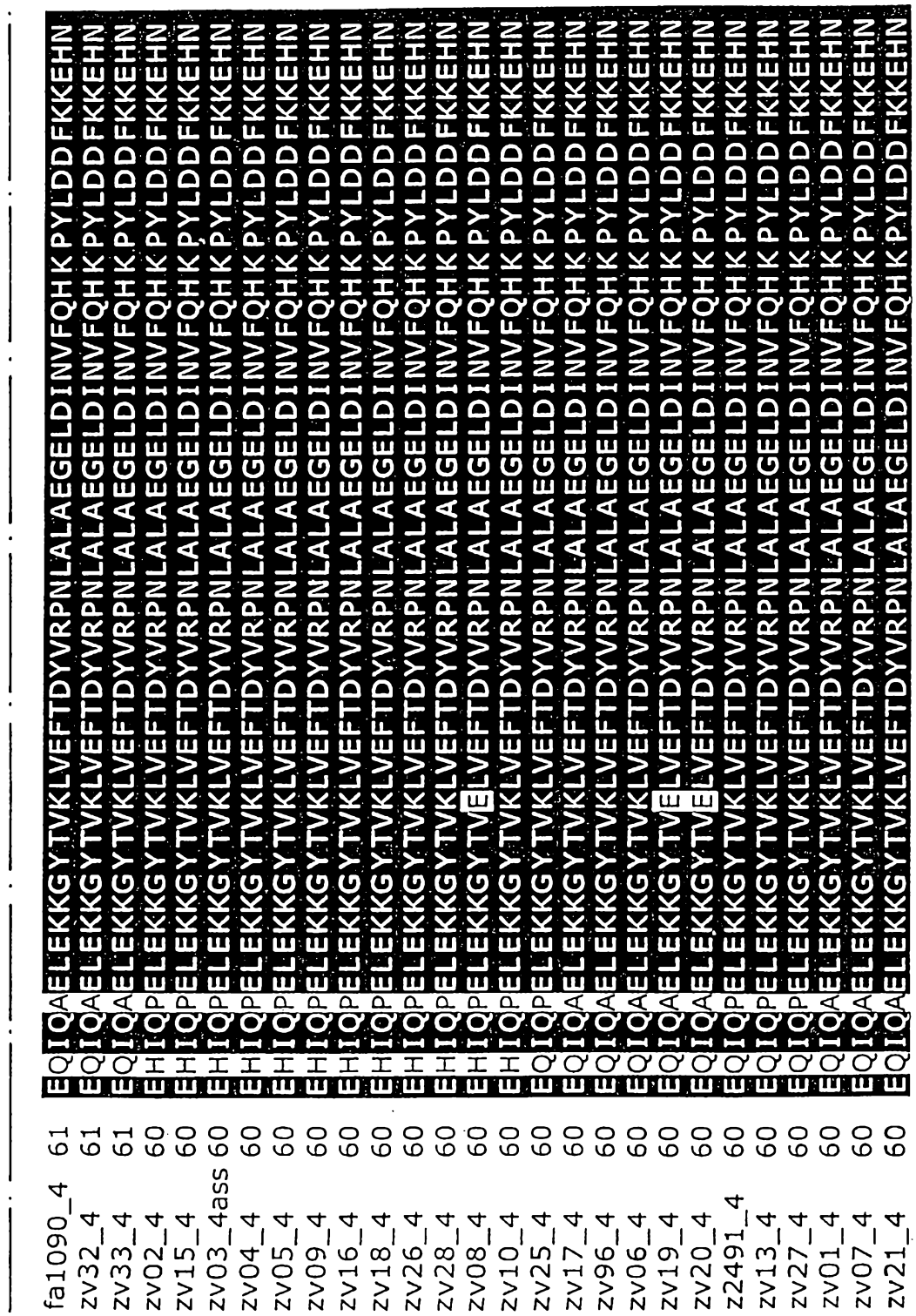
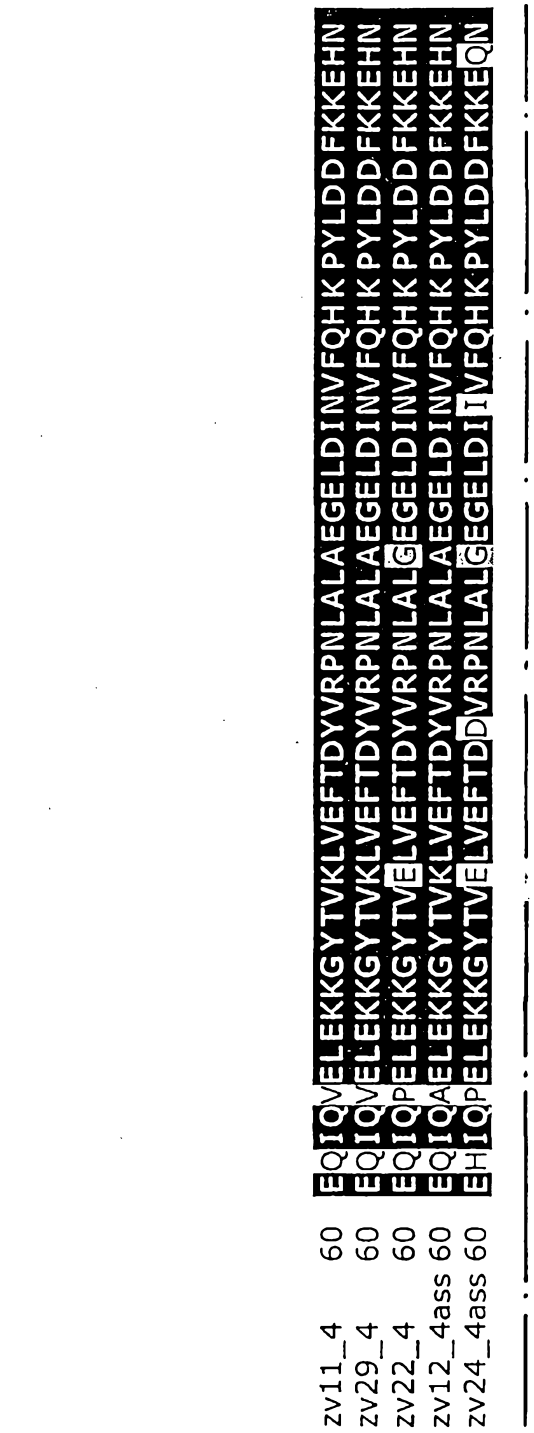
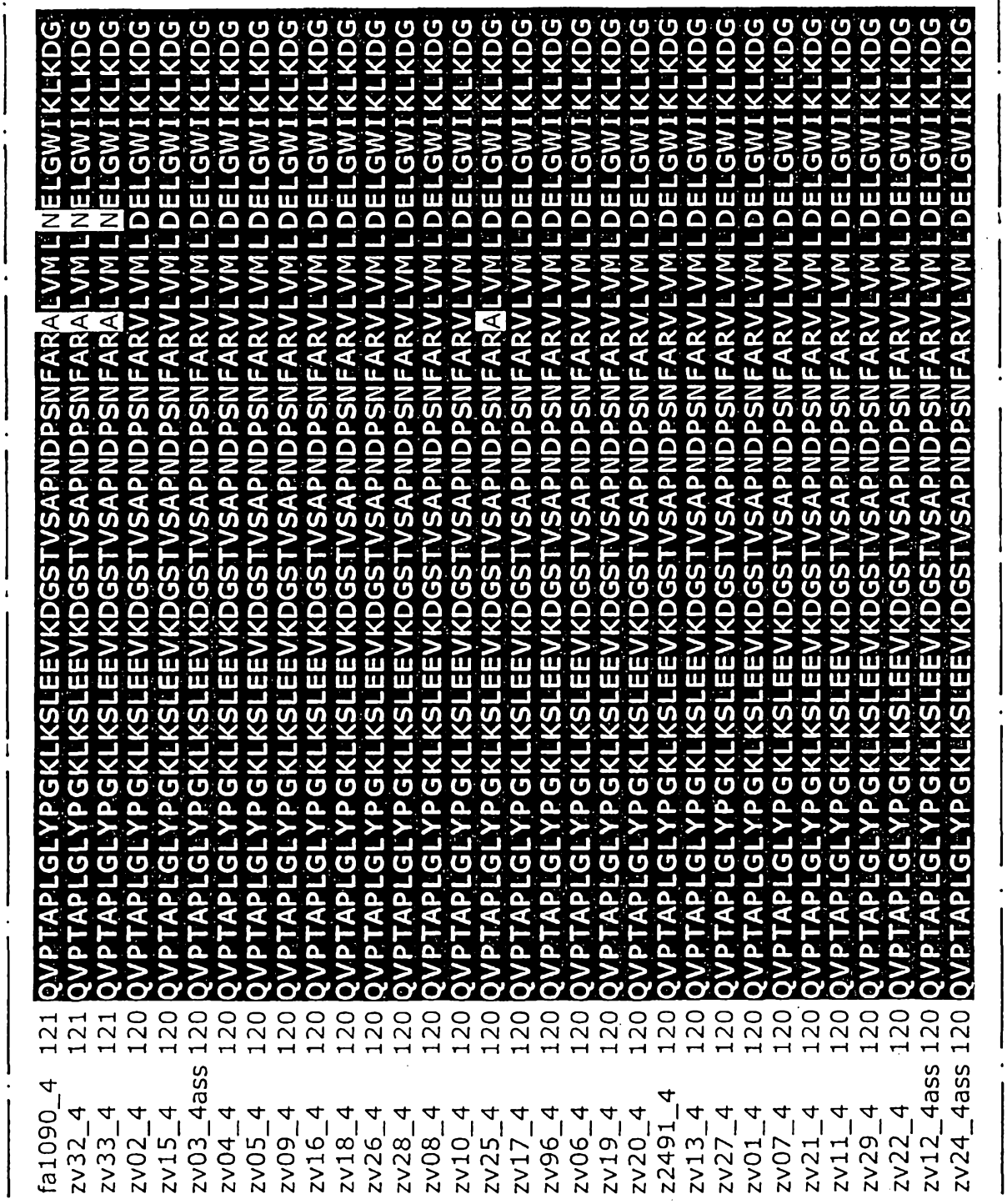


FIG. 2B


zv11_4 60 EQIQVELEKKGYTVKLVEFTDYVRPNLALAEGELDINVFQHKPYLDDFKKEHN
zv29_4 60 EQIQVELEKKGYTVKLVEFTDYVRPNLALAEGELDINVFQHKPYLDDFKKEHN
zv22_4 60 EQIQPELEKKGYTVELVEFTDYVRPNLALGEGELDINVFQHKPYLDDFKKEHN
zv12_4ass 60 EQIQAELEKKGYTVKLVEFTDYVRPNLALAEGELDINVFQHKPYLDDFKKEHN
zv24_4ass 60 EHIQPELEKKGYTVELVEFTDDVRPNLALGEGELDIIVFQHKPYLDDFKKEQN

15/97

FIG. 2B(CONTD.)



16/97

FIG. 2B(CONTD.)

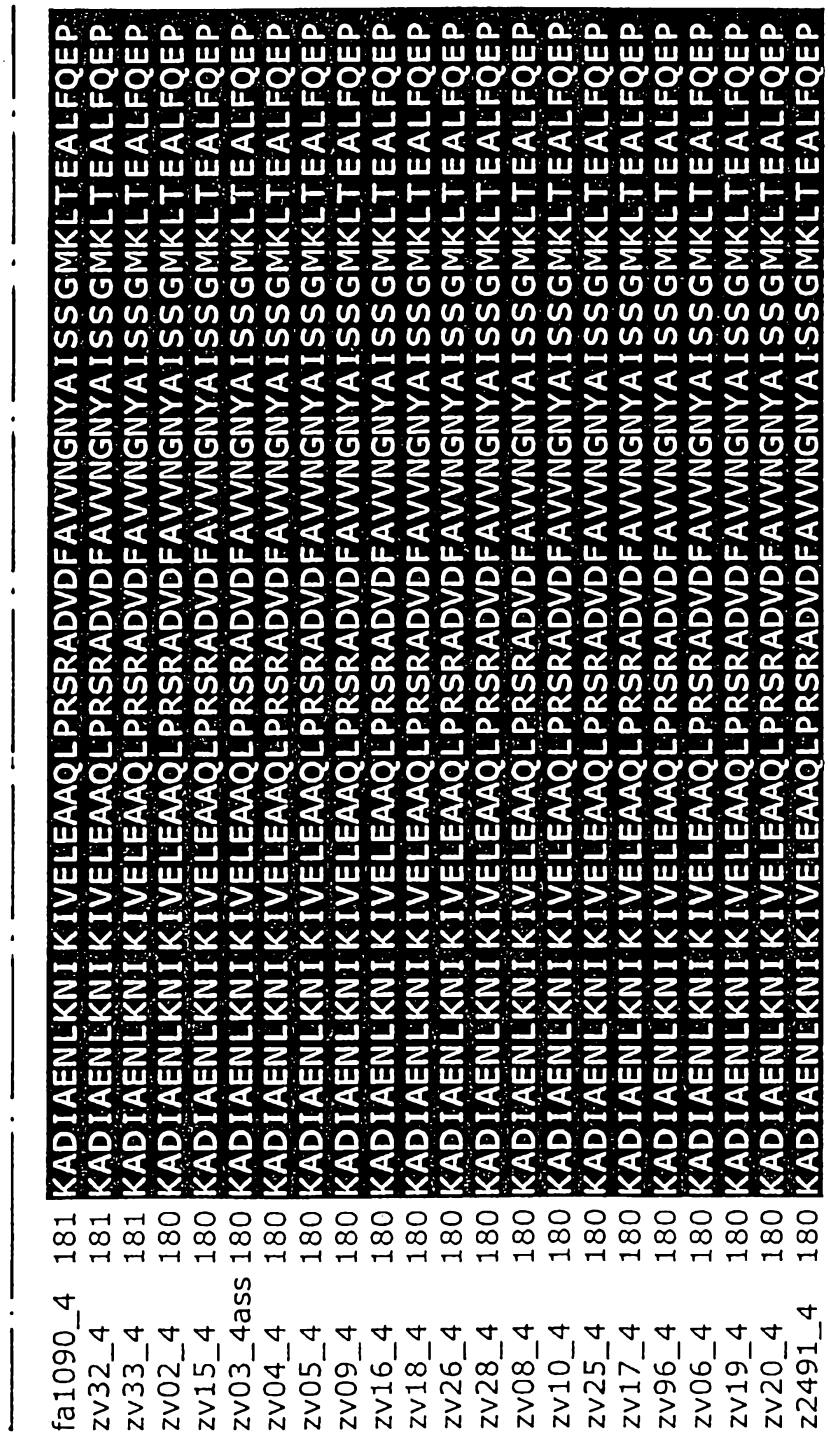


FIG. 2C

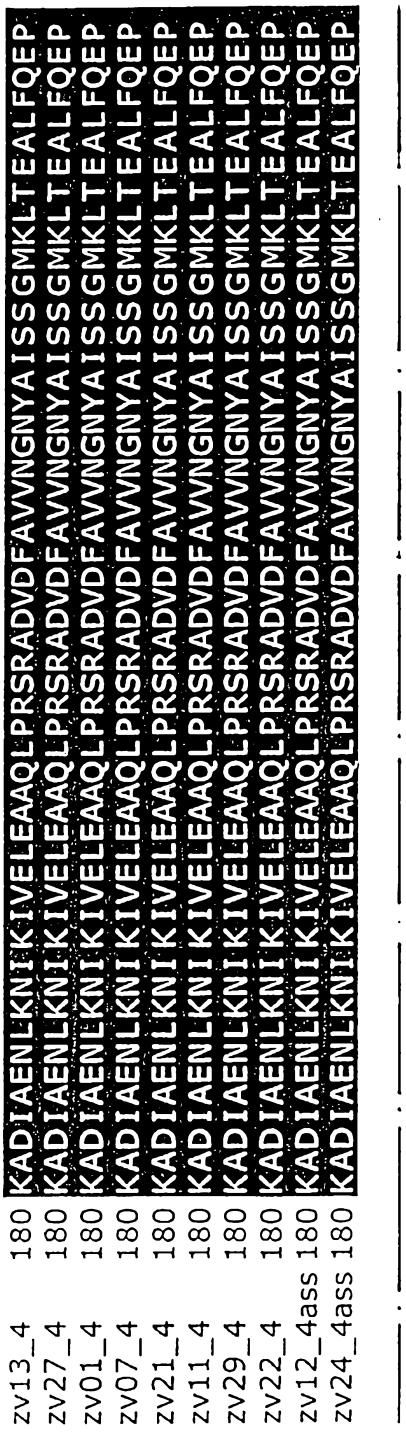

zv13_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv27_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv01_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv07_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv21_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv11_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv29_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv22_4 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv12_4ass 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP
zv24_4ass 180 KADIAENLKNIKIVELEAAQLPRSRADVDFAVVNGNYAISSGMKLTEALFQEP

FIG. 2C(CONTD.)

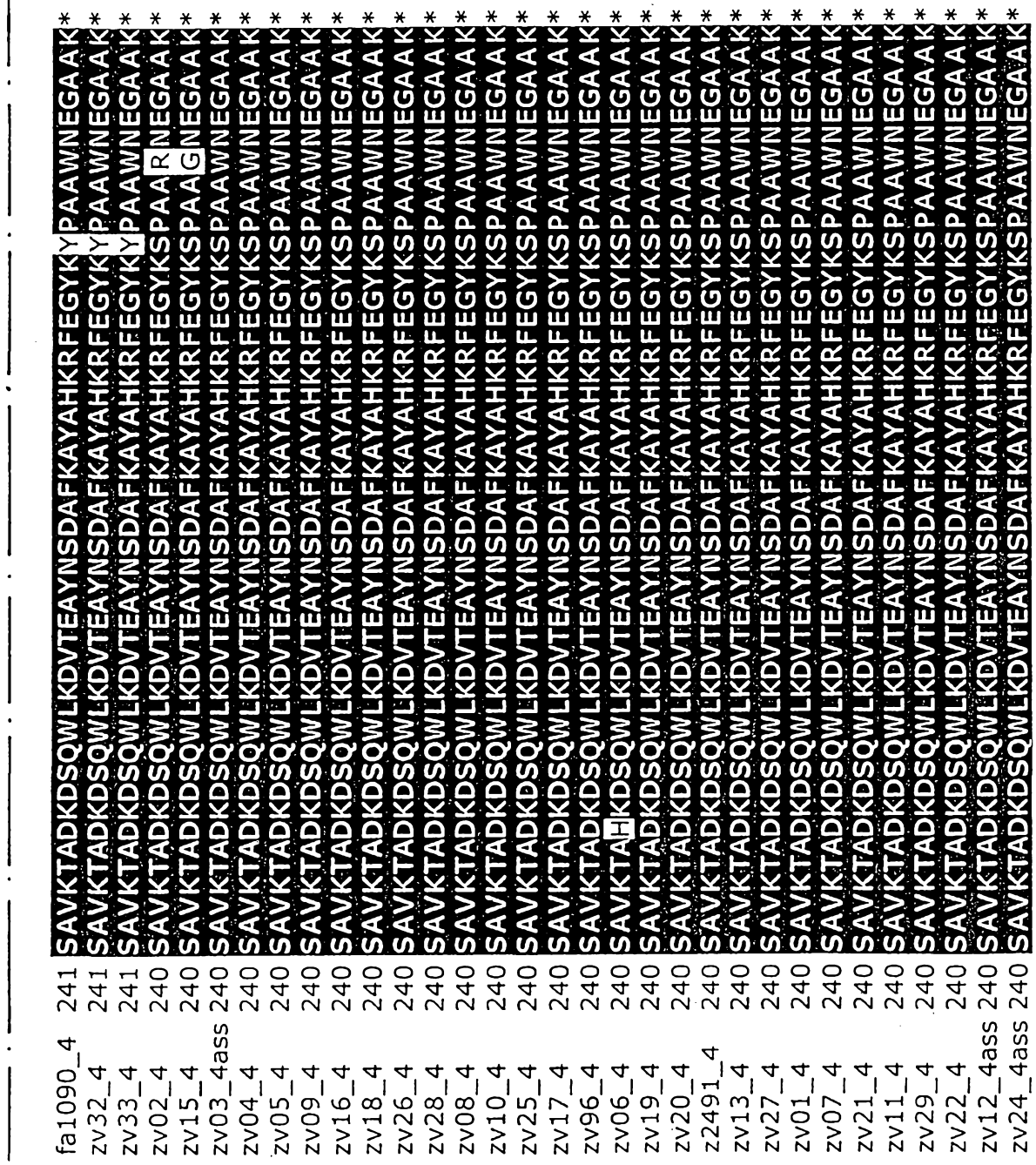

fa1090_4 241 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKYPAAWNEGAAK *
zv32_4 241 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKYPAAWNEGAAK *
zv33_4 241 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKYPAAWNEGAAK *
zv02_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAARNEGAAK *
zv15_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAGNEGAAK *
zv03_4ass 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv04_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv05_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv09_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv16_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv18_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv26_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv28_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv08_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv10_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv25_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv17_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv96_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv06_4 240 SAVKTAHKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv19_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv20_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
z2491_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv13_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv27_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv01_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv07_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv21_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv11_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv29_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv22_4 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv12_4ass 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *
zv24_4ass 240 SAVKTADKDSQWLKDVTEAYNSDAFKAYAHKRFEGYKSPAAWNEGAAK *

FIG. 3A

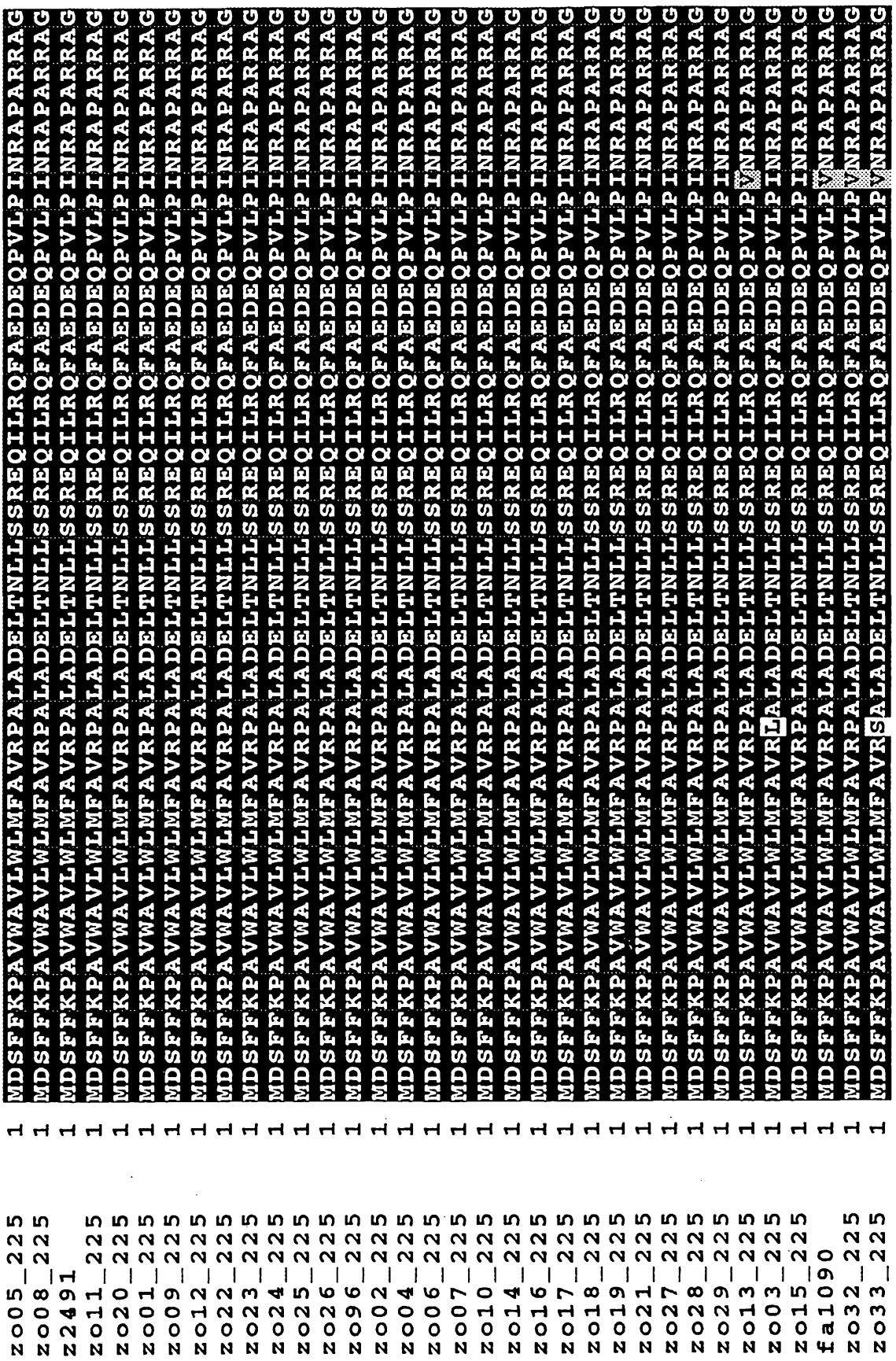

z005_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z008_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z2491 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z011_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z020_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z001_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z009_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z012_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z022_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z023_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z024_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z025_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z026_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z096_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z002_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z004_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z006_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z007_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z010_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z014_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z016_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z017_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z018_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z019_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z021_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z027_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z028_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z029_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z013_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPVNRAPARRAG
z003_225 1 MDSFFKPAVWAVLWLMFAVRLALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
z015_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPINRAPARRAG
fa1090 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPVNRAPARRAG
z032_225 1 MDSFFKPAVWAVLWLMFAVRPALADELTNLLSSREQILRQFAEDEQPVLPVNRAPARRAG
z033_225 1 MDSFFKPAVWAVLWLMFAVRSALADELTNLLSSREQILRQFAEDEQPVLPVNRAPARRAG

FIG. 3A(CONTD.)

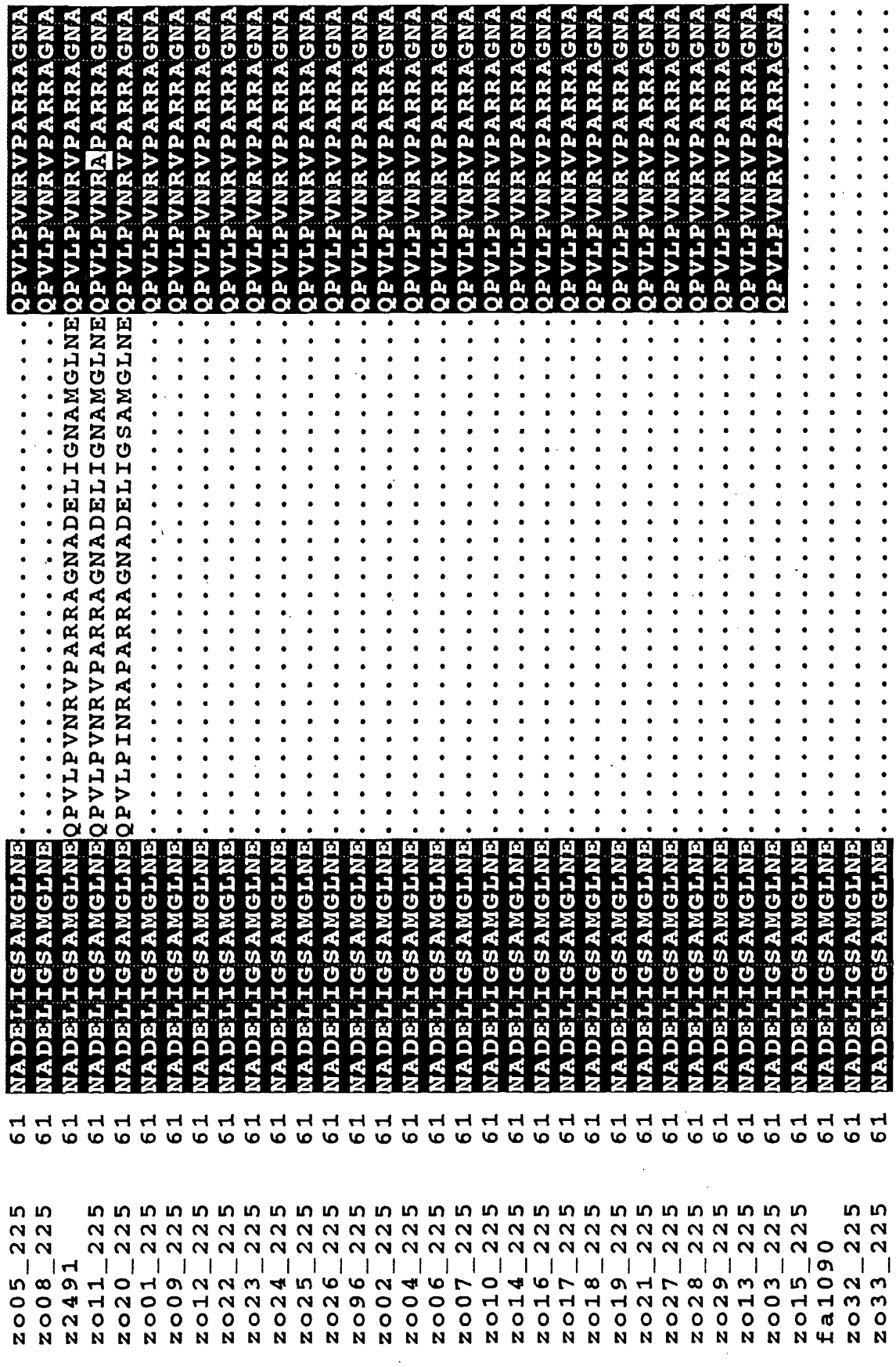

z005_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z008_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z2491 61 NADELIGSAMGLNE QPVLPVNRVPARRAGNADELIGNAMGLNE QPVLPVNRVPARRAGNA
z011_225 61 NADELIGSAMGLNE QPVLPVNRVPARRAGNADELIGNAMGLNE QPVLPVNRAPARRAGNA
z020_225 61 NADELIGSAMGLNE QPVLPINRAPARRAGNADELIGSAMGLNE QPVLPVNRVPARRAGNA
z001_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z009_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z012_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z022_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z023_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z024_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z025_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z026_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z096_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z002_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z004_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z006_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z007_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z010_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z014_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z016_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z017_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z018_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z019_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z021_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z027_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z028_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z029_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z013_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z003_225 61 NADELIGSAMGLNE ..... QPVLPVNRVPARRAGNA
z015_225 61 NADELIGSAMGLNE .....
fa1090 61 NADELIGSAMGLNE .....
z032_225 61 NADELIGSAMGLNE .....
z033_225 61 NADELIGSAMGLNE .....

FIG. 3B

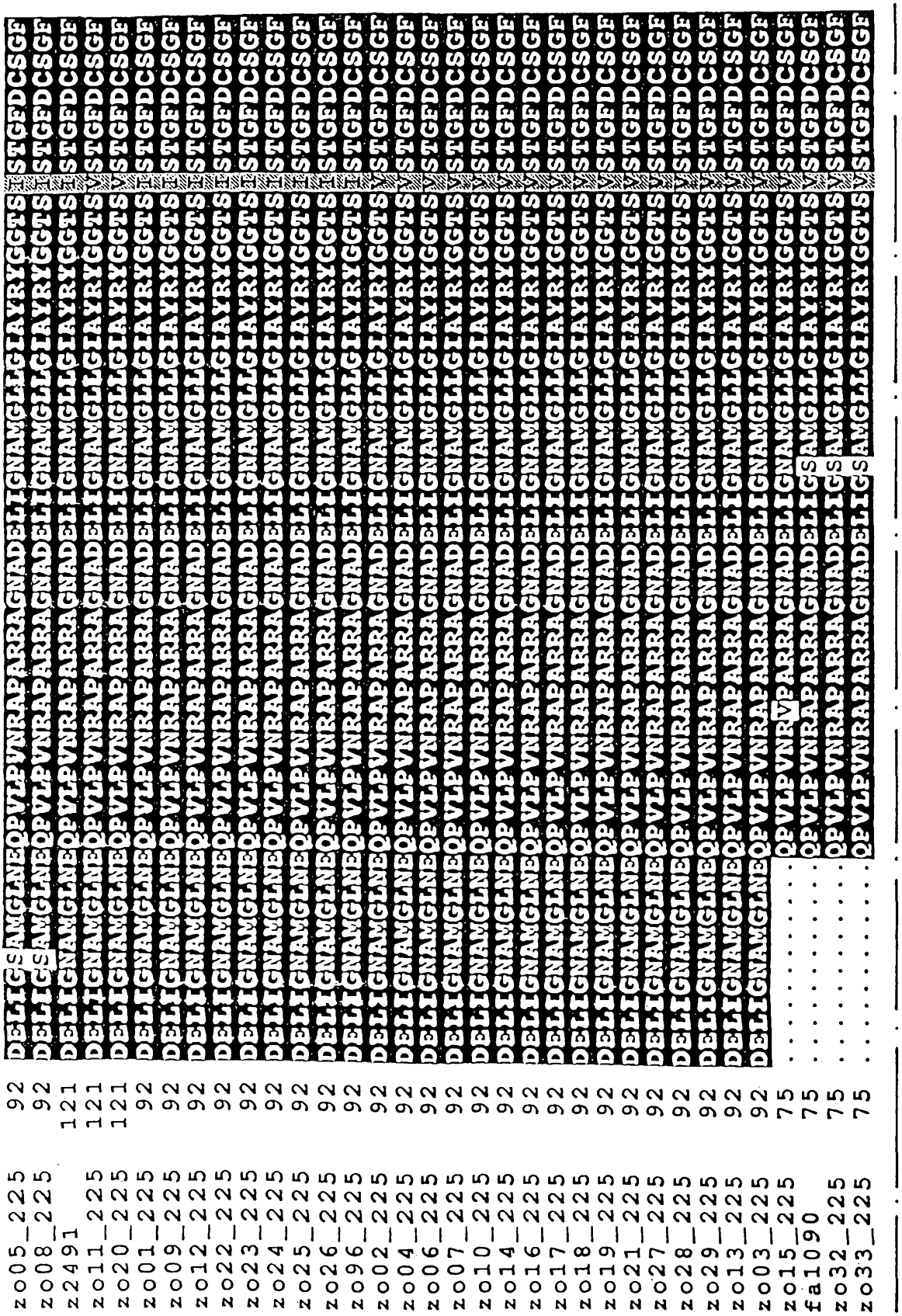


FIG. 3B(CONTD.)

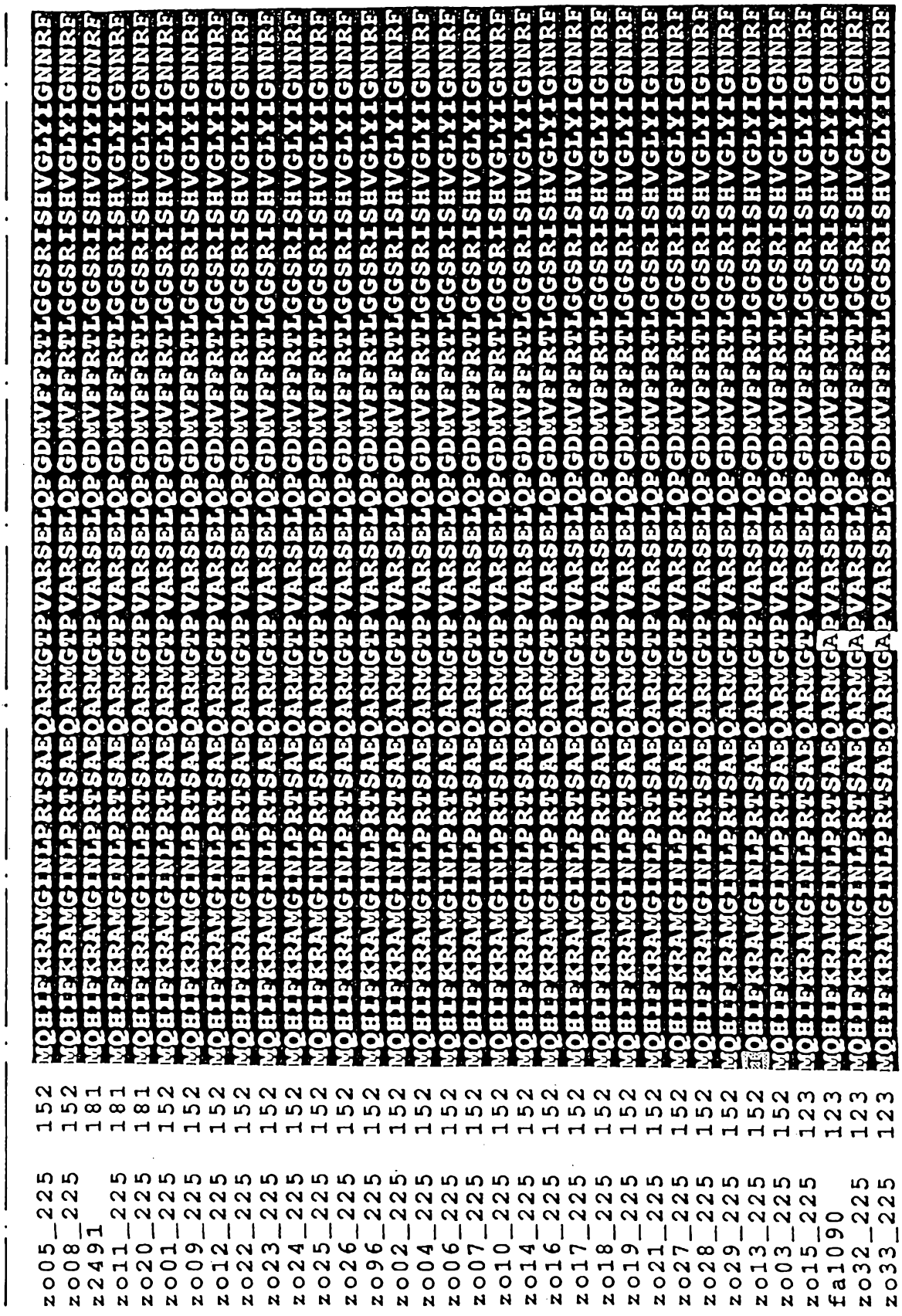


FIG. 3C

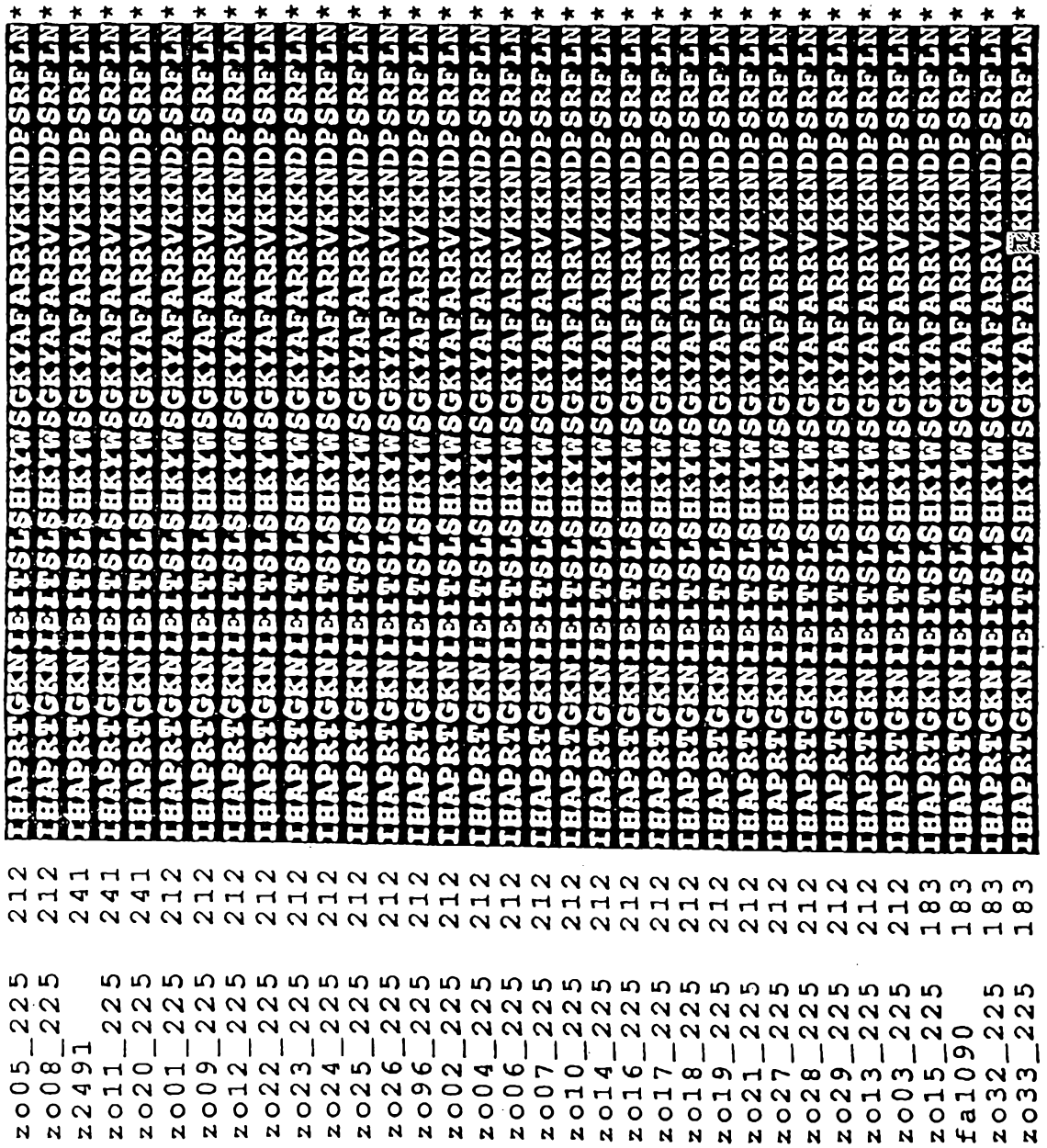


FIG. 4A

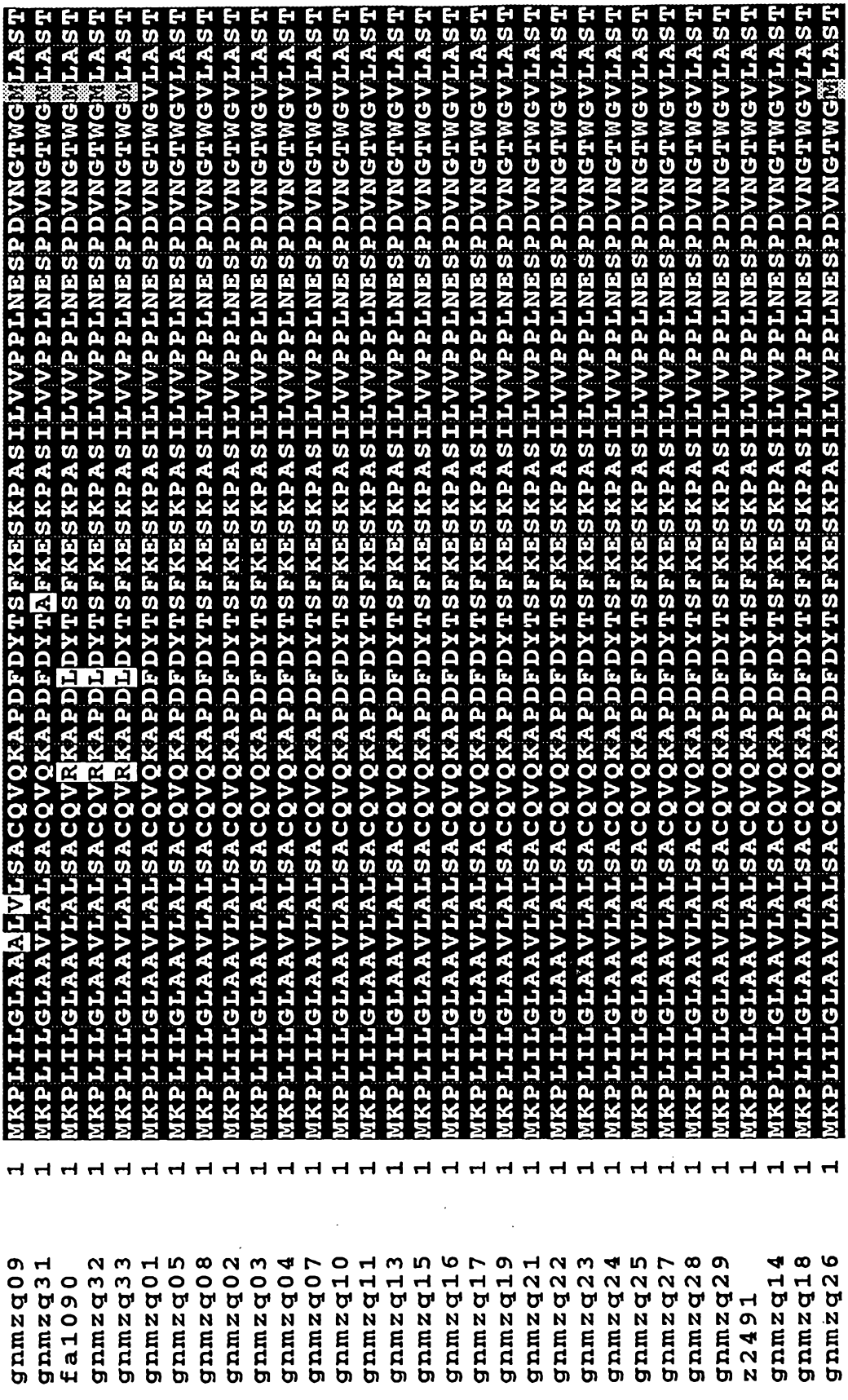

gnmzq09
gnmzq31
fa1090
gnmzq32
gnmzq33
gnmzq01
gnmzq05
gnmzq08
gnmzq02
gnmzq03
gnmzq04
gnmzq07
gnmzq10
gnmzq11
gnmzq13
gnmzq15
gnmzq16
gnmzq17
gnmzq19
gnmzq21
gnmzq22
gnmzq23
gnmzq24
gnmzq25
gnmzq27
gnmzq28
gnmzq29
z2491
gnmzq14
gnmzq18
gnmzq26

FIG. 4A(CONTD.)

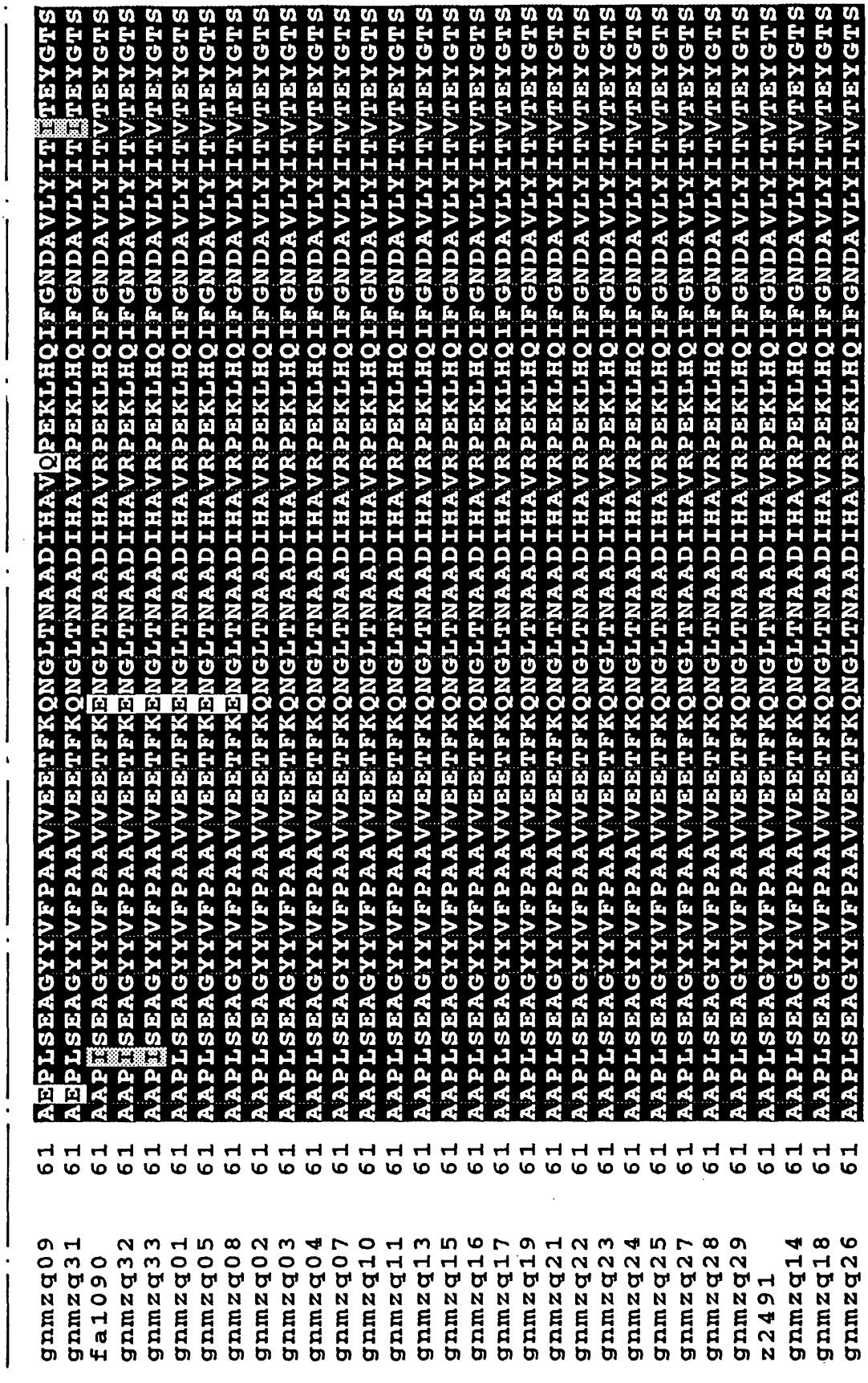

gnmzq09 61 AEPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVQPEKLHQIFGNDAVLYITTEYGTS
gnmzq31 61 AEPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTEYGTS
fa1090 61 AAPLSEAGYYVFPAAVVEETFKENGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq32 61 AAPLSEAGYYVFPAAVVEETFKENGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq33 61 AAPLSEAGYYVFPAAVVEETFKENGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq01 61 AAPLSEAGYYVFPAAVVEETFKENGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq05 61 AAPLSEAGYYVFPAAVVEETFKENGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq08 61 AAPLSEAGYYVFPAAVVEETFKENGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq02 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq03 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq04 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq07 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq10 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq11 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq13 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq15 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq16 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq17 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq19 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq21 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq22 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq23 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq24 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq25 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq27 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq28 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq29 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
z2491 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq14 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq18 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS
gnmzq26 61 AAPLSEAGYYVFPAAVVEETFKQNGLTNAADIHAVRPEKLHQIFGNDAVLYITTVTEYGTS

FIG. 4B

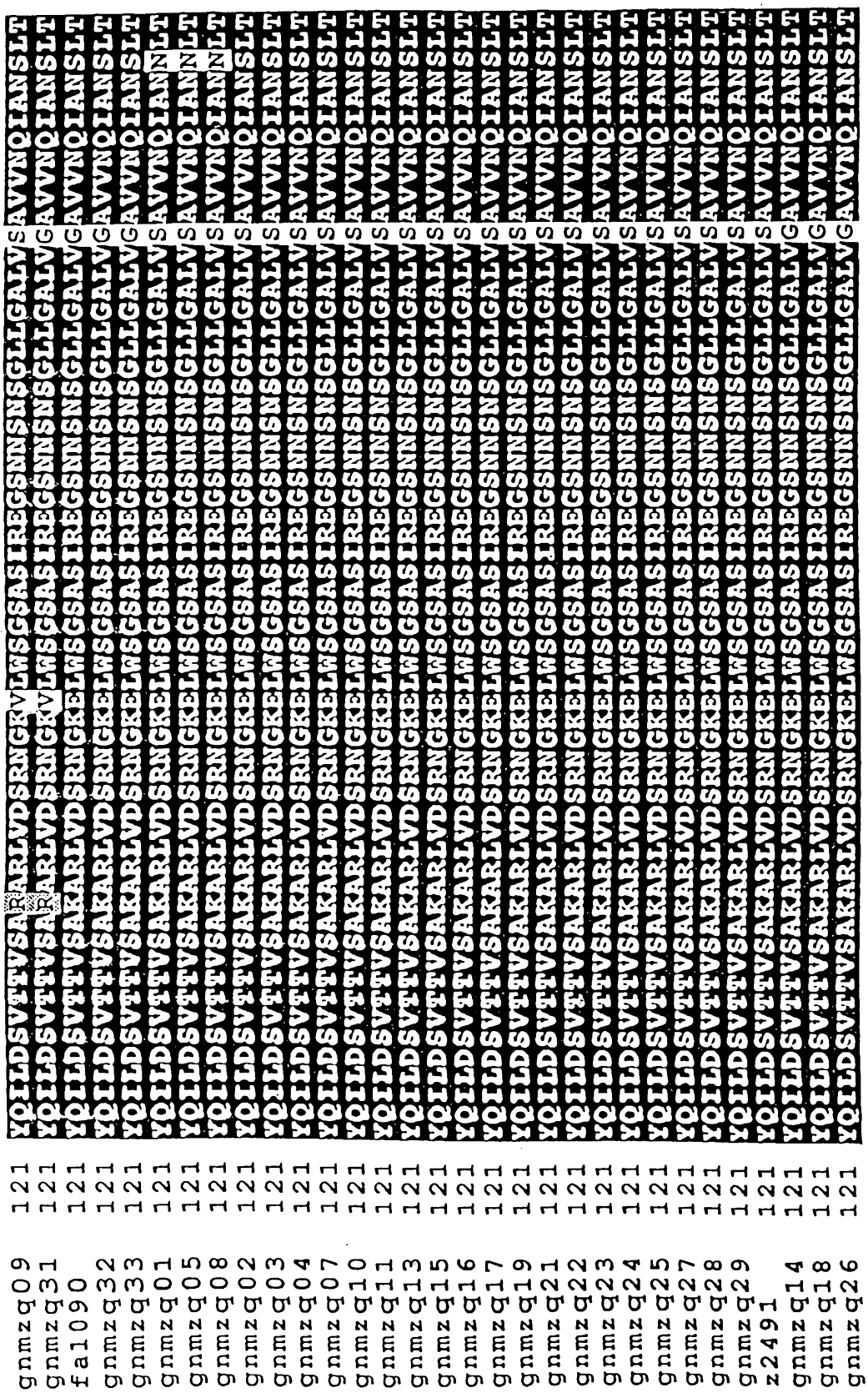

gnmzq09 121 YQILDSVTTVSAKARLVDSRNGKVLWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq31 121 YQILDSVTTVSAKARLVDSRNGKVLWSGSASIREGSNNSNSGLLGALVGAVVNQIANSLT
fa1090 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVGAVVNQIANSLT
gnmzq32 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVGAVVNQIANSLT
gnmzq33 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVGAVVNQIANSLT
gnmzq01 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANNLT
gnmzq05 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANNLT
gnmzq08 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANNLT
gnmzq02 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq03 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq04 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq07 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq10 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq11 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq13 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq15 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq16 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq17 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq19 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq21 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq22 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq23 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq24 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq25 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq27 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq28 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq29 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
z2491 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVSAVVNQIANSLT
gnmzq14 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVGAVVNQIANSLT
gnmzq18 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVGAVVNQIANSLT
gnmzq26 121 YQILDSVTTVSAKARLVDSRNGKELWSGSASIREGSNNSNSGLLGALVGAVVNQIANSLT

FIG. 4B(CONTD.)

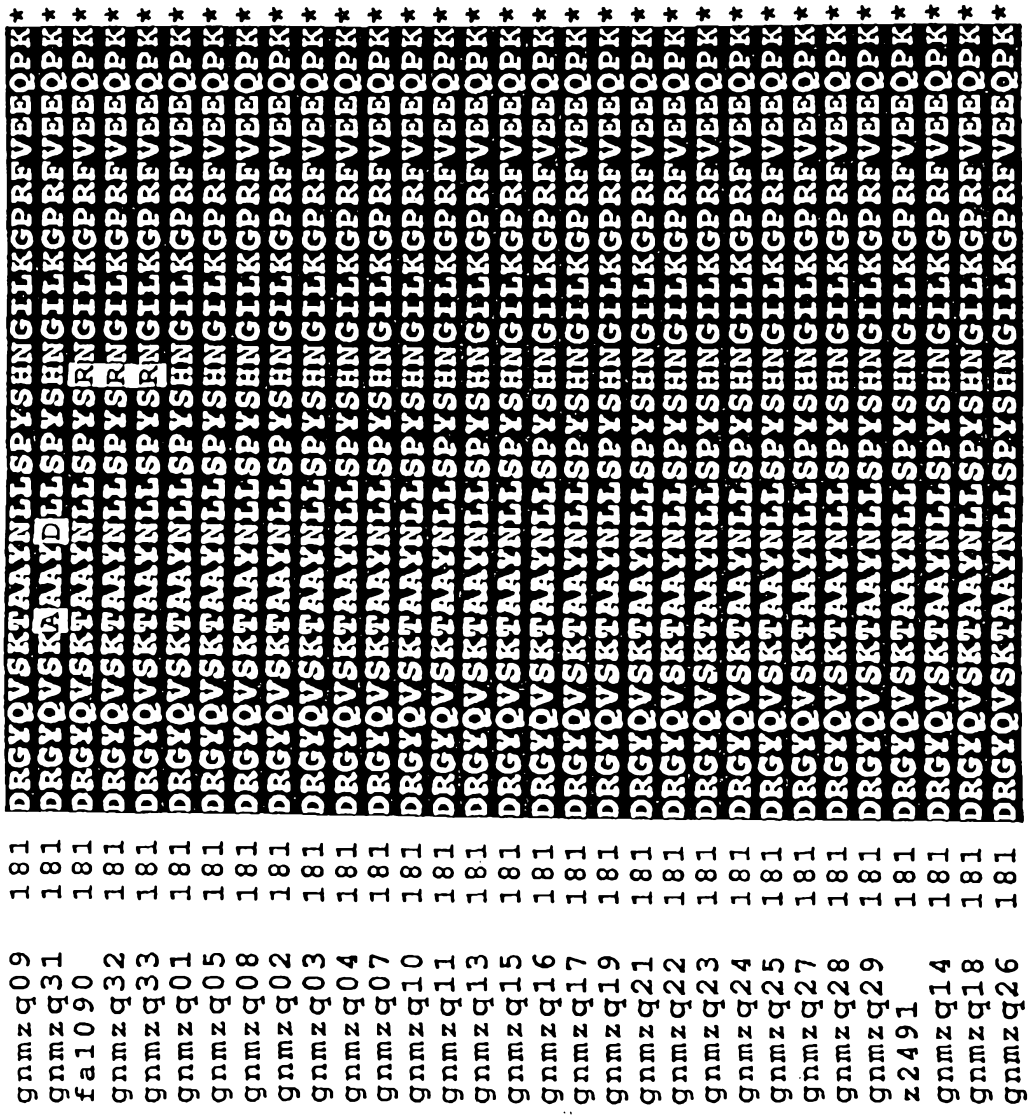

gnmzq09 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq31 181 DRGYQVSKTAAYDLLSPYSHNGILKGPRFVEEQPK *
fa1090 181 DRGYQVSKTAAYNLLSPYSRNGILKGPRFVEEQPK *
gnmzq32 181 DRGYQVSKTAAYNLLSPYSRNGILKGPRFVEEQPK *
gnmzq33 181 DRGYQVSKTAAYNLLSPYSRNGILKGPRFVEEQPK *
gnmzq01 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq05 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq08 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq02 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq03 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq04 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq07 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq10 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq11 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq13 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq15 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq16 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq17 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq19 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq21 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq22 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq23 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq24 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq25 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq27 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq28 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq29 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
z2491 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq14 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq18 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *
gnmzq26 181 DRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK *

FIG.5

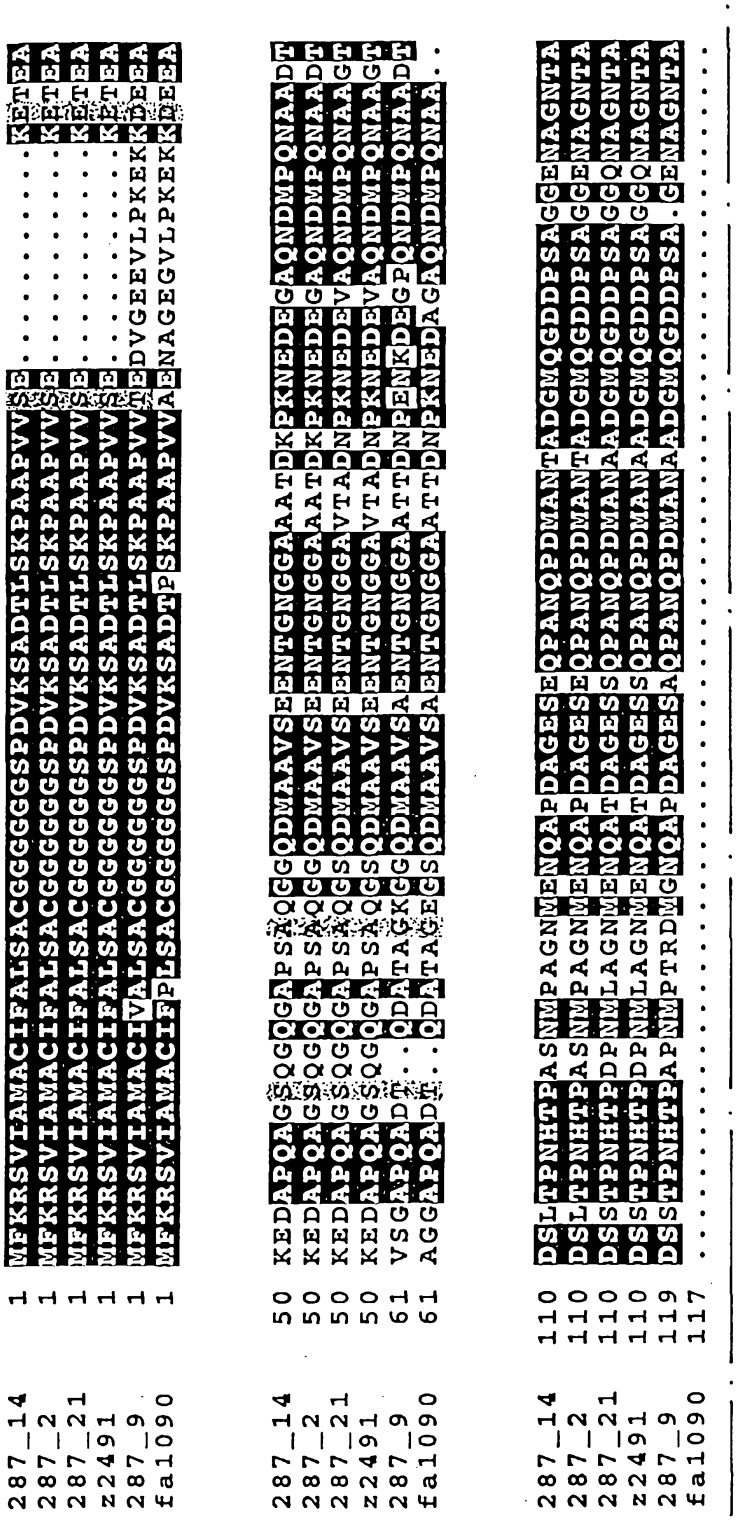


FIG. 5(CONTD.)

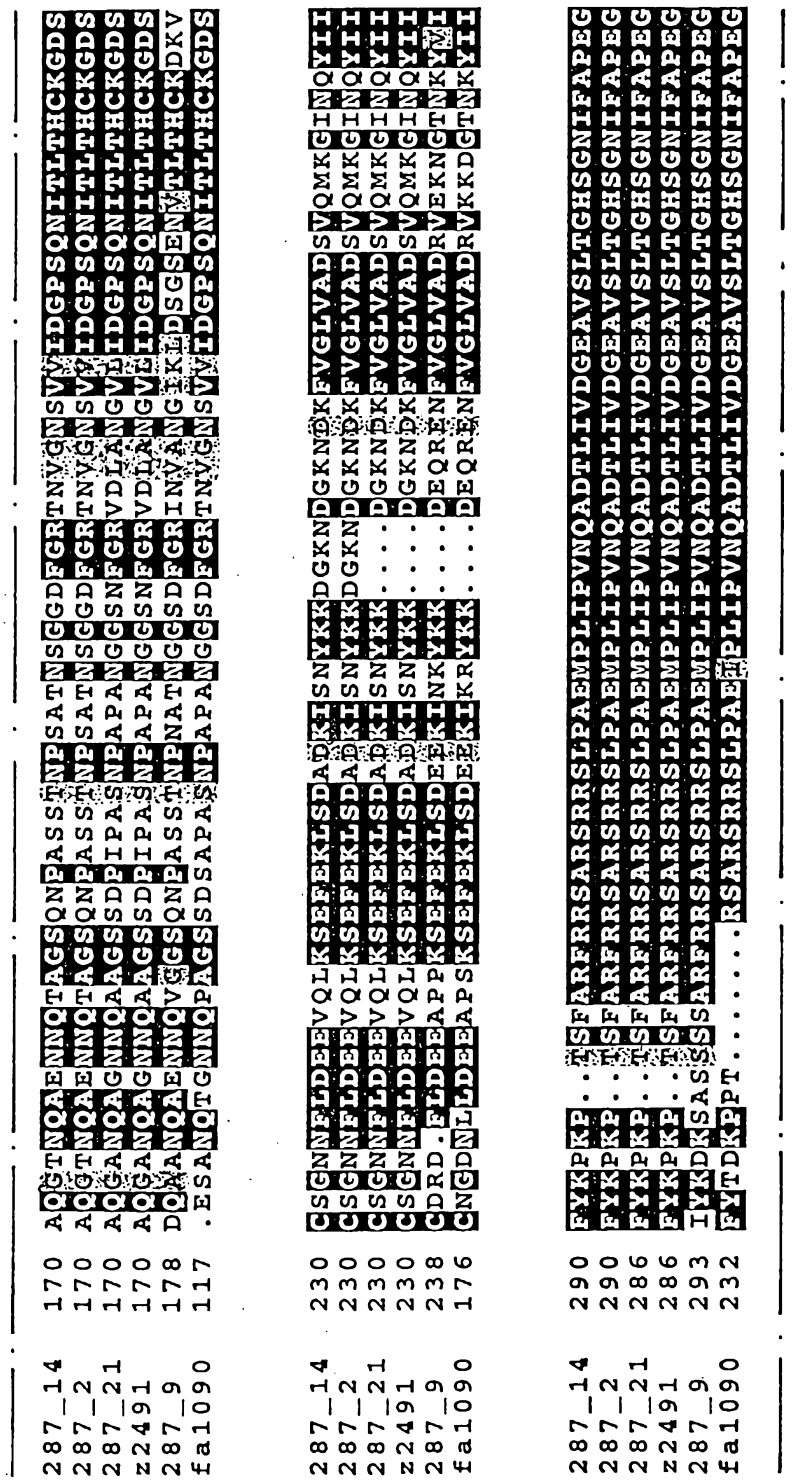


FIG. 5(CONTD.)

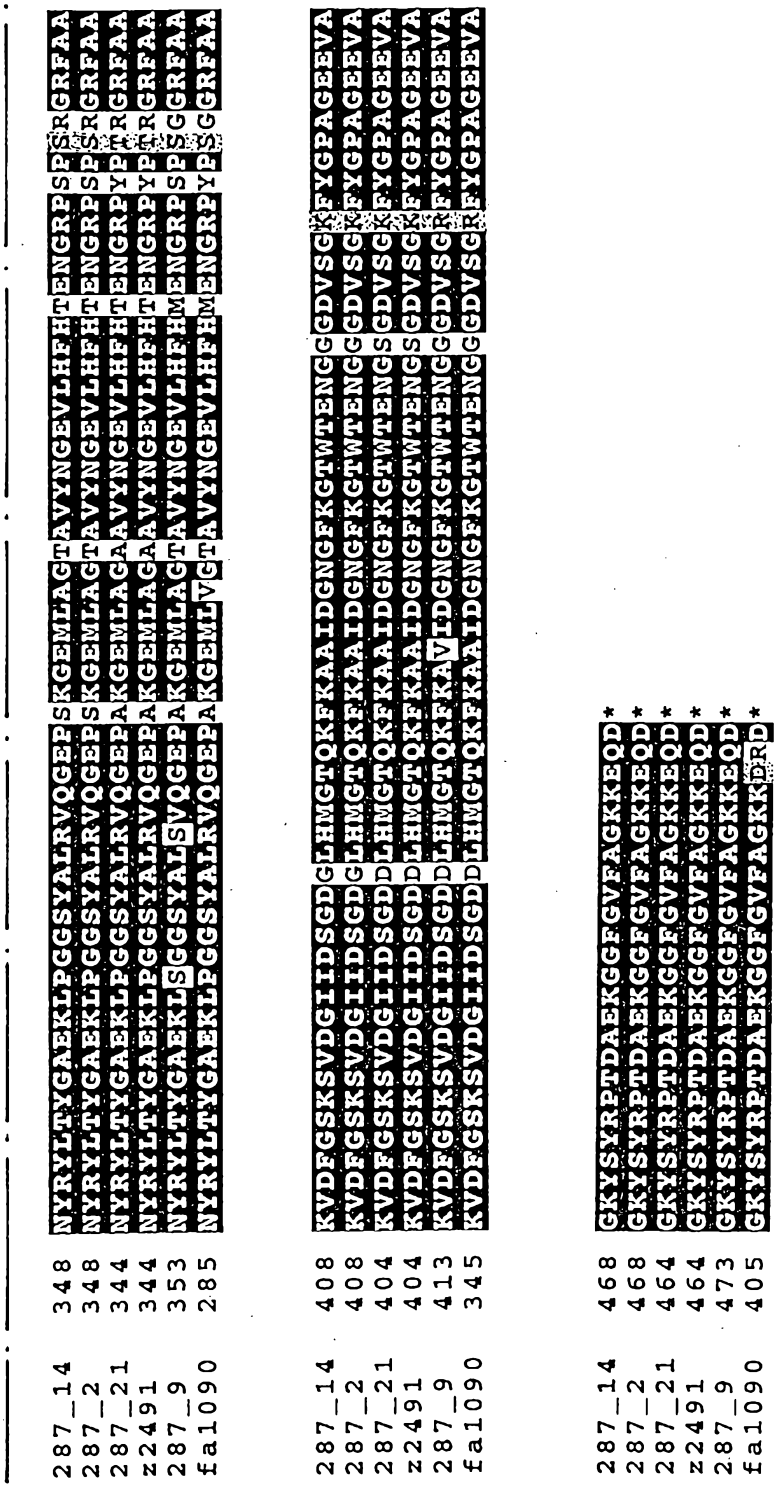

287_14 348 NYRYLTYGAEKLPGGSYALRVQGEPSKGEMLAGTAVYNGEVLHFHTENGRPSPSRGRFAA
287_2 348 NYRYLTYGAEKLPGGSYALRVQGEPSKGEMLAGTAVYNGEVLHFHTENGRPSPSRGRFAA
287_21 344 NYRYLTYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPPRRGRFAA
z2491 344 NYRYLTYGAEKLPGGSYALRVQGEPAKGEMLAGAAVYNGEVLHFHTENGRPYPPRRGRFAA
287_9 353 NYRYLTYGAEKLSGGSYALSVQGEPAKGEMLAGTAVYNGEVLHFHMENGRPSPSSGGRFAA
fa1090 285 NYRYLTYGAEKLPGGSYALRVQGEPAKGEMLVGTAVYNGEVLHFHMENGRPYPSSGGRFAA
287_14 408 KVDFGSKSVDGIIDSGDGLHMGTQKFKAAIDGNGFKGTWTENGGGDVSGKFYGPAGEEVA
287_2 408 KVDFGSKSVDGIIDSGDGLHMGTQKFKAAIDGNGFKGTWTENGGGDVSGRFYGPAGEEVA
287_21 404 KVDFGSKSVDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVA
z2491 404 KVDFGSKSVDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVA
287_9 413 KVDFGSKSVDGIIDSGDDLHMGTQKFKAVIDGNGFKGTWTENGGGDVSGKFYGPAGEEVA
fa1090 345 KVDFGSKSVDGIIDSGDDLHMGTQKFKAAIDGNGFKGTWTENGGGDVSGRFYGPAGEEVA
287_14 468 GKYSYRPTDAEKGGFGVFAGKKEQD*
287_2 468 GKYSYRPTDAEKGGFGVFAGKKEQD*
287_21 464 GKYSYRPTDAEKGGFGVFAGKKEQD*
z2491 464 GKYSYRPTDAEKGGFGVFAGKKEQD*
287_9 473 GKYSYRPTDAEKGGFGVFAGKKEQD*
fa1090 405 GKYSYRPTDAEKGGFGVFAGKKDRD*

FIG. 6A

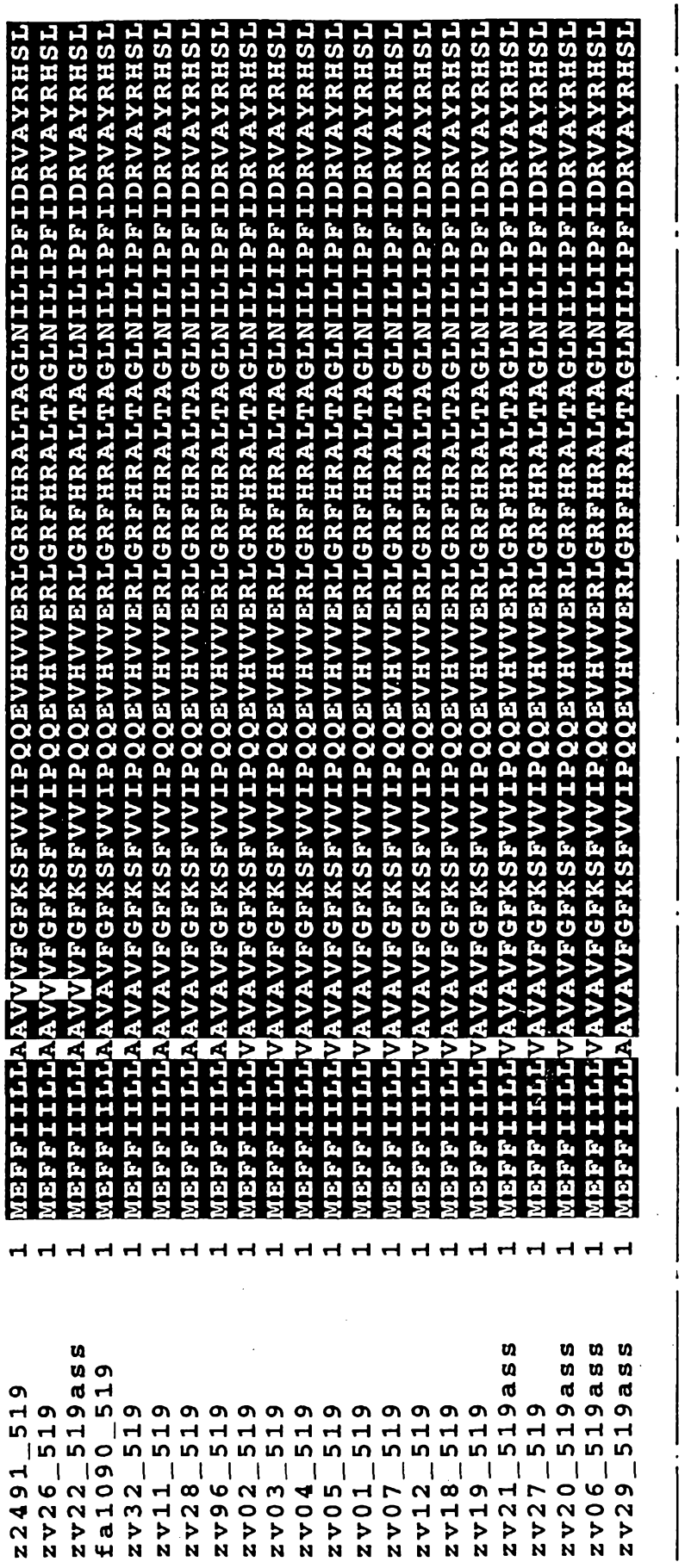

z2491_519
zv26_519
zv22_519ass
fa1090_519
zv32_519
zv11_519
zv28_519
zv96_519
zv02_519
zv03_519
zv04_519
zv05_519
zv01_519
zv07_519
zv12_519
zv18_519
zv19_519
zv21_519ass
zv27_519
zv20_519ass
zv06_519ass
zv29_519ass

FIG. 6A(CONTD.)

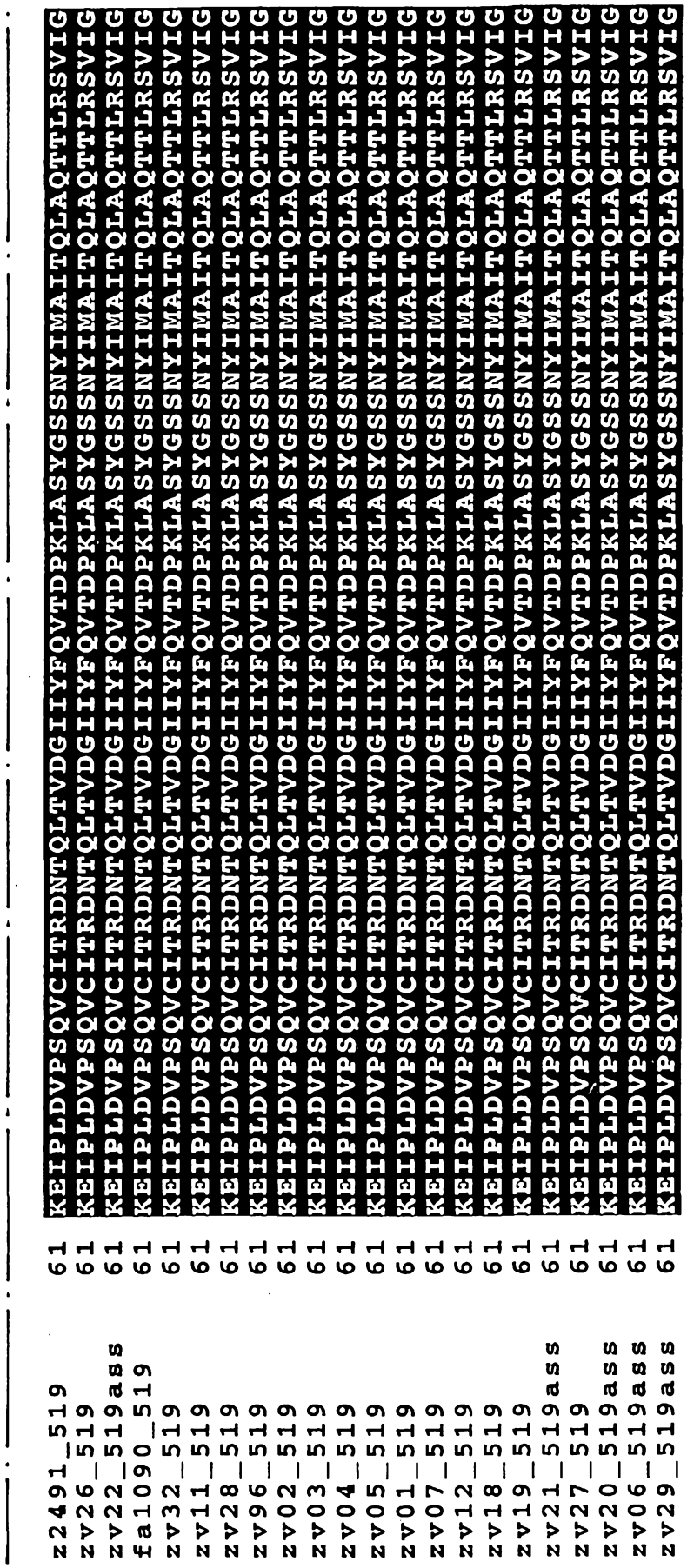

z2491_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv26_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv22_519ass 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
fa1090_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv32_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv11_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv28_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv96_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv02_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv03_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv04_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv05_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv01_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv07_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv12_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv18_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv19_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv21_519ass 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv27_519 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv20_519ass 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv06_519ass 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG
zv29_519ass 61 KEIPLDVPSQVCITRDNTQLTVDGIIYFQVTDPKLASYGSSNYIMAITQLAQTTLRSVIG

FIG. 6A(CONTD.)

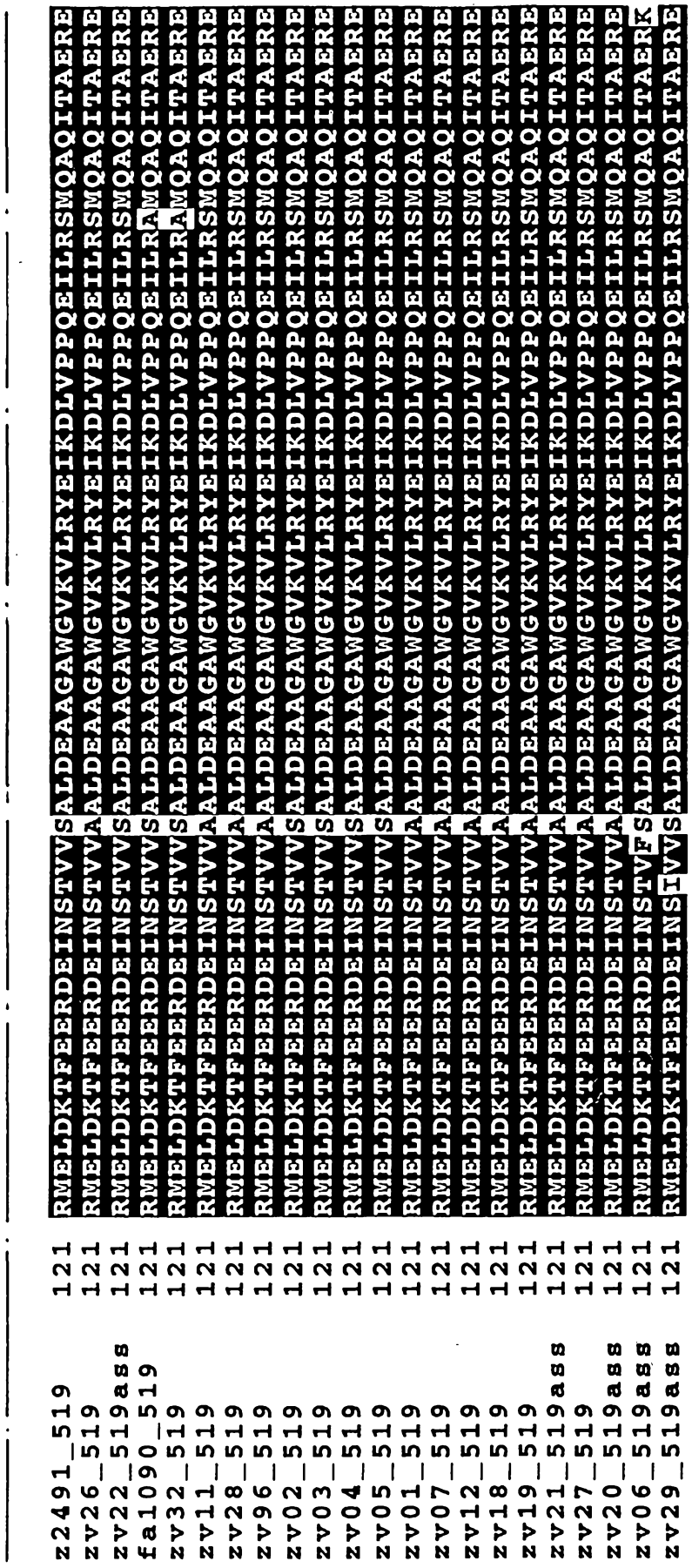

z2491_519 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv26_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv22_519ass 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
fa1090_519 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRAMQAQITAERE
zv32_519 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRAMQAQITAERE
zv11_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv28_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv96_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv02_519 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv03_519 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv04_519 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv05_519 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv01_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv07_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv12_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv18_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv19_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv21_519ass 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv27_519 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv20_519ass 121 RMELDKTFEERDEINSTVVAALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE
zv06_519ass 121 RMELDKTFEERDEINSTVFSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERK
zv29_519ass 121 RMELDKTFEERDEINSTVVSALDEAAGAWGVKVLRYEIKDLVPPQEILRSMQAQITAERE

FIG. 6B

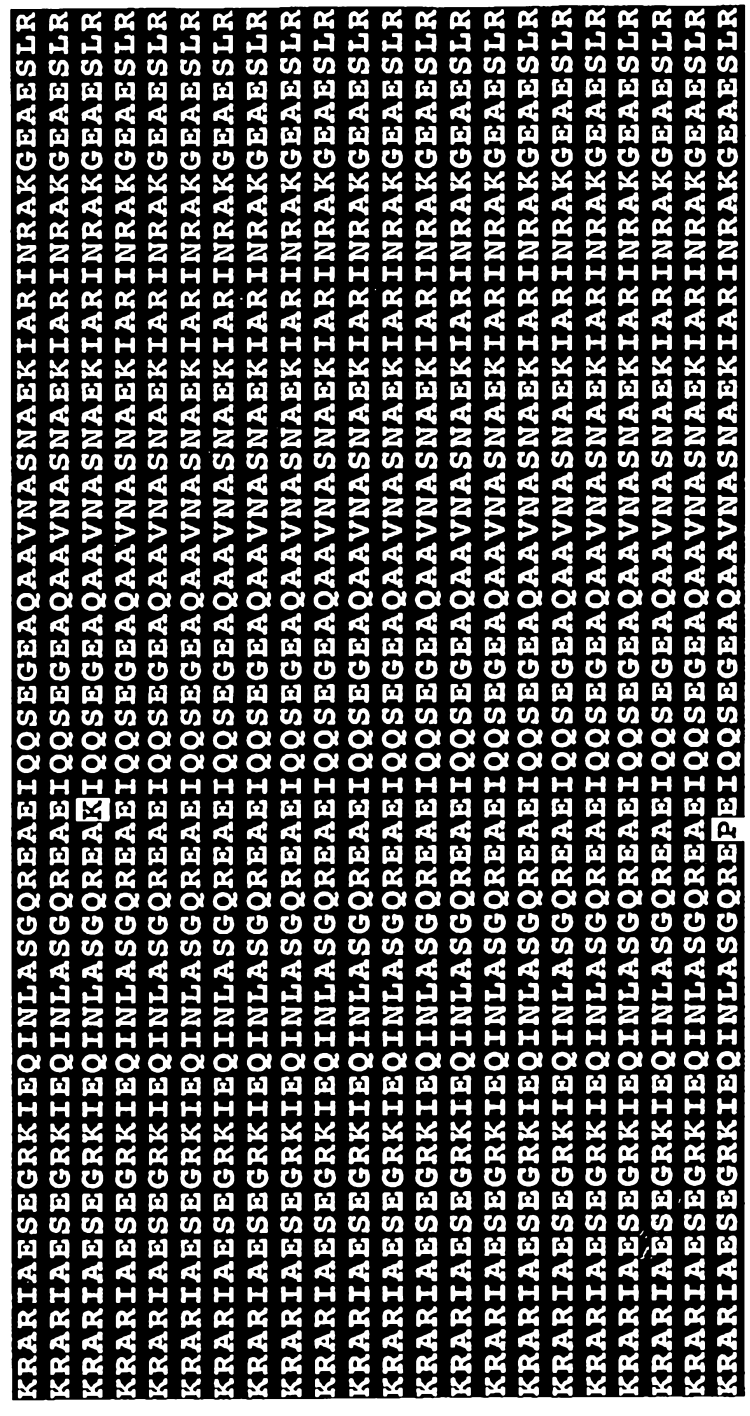

z2491_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv26_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv22_519ass 181 KRARIAESEGRKIEQINLASGQREAKIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
fa1090_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv32_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv11_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv28_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv96_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv02_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv03_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv04_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv05_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv01_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv07_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv12_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv18_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv19_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv21_519ass 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv27_519 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv20_519ass 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv06_519ass 181 KRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
zv29_519ass 181 KRARIAESEGRKIEQINLASGQREPEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR

FIG. 6B(CONTD.)

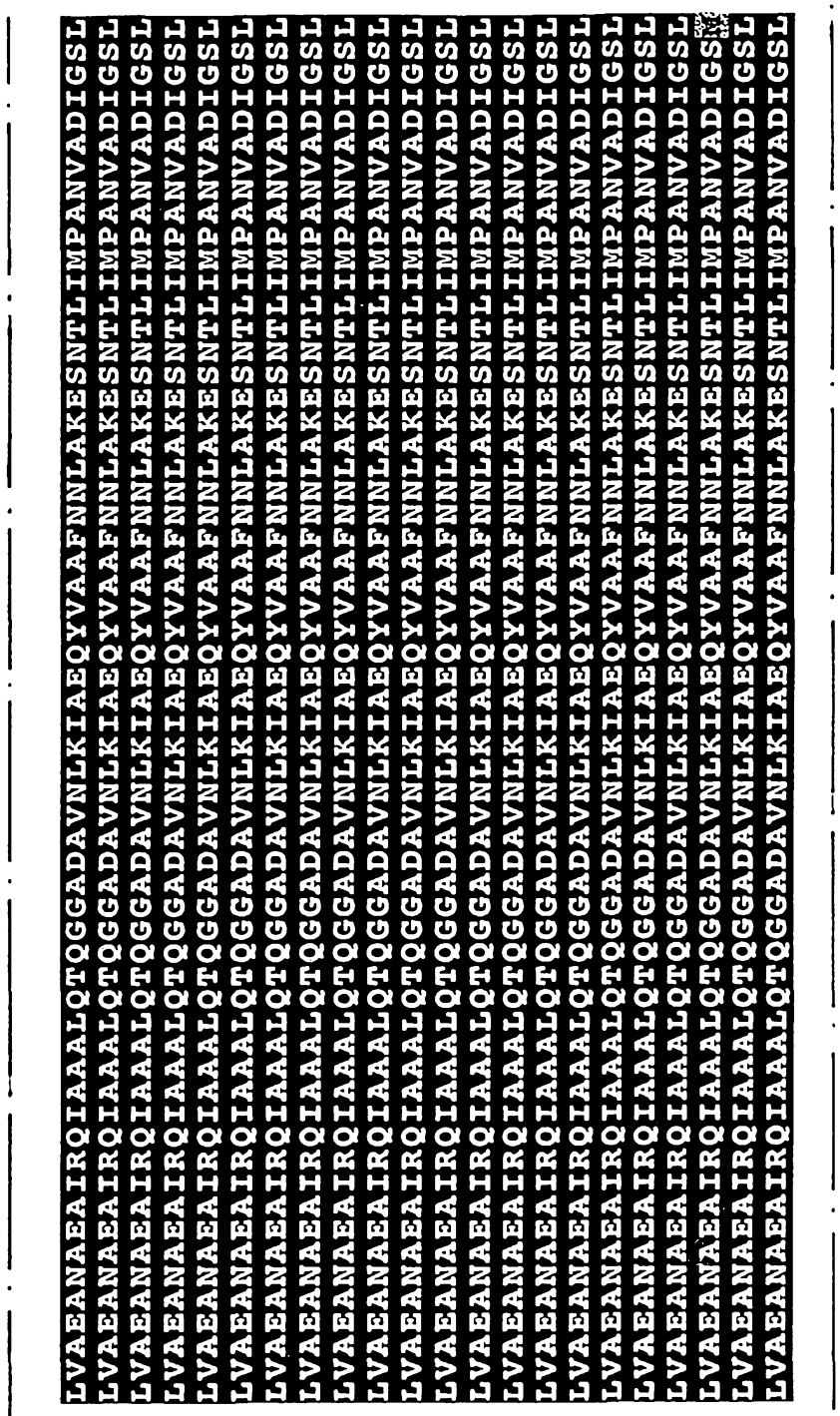

z2491_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv26_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv22_519ass 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
fa1090_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv32_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv11_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv28_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv96_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv02_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv03_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv04_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv05_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv01_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv07_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv12_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv18_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv19_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv21_519ass 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv27_519 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv20_519ass 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv06_519ass 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL
zv29_519ass 241 LVAEANAEAIRQIAAALQTQGGADAVNLKIAEQYVAAFNNLAKESNTLIMPANVADIGSL

FIG. 6B(CONTD.)

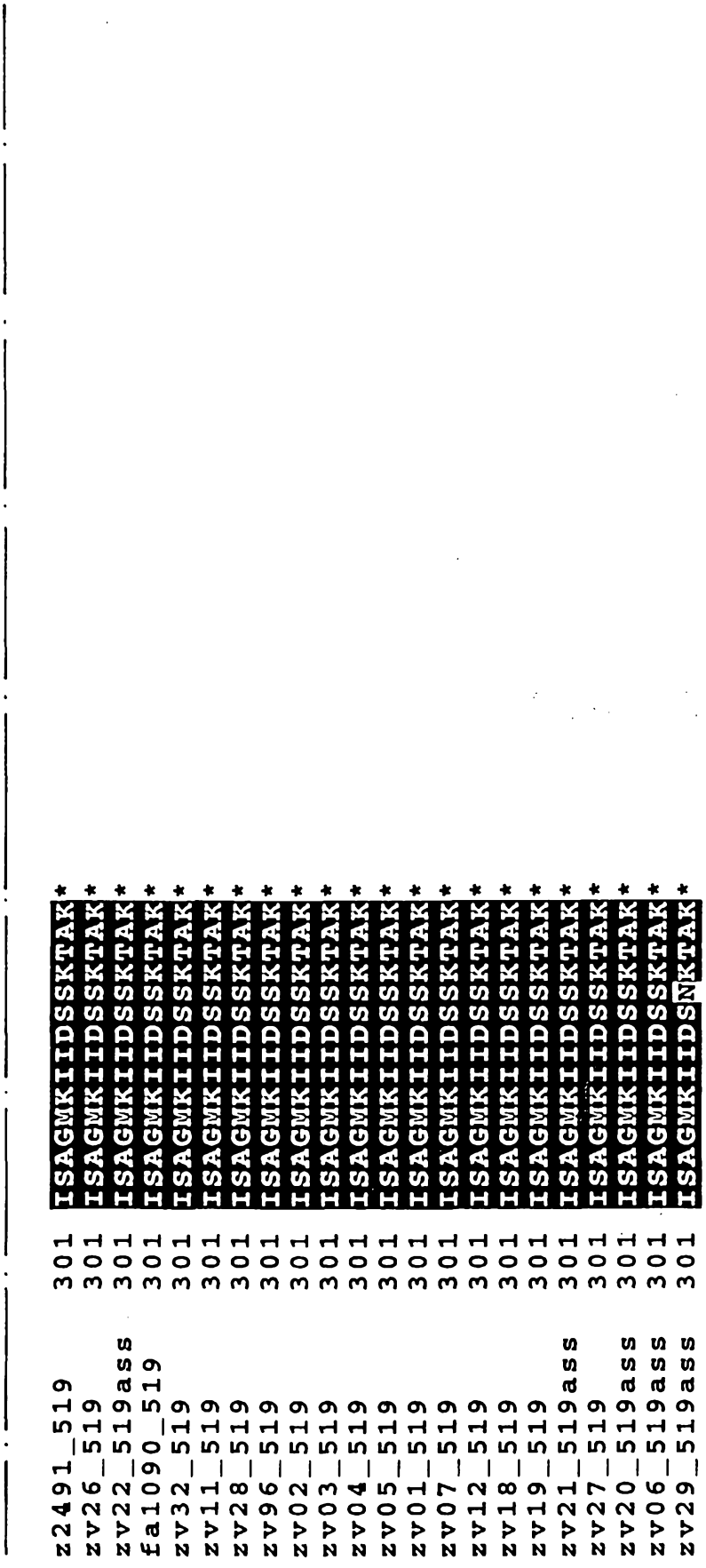

z2491_519 301 ISAGMKIIDSSKTAK*
zv26_519 301 ISAGMKIIDSSKTAK*
zv22_519ass 301 ISAGMKIIDSSKTAK*
fa1090_519 301 ISAGMKIIDSSKTAK*
zv32_519 301 ISAGMKIIDSSKTAK*
zv11_519 301 ISAGMKIIDSSKTAK*
zv28_519 301 ISAGMKIIDSSKTAK*
zv96_519 301 ISAGMKIIDSSKTAK*
zv02_519 301 ISAGMKIIDSSKTAK*
zv03_519 301 ISAGMKIIDSSKTAK*
zv04_519 301 ISAGMKIIDSSKTAK*
zv05_519 301 ISAGMKIIDSSKTAK*
zv01_519 301 ISAGMKIIDSSKTAK*
zv07_519 301 ISAGMKIIDSSKTAK*
zv12_519 301 ISAGMKIIDSSKTAK*
zv18_519 301 ISAGMKIIDSSKTAK*
zv19_519 301 ISAGMKIIDSSKTAK*
zv21_519ass 301 ISAGMKIIDSSKTAK*
zv27_519 301 ISAGMKIIDSSKTAK*
zv20_519ass 301 ISAGMKIIDSSKTAK*
zv06_519ass 301 ISAGMKIIDSSKTAK*
zv29_519ass 301 ISAGMKIIDSNKTAK*

FIG. 7A

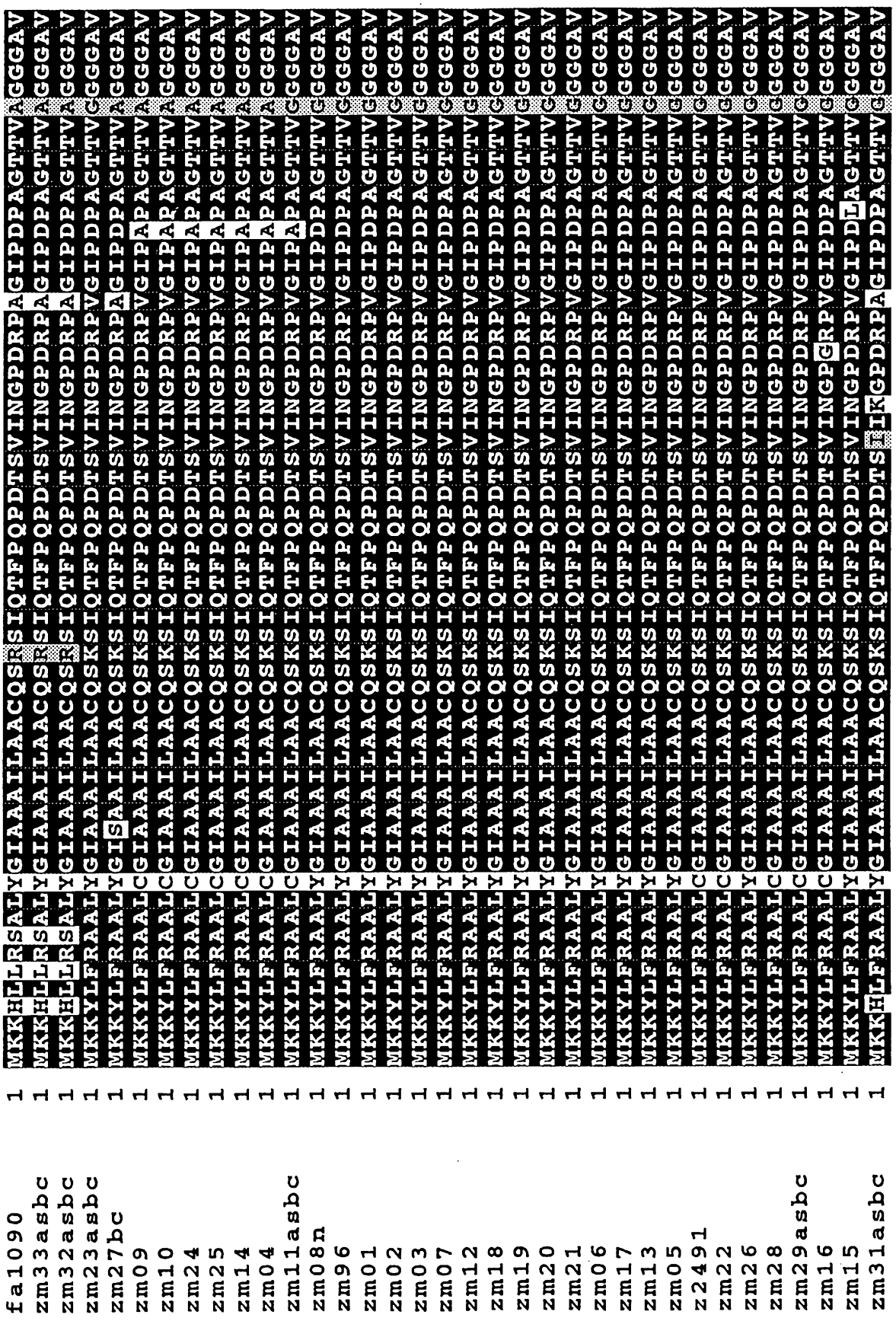


FIG. 7A (CONTD.)


fa1090 61 YTVVPHLSMPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKRFFER
zm33asbc 61 YTVVPHLSMPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPIHSFQAKRFFER
zm32asbc 61 YTVVPHLSMPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKRFFER
zm23asbc 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm27bc 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm09 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm10 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm24 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm25 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm14 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm04 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm11asbc 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSVQAKQFFER
zm08n 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm96 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm01 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm02 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm03 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm07 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm12 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm18 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm19 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm20 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm21 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm06 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm17 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm13 61 YTVVPHLSLPHWAEQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm05 61 YTVVPHLSLPHWAAQDFAKSLQSFRLSCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
z2491 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSVQAKQFFER
zm22 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSVQAKQFFER
zm26 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSVQAKQFFER
zm28 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm29asbc 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm16 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER
zm15 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNEQGWQDVCAQAFQTPVHSFQAKQFFER
zm31asbc 61 YTVVPHLSLPHWAAQDFAKSLQSFRLGCANLKNRQGWQDVCAQAFQTPVHSFQAKQFFER

FIG. 7B

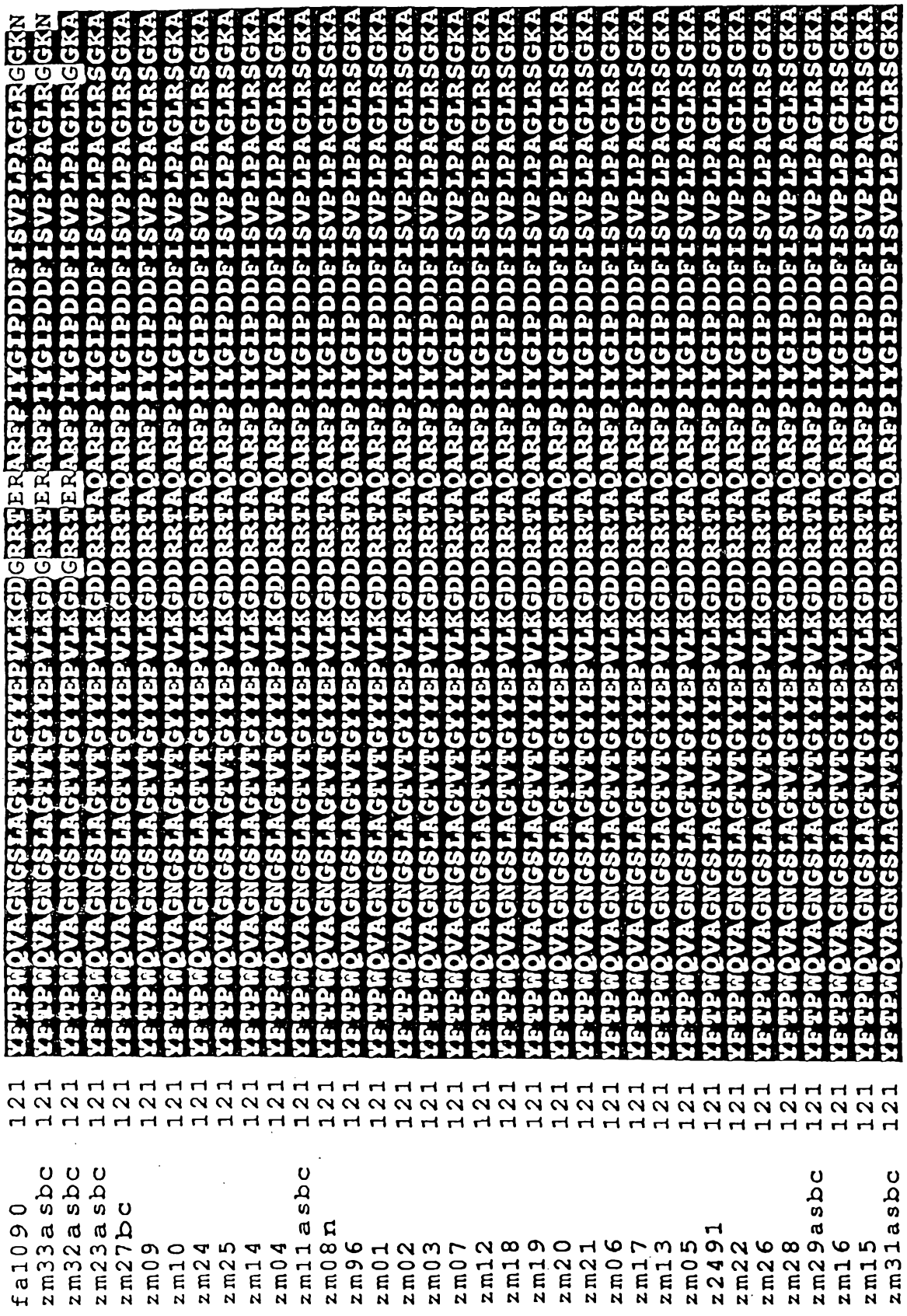

fa1090 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDGRRTERARFPIYGIPDDFISVPLPAGLRGGKN
zm33asbc 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDGRRTERARFPIYGIPDDFISVPLPAGLRGGKN
zm32asbc 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDGRRTERARFPIYGIPDDFISVPLPAGLRGGKA
zm23asbc 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm27bc 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm09 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm10 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm24 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm25 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm14 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm04 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm11asbc 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm08n 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm96 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm01 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm02 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm03 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm07 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm12 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm18 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm19 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm20 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm21 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm06 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm17 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm13 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm05 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
z2491 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm22 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm26 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm28 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm29asbc 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm16 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm15 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA
zm31asbc 121 YETPWQVAGNGSLAGTVTGYYEPVLKGDDRRTAQARFPIYGIPDDFISVPLPAGLRSGKA

FIG. 7B(CONTD.)

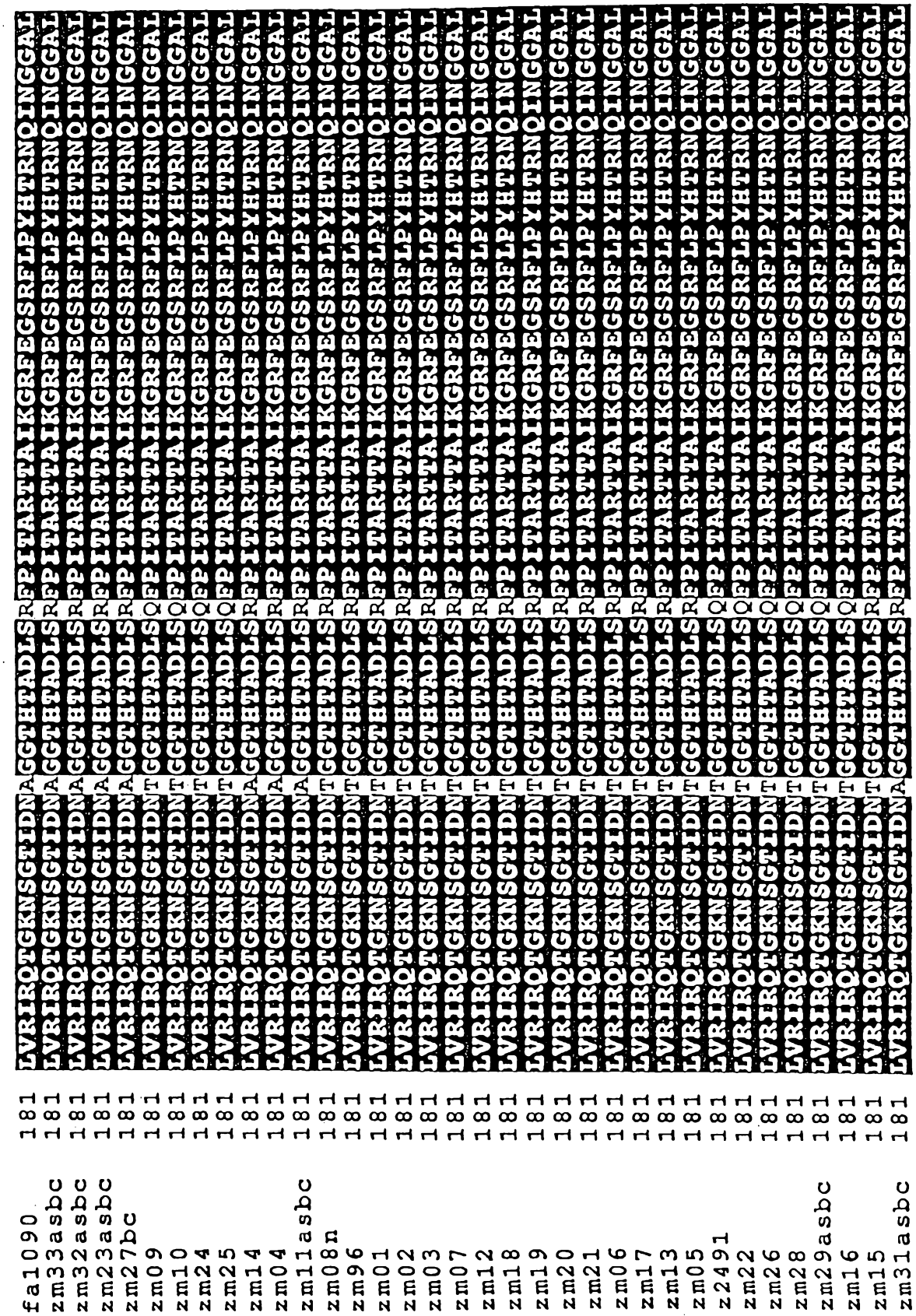

fa1090 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm33asbc 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm32asbc 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm23asbc 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm27bc 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm09 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm10 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm24 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm25 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm14 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm04 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm11asbc 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm08n 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm96 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm01 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm02 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm03 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm07 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm12 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm18 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm19 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm20 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm21 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm06 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm17 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm13 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm05 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
z2491 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm22 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm26 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm28 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm29asbc 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm16 181 LVRIRQTGKNSGTIDNTGGTHTADLSQFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm15 181 LVRIRQTGKNSGTIDNTGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL
zm31asbc 181 LVRIRQTGKNSGTIDNAGGTHTADLSRFPITARTTAIKGRFEGSRFLPYHTRNQINGGAL

FIG. 7C

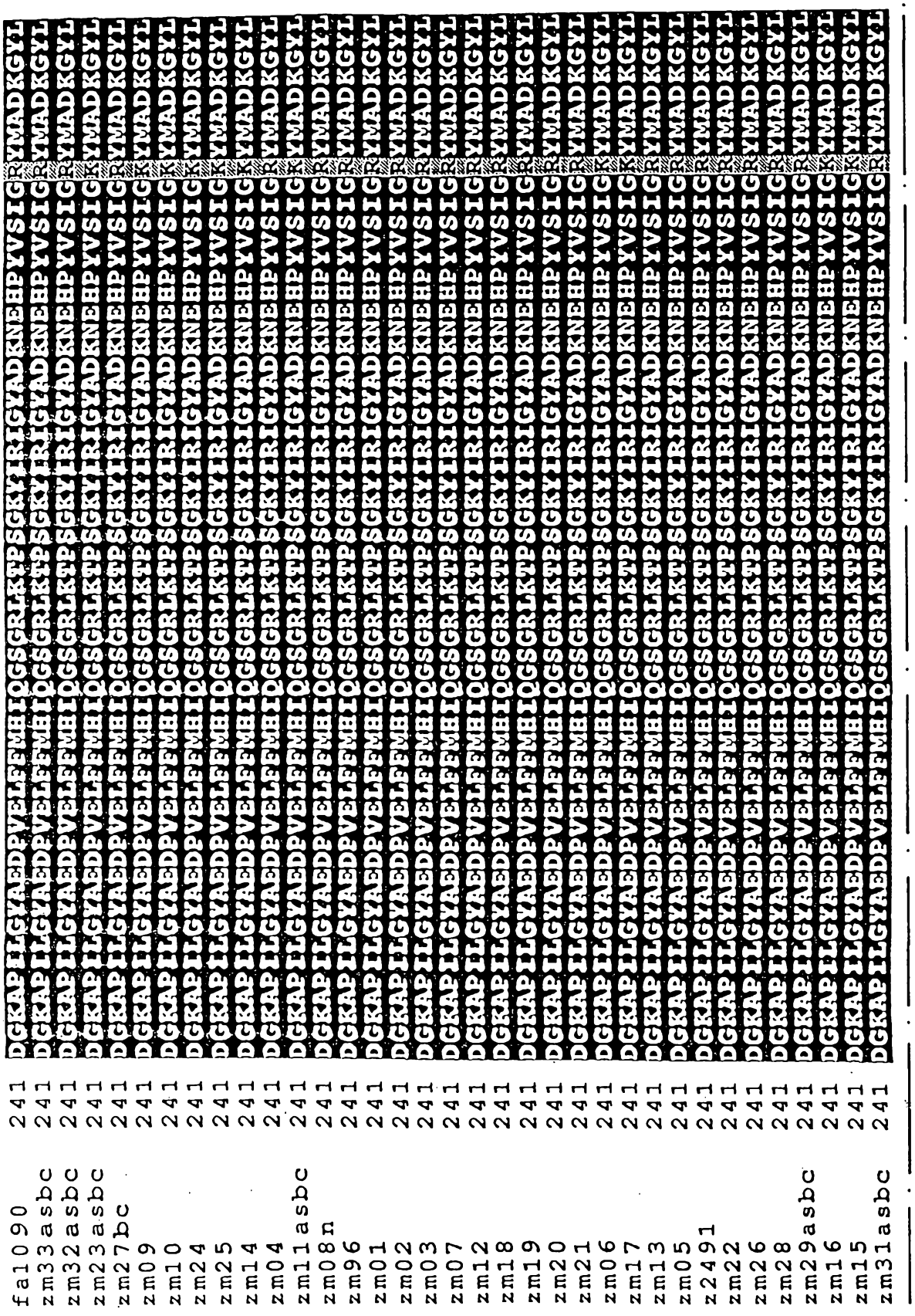

fa1090
zm33asbc
zm32asbc
zm23asbc
zm27bc
zm09
zm10
zm24
zm25
zm14
zm04
zm11asbc
zm08n
zm96
zm01
zm02
zm03
zm07
zm12
zm18
zm19
zm20
zm21
zm06
zm17
zm13
zm05
z2491
zm22
zm26
zm28
zm29asbc
zm16
zm15
zm31asbc

FIG. 7C(CONTD.)

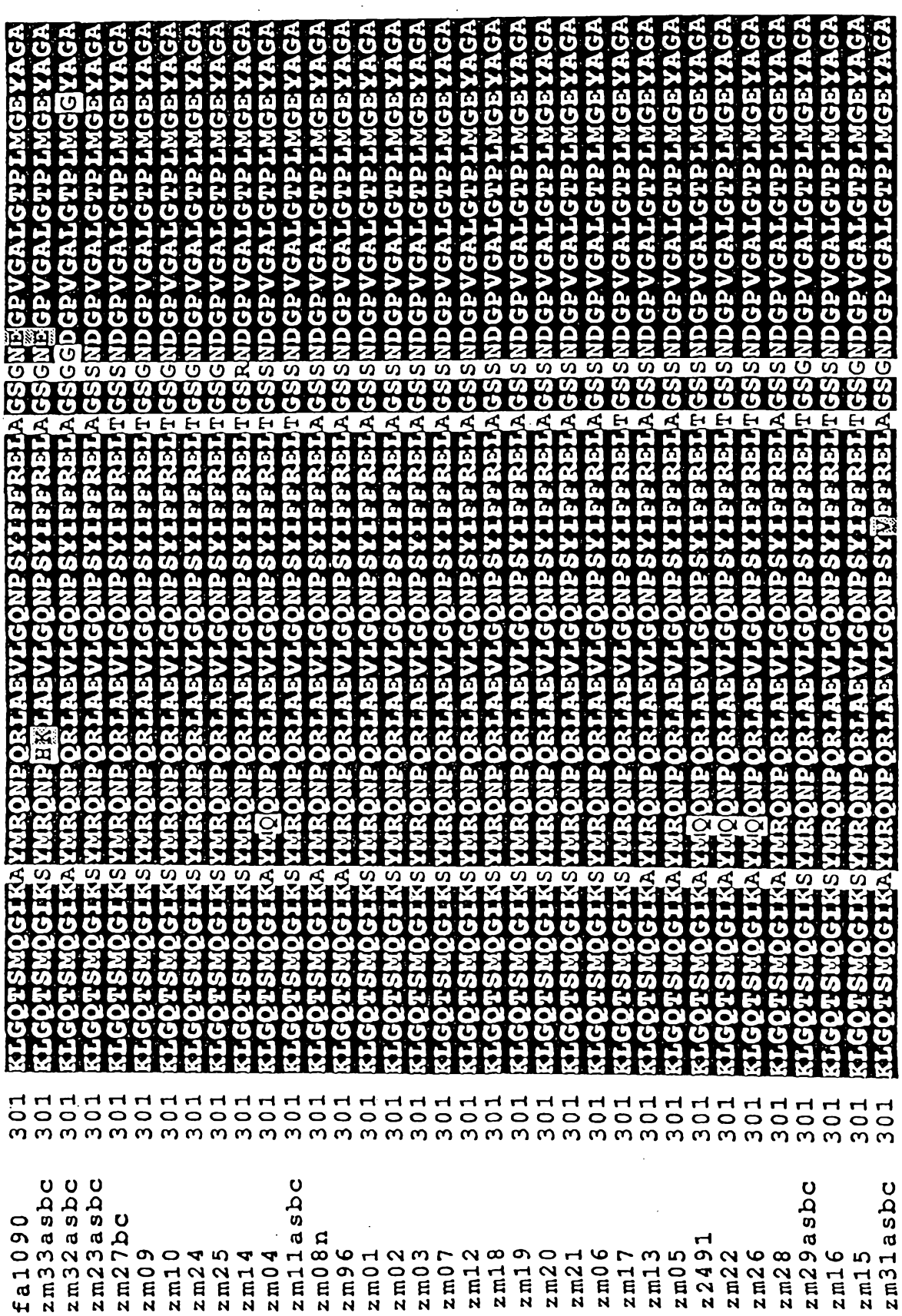


FIG.7D

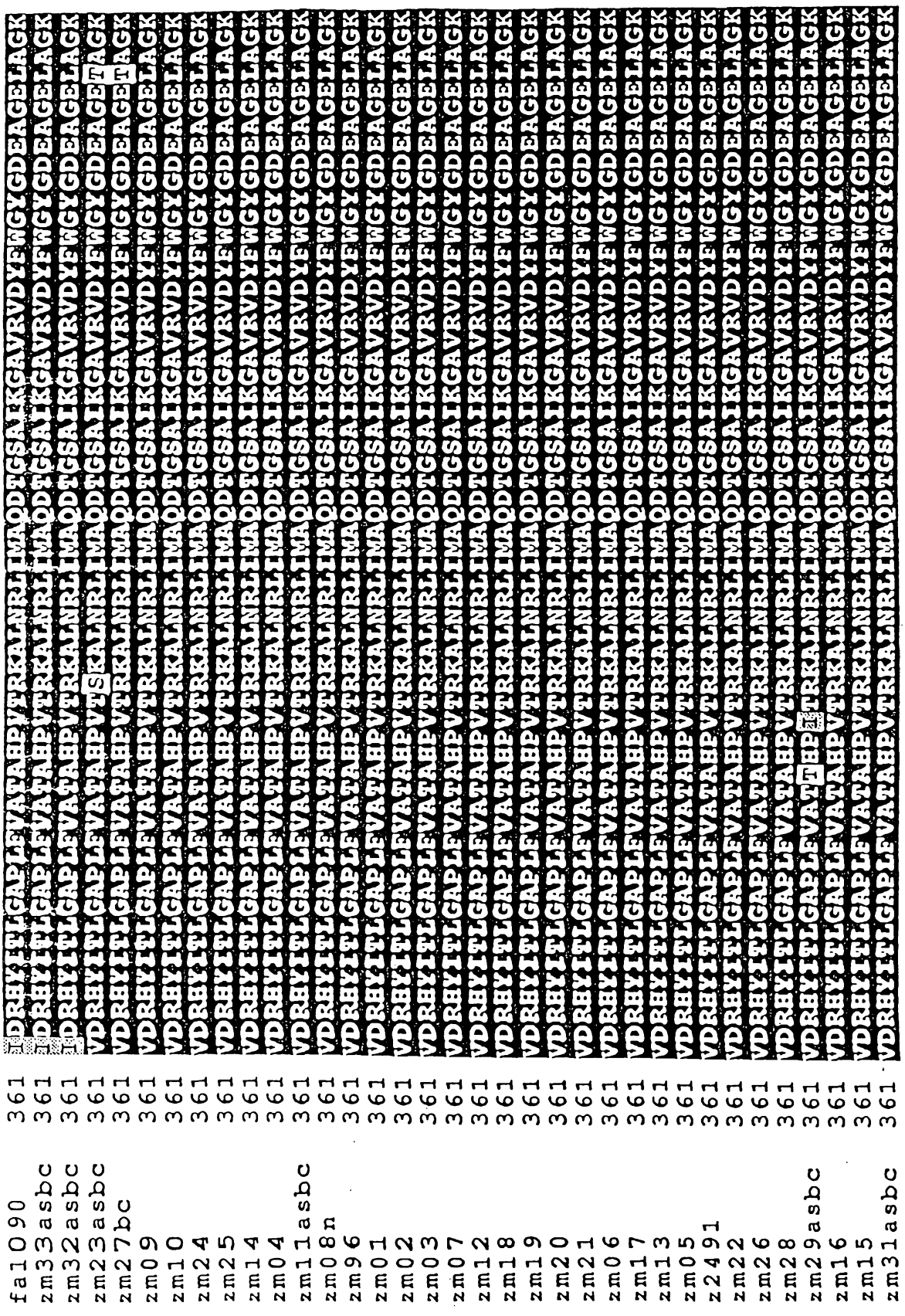


FIG. 7D(CONTD.)

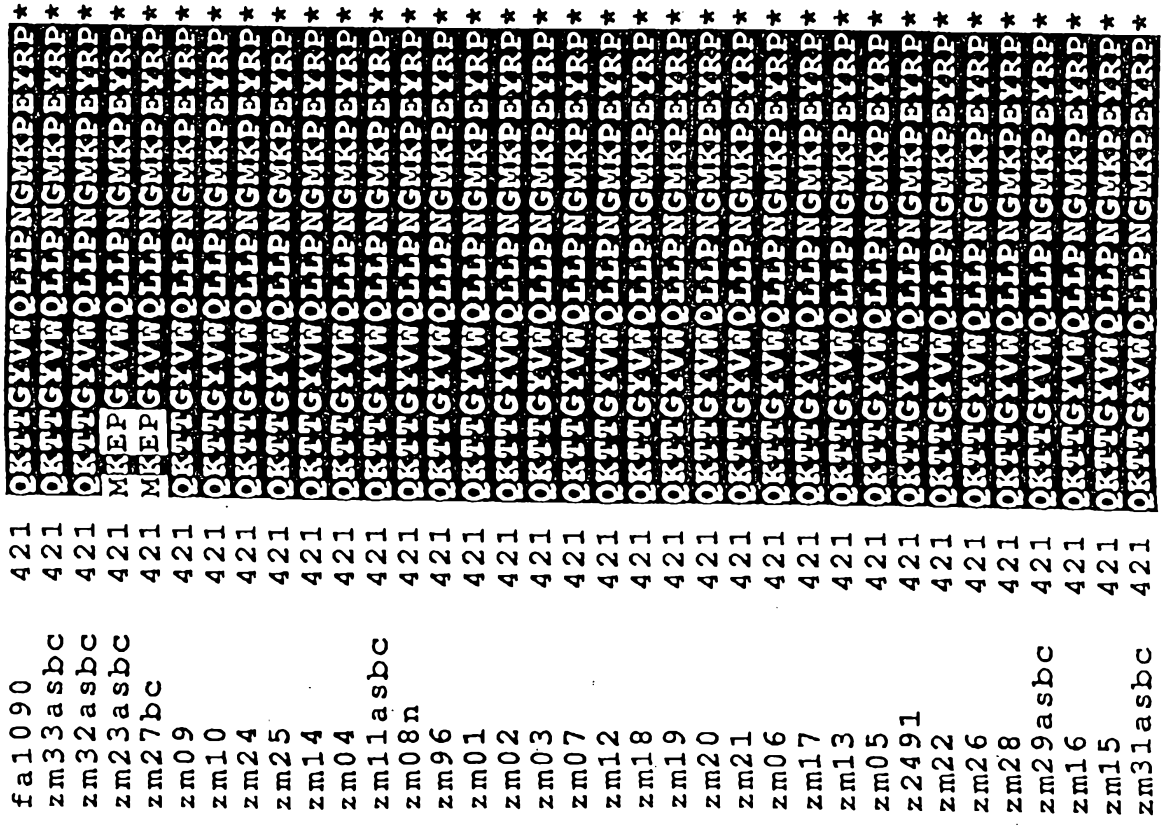

fa1090 421 QKTTGYVWQLLPNGMKPEYRP *
zm33asbc 421 QKTTGYVWQLLPNGMKPEYRP *
zm32asbc 421 QKTTGYVWQLLPNGMKPEYRP *
zm23asbc 421 MKEPGYVWQLLPNGMKPEYRP *
zm27bc 421 MKEPGYVWQLLPNGMKPEYRP *
zm09 421 QKTTGYVWQLLPNGMKPEYRP *
zm10 421 QKTTGYVWQLLPNGMKPEYRP *
zm24 421 QKTTGYVWQLLPNGMKPEYRP *
zm25 421 QKTTGYVWQLLPNGMKPEYRP *
zm14 421 QKTTGYVWQLLPNGMKPEYRP *
zm04 421 QKTTGYVWQLLPNGMKPEYRP *
zm11asbc 421 QKTTGYVWQLLPNGMKPEYRP *
zm08n 421 QKTTGYVWQLLPNGMKPEYRP *
zm96 421 QKTTGYVWQLLPNGMKPEYRP *
zm01 421 QKTTGYVWQLLPNGMKPEYRP *
zm02 421 QKTTGYVWQLLPNGMKPEYRP *
zm03 421 QKTTGYVWQLLPNGMKPEYRP *
zm07 421 QKTTGYVWQLLPNGMKPEYRP *
zm12 421 QKTTGYVWQLLPNGMKPEYRP *
zm18 421 QKTTGYVWQLLPNGMKPEYRP *
zm19 421 QKTTGYVWQLLPNGMKPEYRP *
zm20 421 QKTTGYVWQLLPNGMKPEYRP *
zm21 421 QKTTGYVWQLLPNGMKPEYRP *
zm06 421 QKTTGYVWQLLPNGMKPEYRP *
zm17 421 QKTTGYVWQLLPNGMKPEYRP *
zm13 421 QKTTGYVWQLLPNGMKPEYRP *
zm05 421 QKTTGYVWQLLPNGMKPEYRP *
z2491 421 QKTTGYVWQLLPNGMKPEYRP *
zm22 421 QKTTGYVWQLLPNGMKPEYRP *
zm26 421 QKTTGYVWQLLPNGMKPEYRP *
zm28 421 QKTTGYVWQLLPNGMKPEYRP *
zm29asbc 421 QKTTGYVWQLLPNGMKPEYRP *
zm16 421 QKTTGYVWQLLPNGMKPEYRP *
zm15 421 QKTTGYVWQLLPNGMKPEYRP *
zm31asbc 421 QKTTGYVWQLLPNGMKPEYRP *

FIG. 8

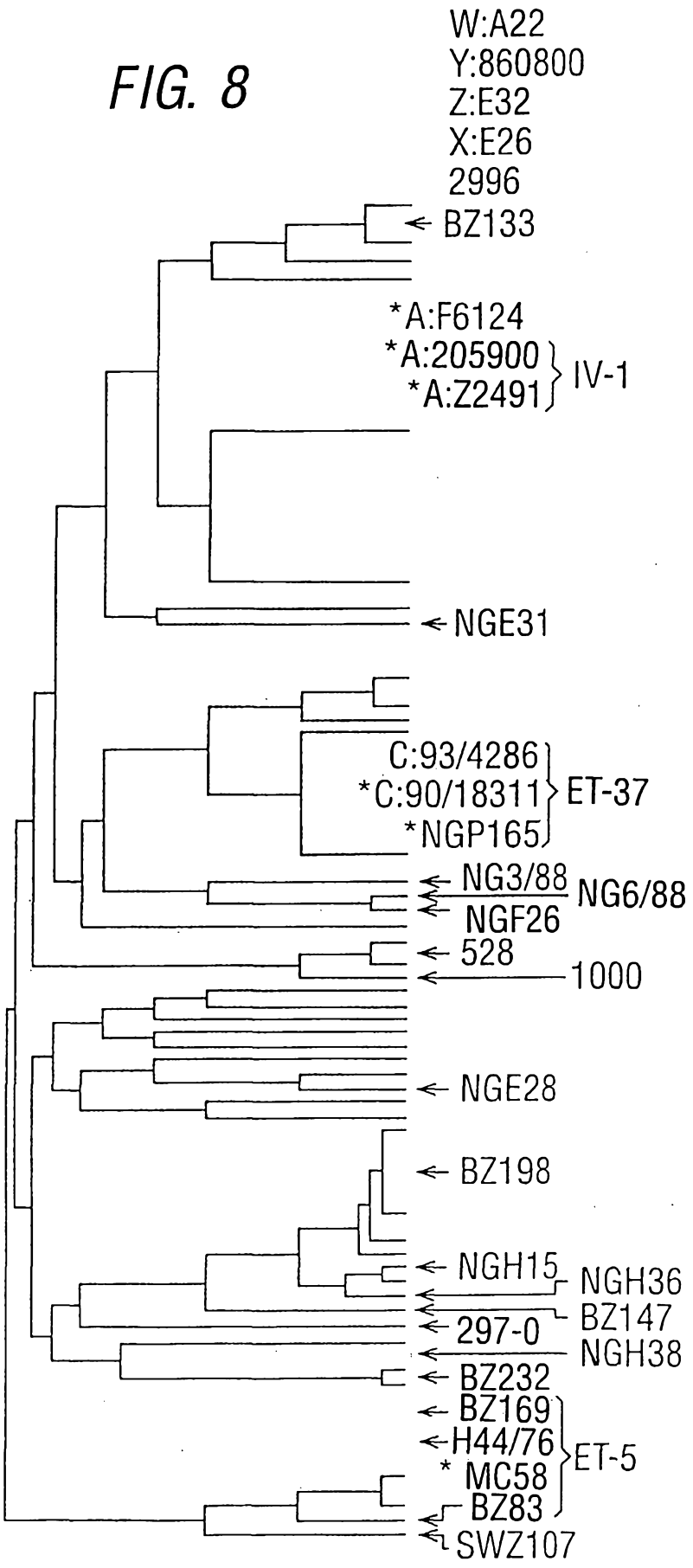

W:A22
Y:860800
Z:E32
X:E26
2996
BZ133
*A:F6124
*A:205900
*A:Z2491
IV-1
NGE31
C:93/4286
*C:90/18311
*NGP165
ET-37
NG3/88
NG6/88
NGF26
528
1000
NGE28
BZ198
NGH15
NGH36
BZ147
297-0
NGH38
BZ232
BZ169
H44/76
* MC58
BZ83
ET-5
SWZ107

FIG. 9a

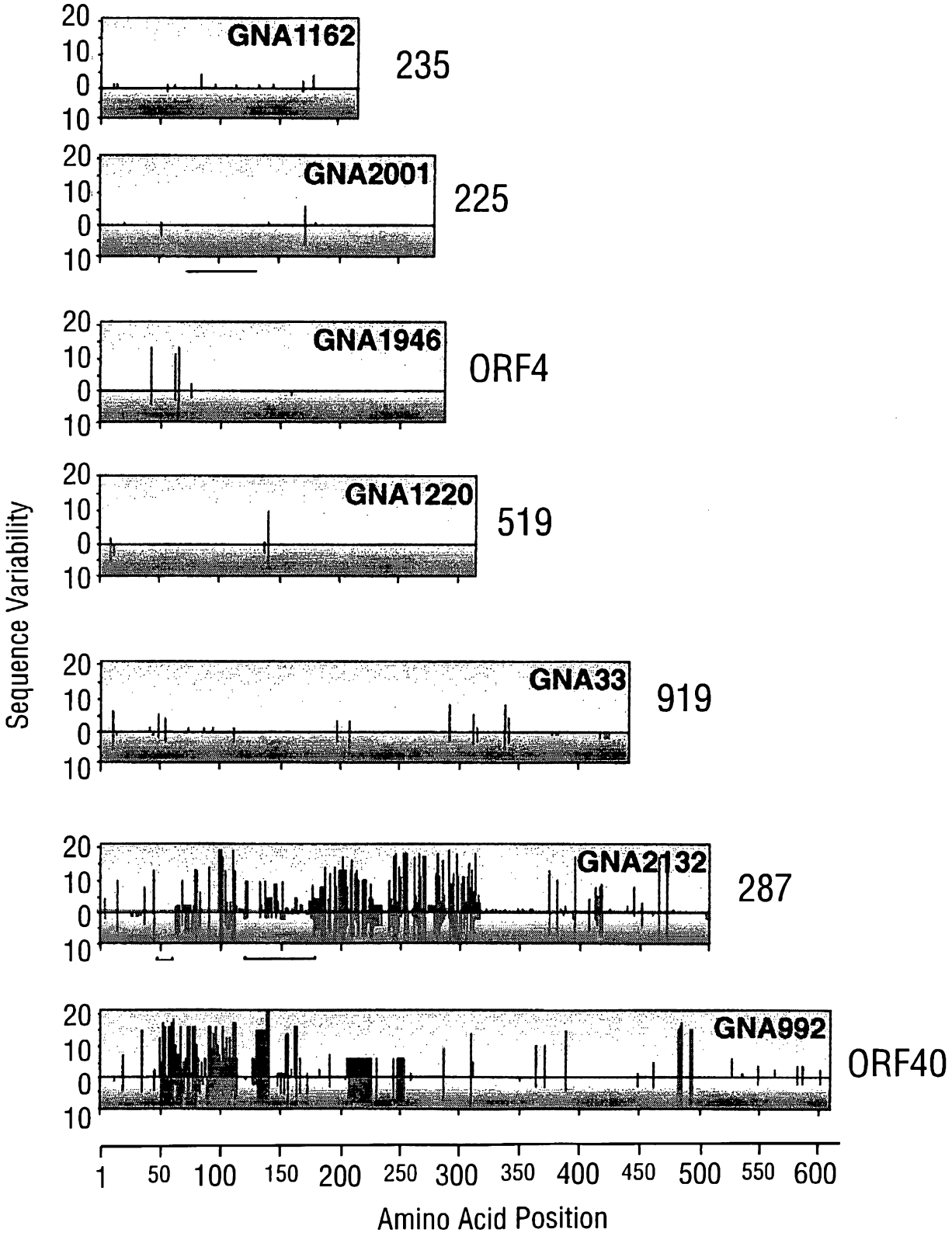

GNA1162
235
GNA2001
225
GNA1946
ORF4
GNA1220
519
GNA33
919
GNA2132
287
GNA992
ORF40
20
10
0
10
Sequence Variability
1
50
100
150
200
250
300
350
400
450
500
550
600
Amino Acid Position

FIG. 9b

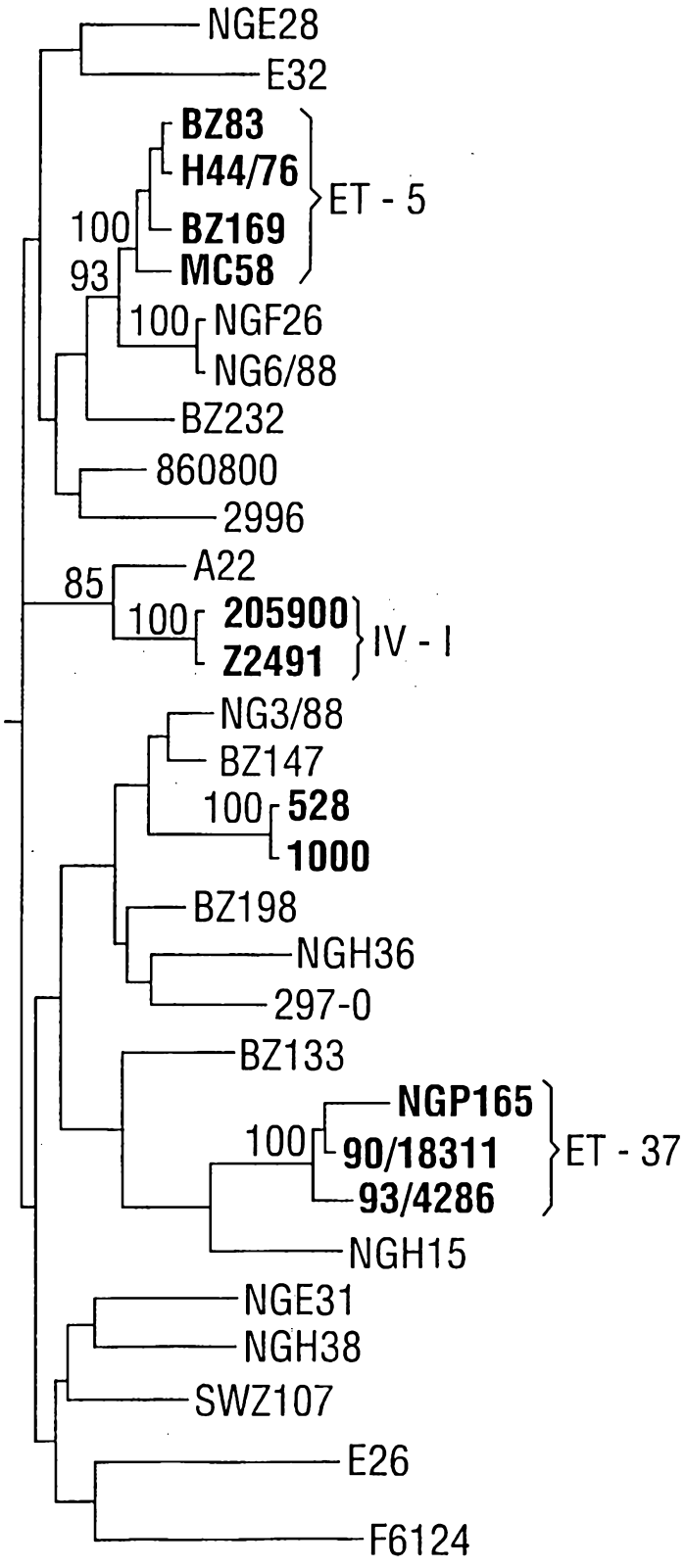

NGE28
E32
BZ83
H44/76
BZ169
MC58
ET - 5
100
93
100
NGF26
NG6/88
BZ232
860800
2996
85
A22
100
205900
Z2491
IV - I
NG3/88
BZ147
100
528
1000
BZ198
NGH36
297-0
BZ133
NGP165
100
90/18311
93/4286
ET - 37
NGH15
NGE31
NGH38
SWZ107
E26
F6124

FIG. 10A

ORF 4

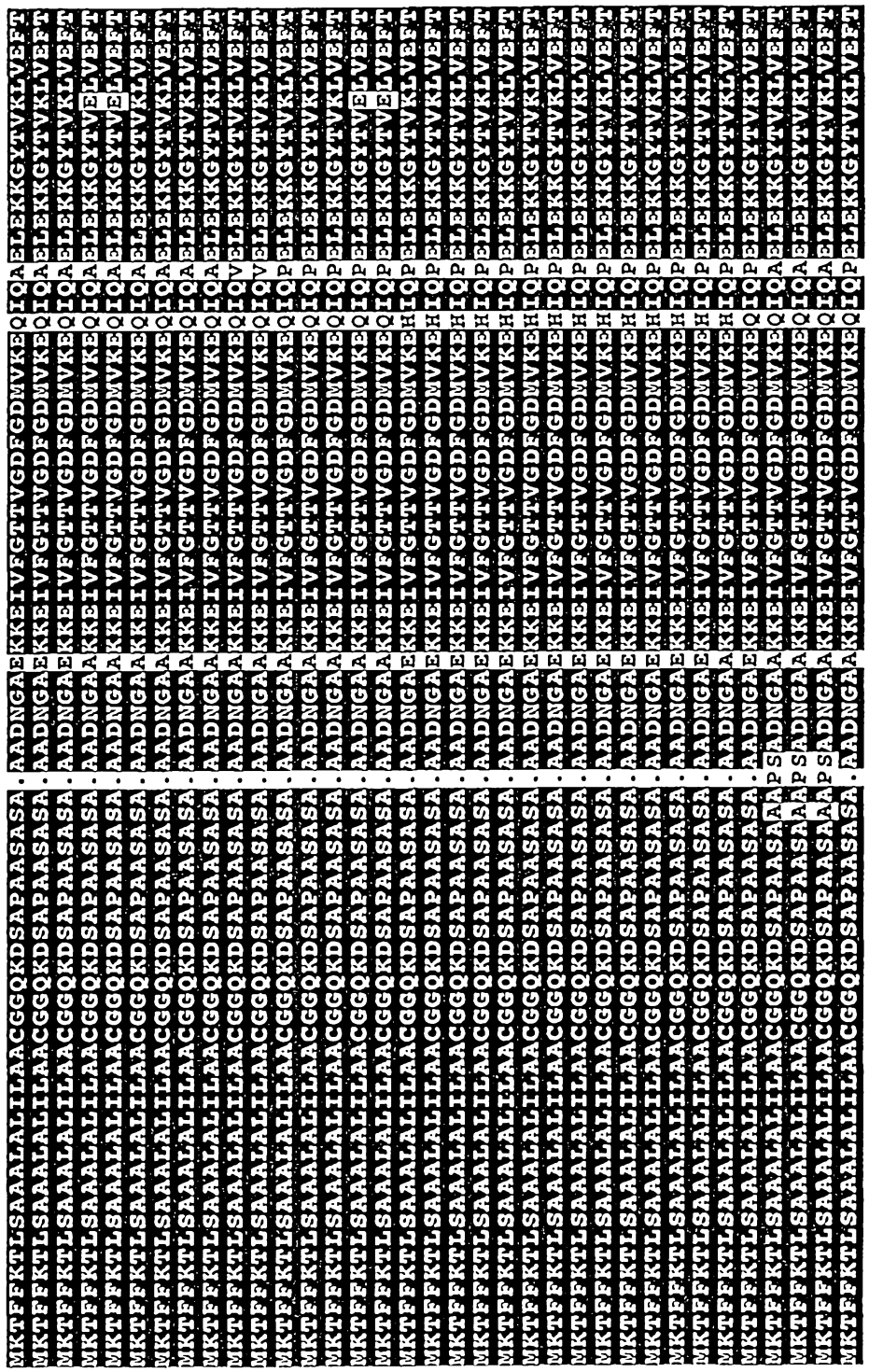


FIG. 10A(CONTD.)

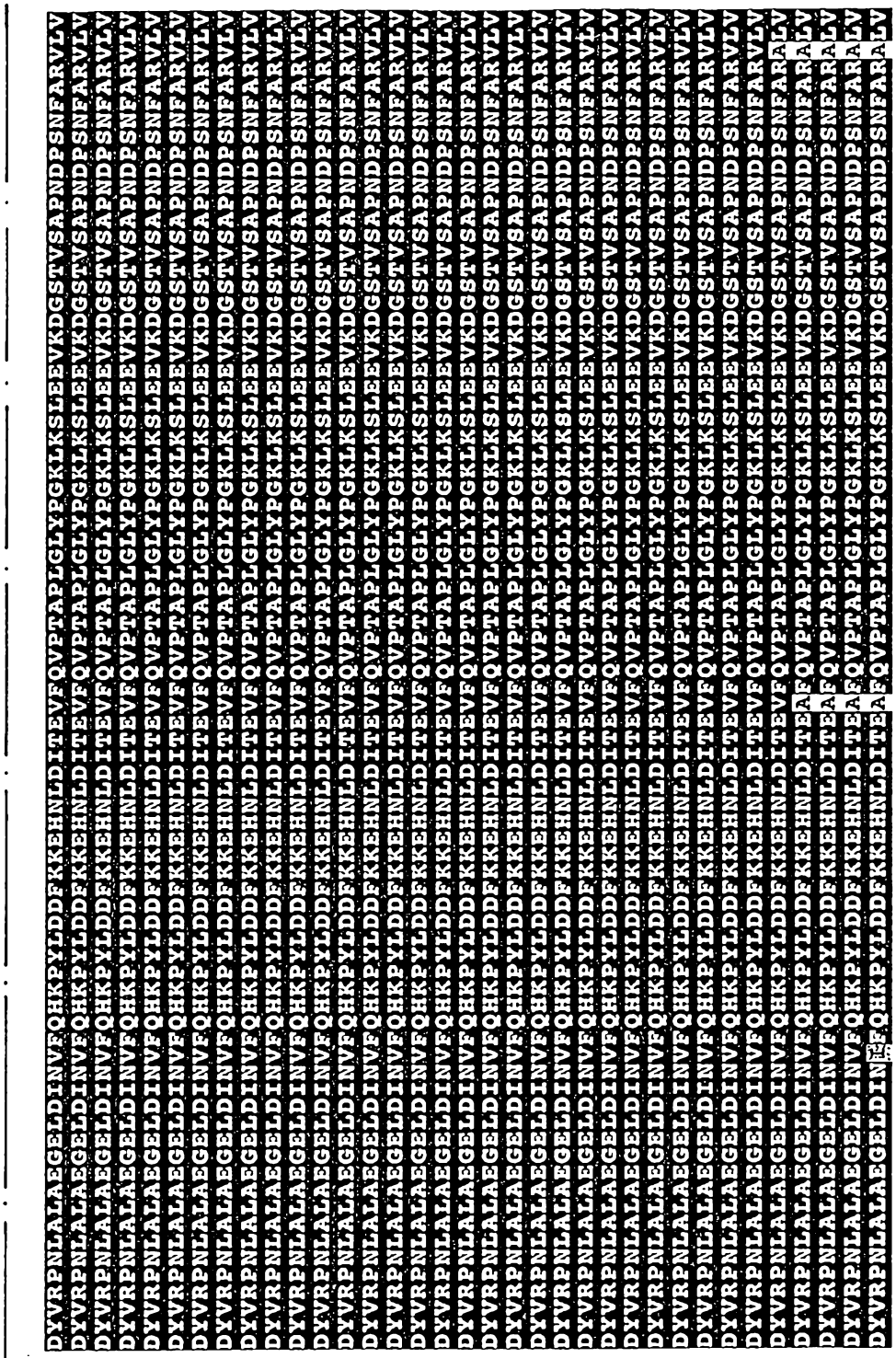
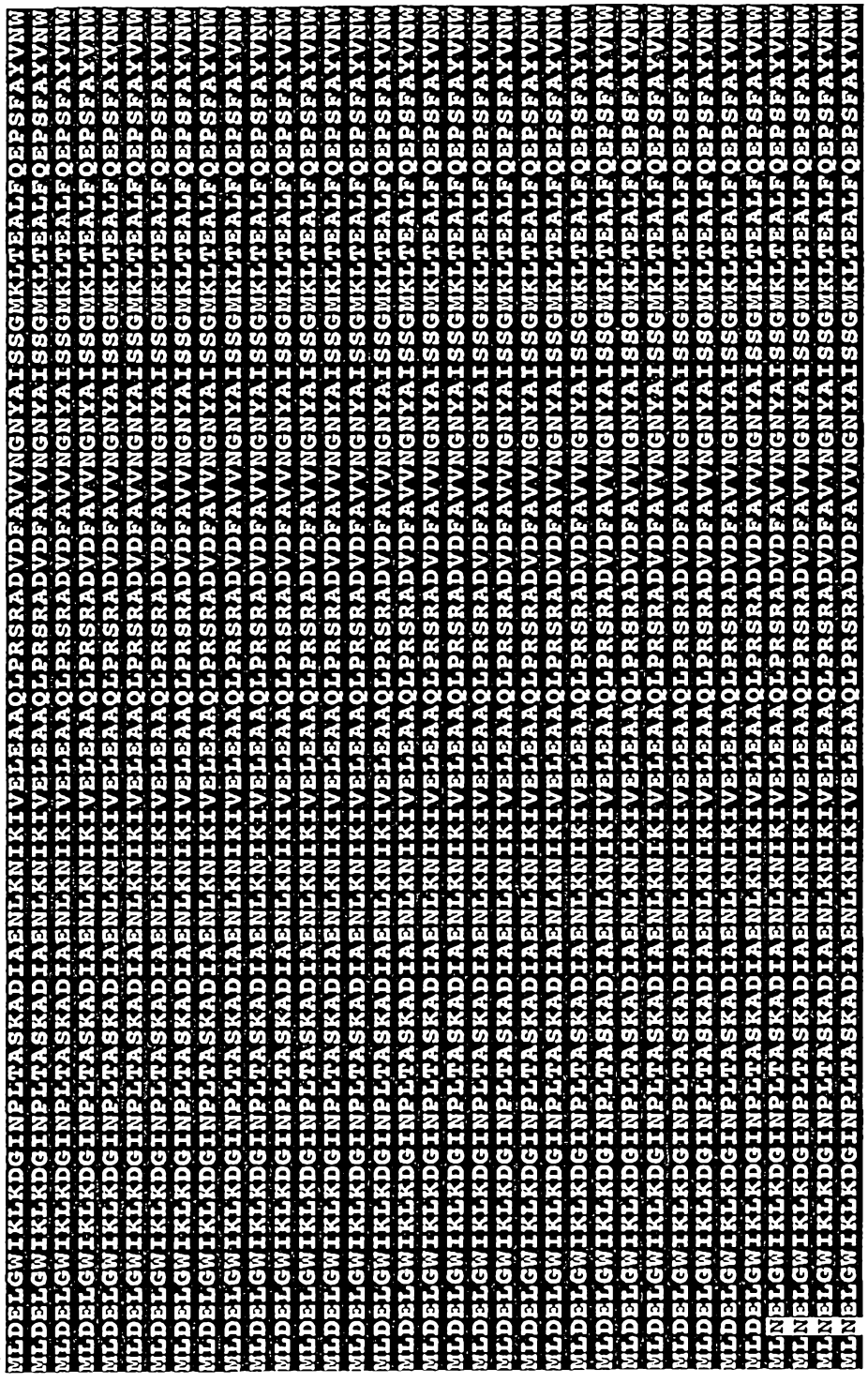


FIG.10B



51/97

FIG. 10B(CONTD.)

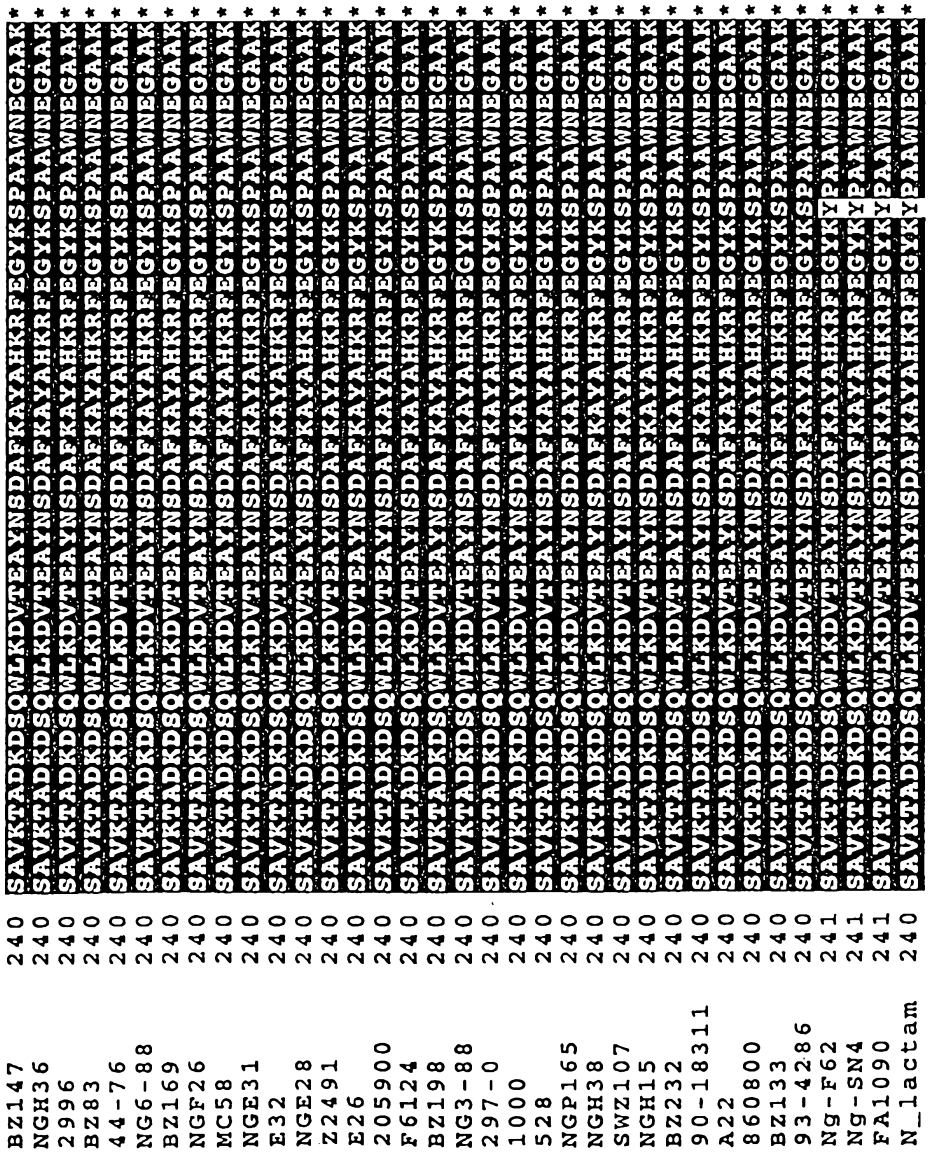


FIG. 11A

ORF 40

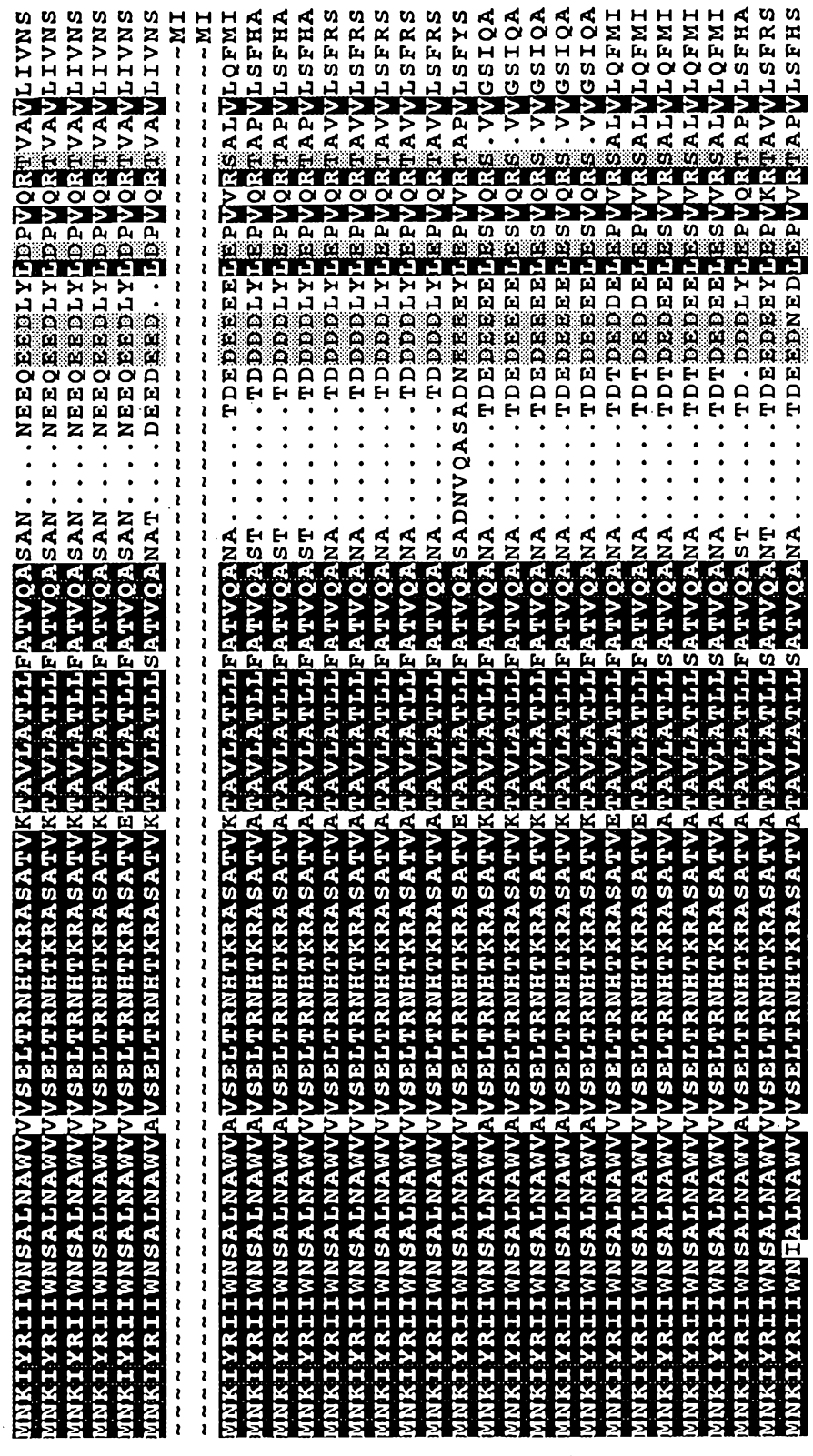


FIG. 11A(CONTD.)

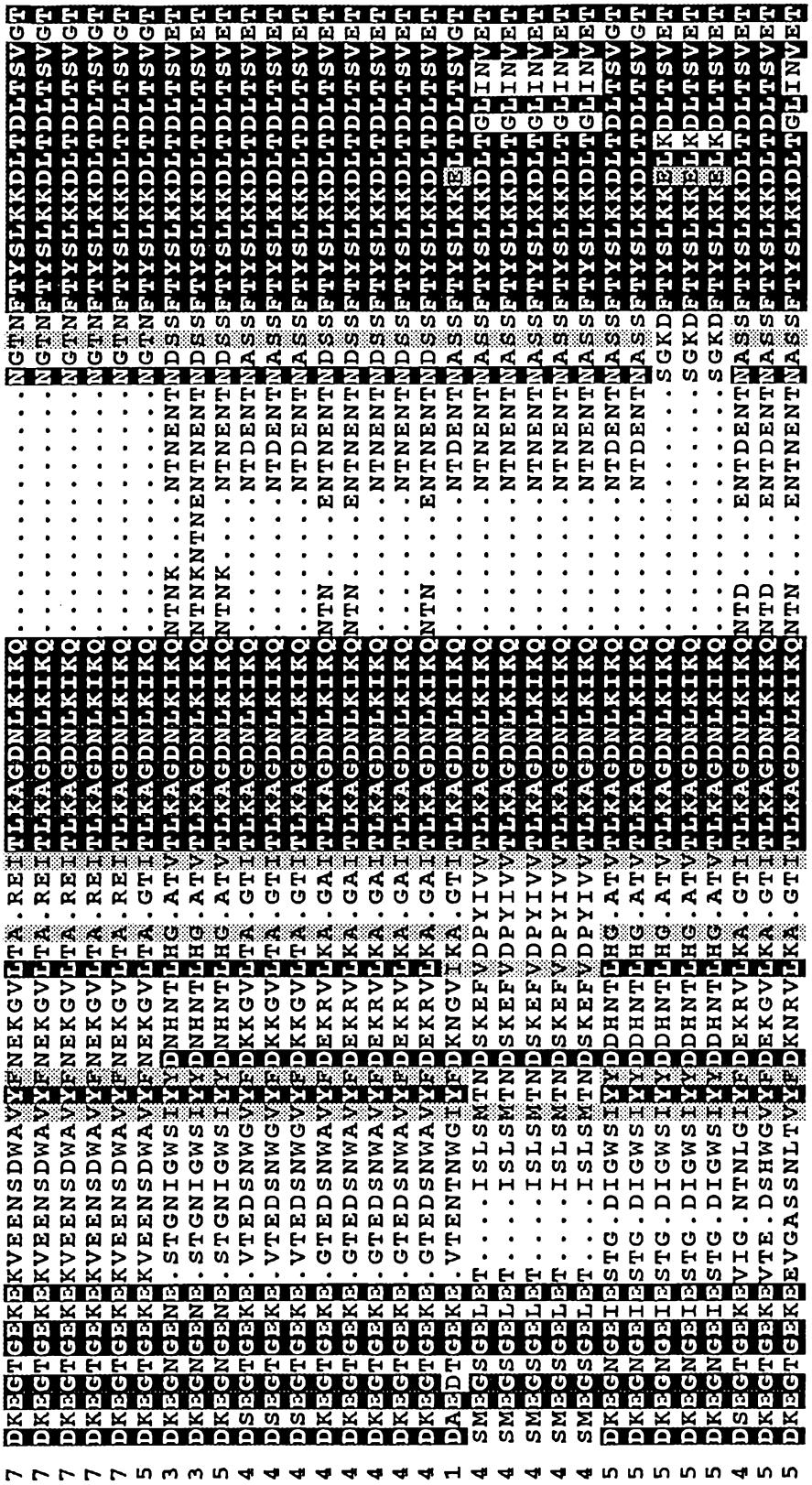


FIG. 11B

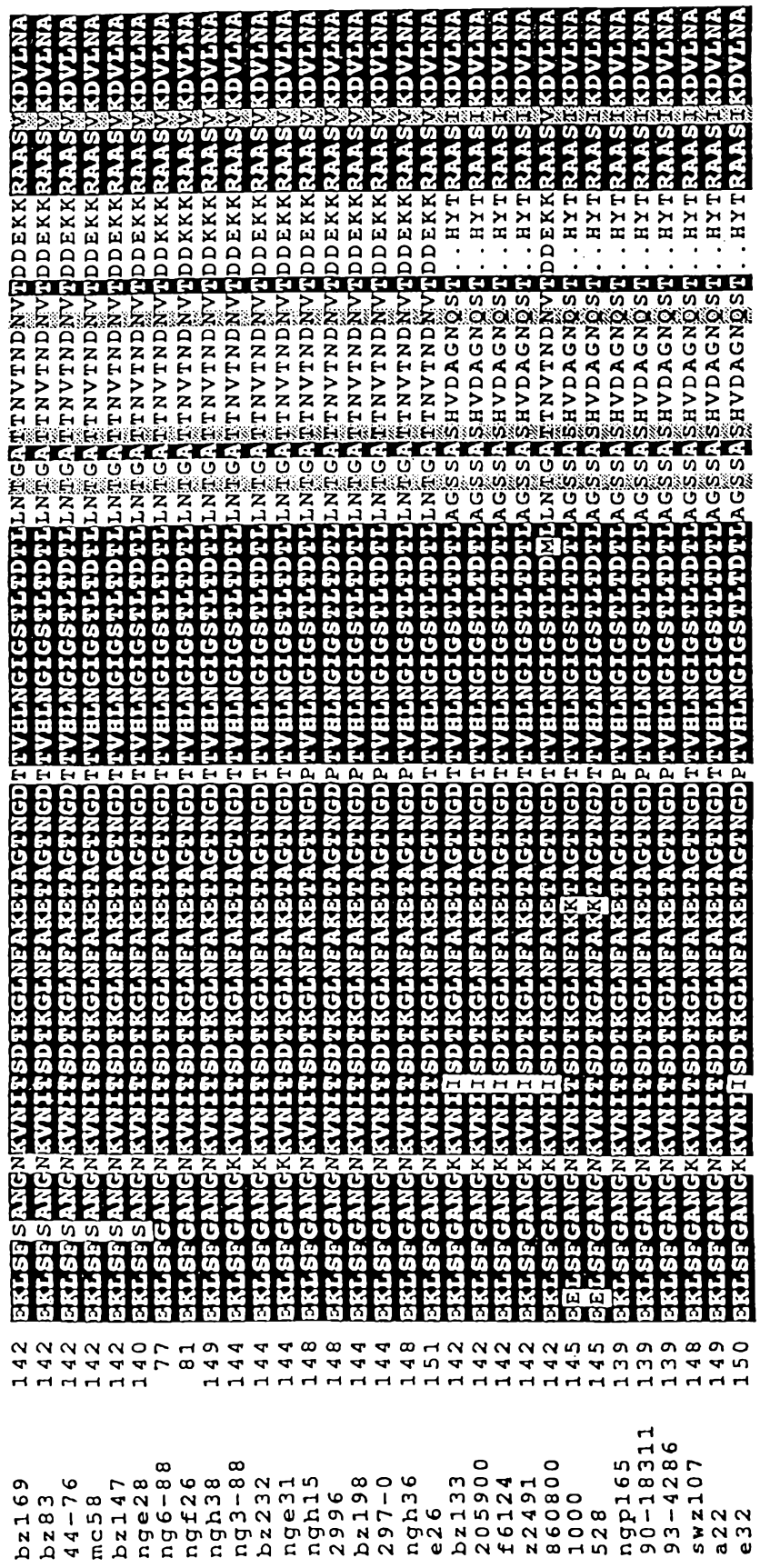


FIG. 11B(CONTD.)

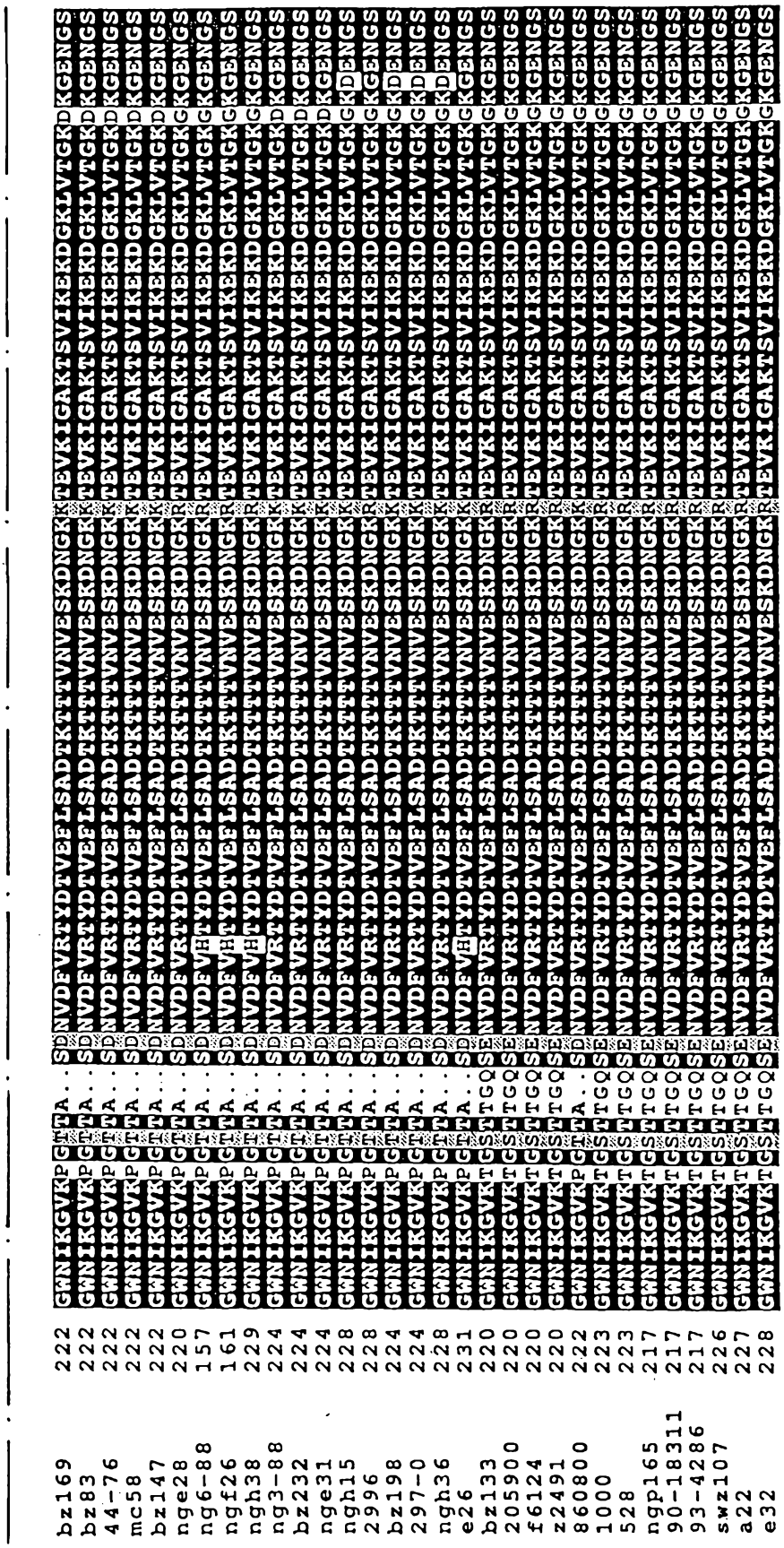


FIG. 11C

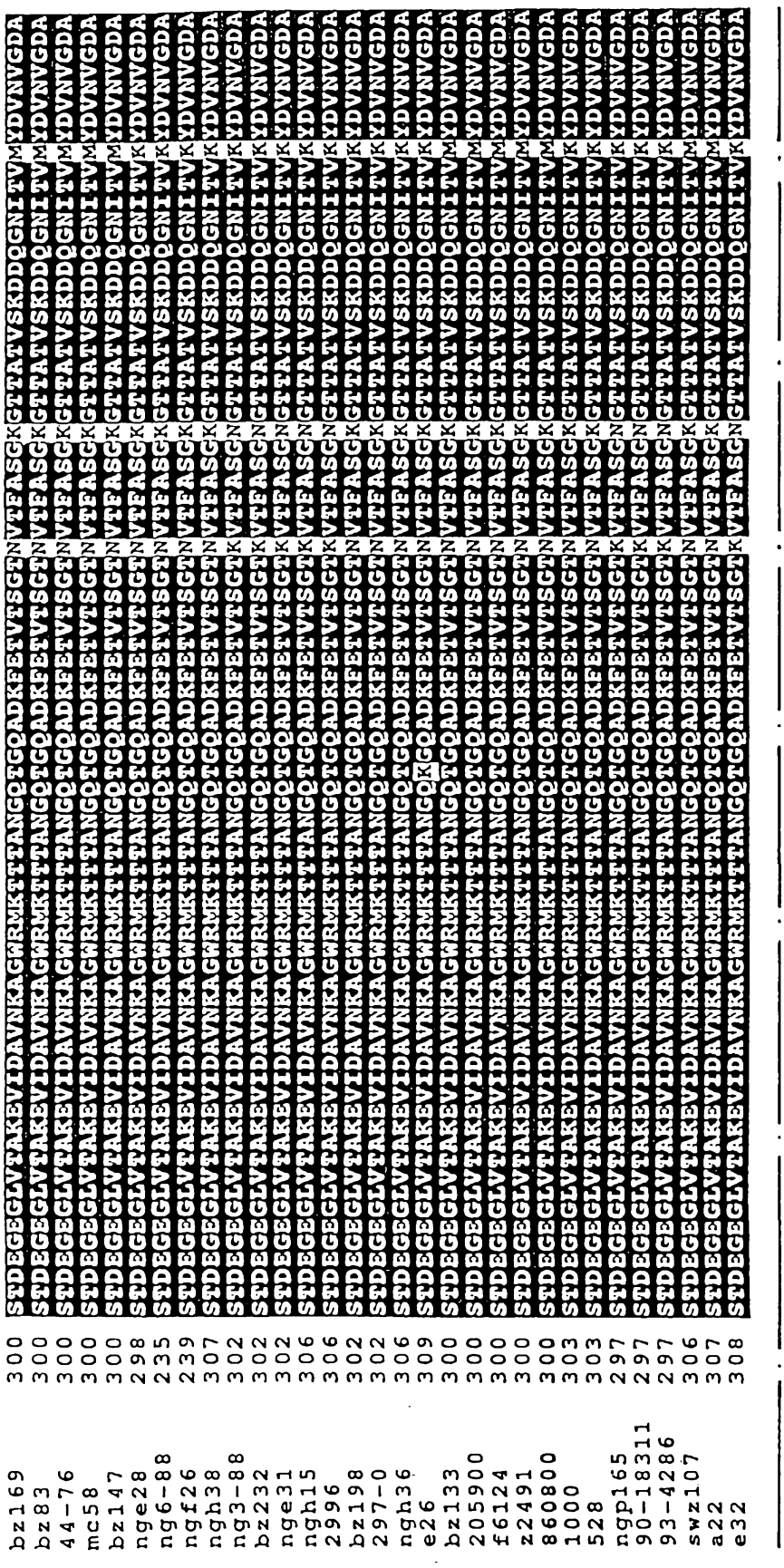

bz169 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
bz83 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
44-76 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
mc58 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
bz147 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
nge28 298 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
ng6-88 235 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
ngf26 239 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
ngh38 307 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
ng3-88 302 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
bz232 302 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
nge31 302 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
ngh15 306 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
2996 306 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
bz198 302 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
297-0 302 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
ngh36 306 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
e26 309 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQKGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
bz133 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
205900 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
f6124 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
z2491 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
860800 300 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
1000 303 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
528 303 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVKYDVNVGDA
ngP165 297 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
90-18311 297 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
93-4286 297 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA
swz107 306 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
a22 307 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTNVTFASGKGTTATVSKDDQGNITVMYDVNVGDA
e32 308 STDEGEGLVTAKEVIDAVNKAGWRMKTTANGQTGQADKFETVTSGTKVTFASGNGTTATVSKDDQGNITVKYDVNVGDA

FIG. 11C(CONTD.)

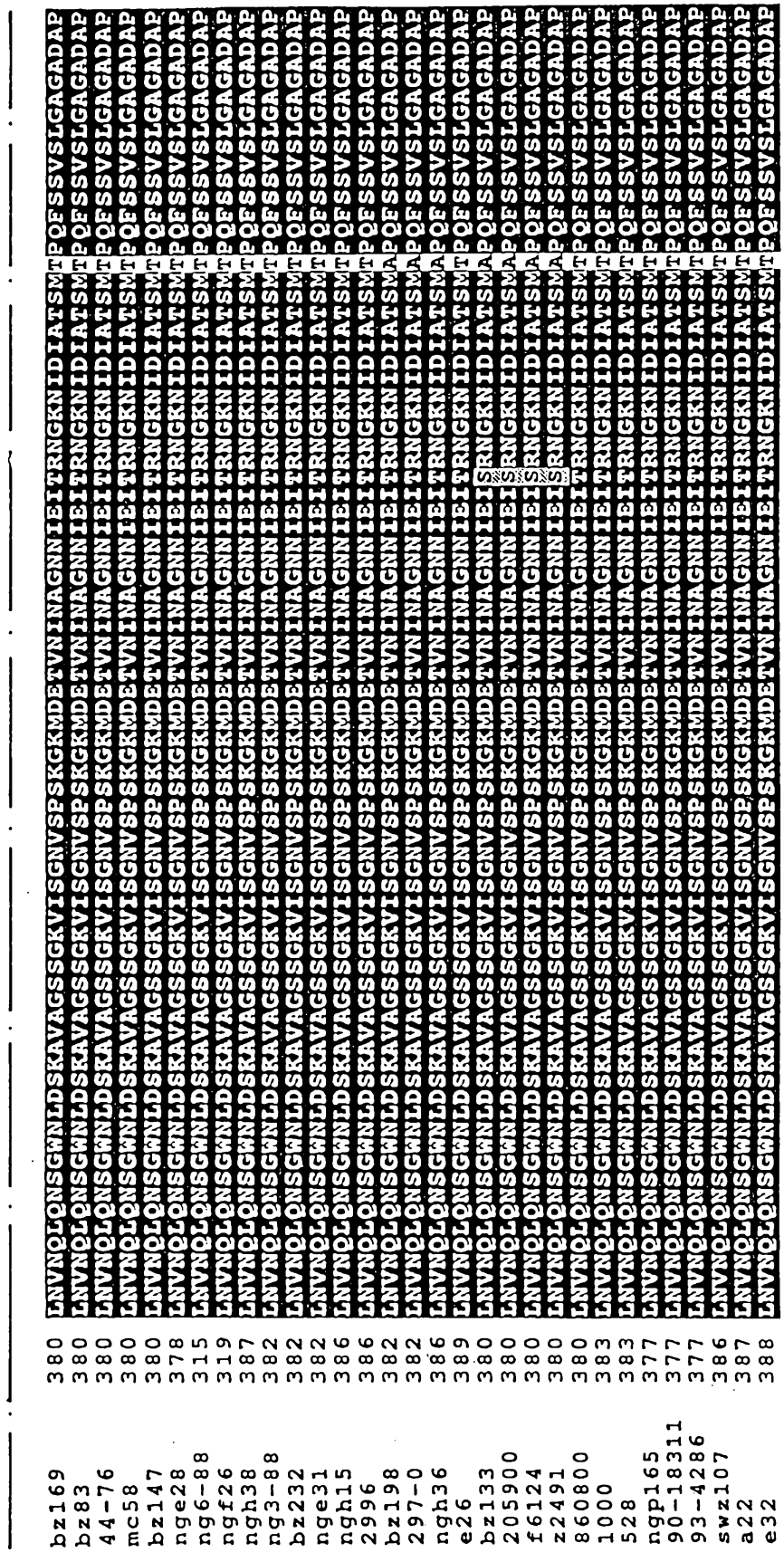
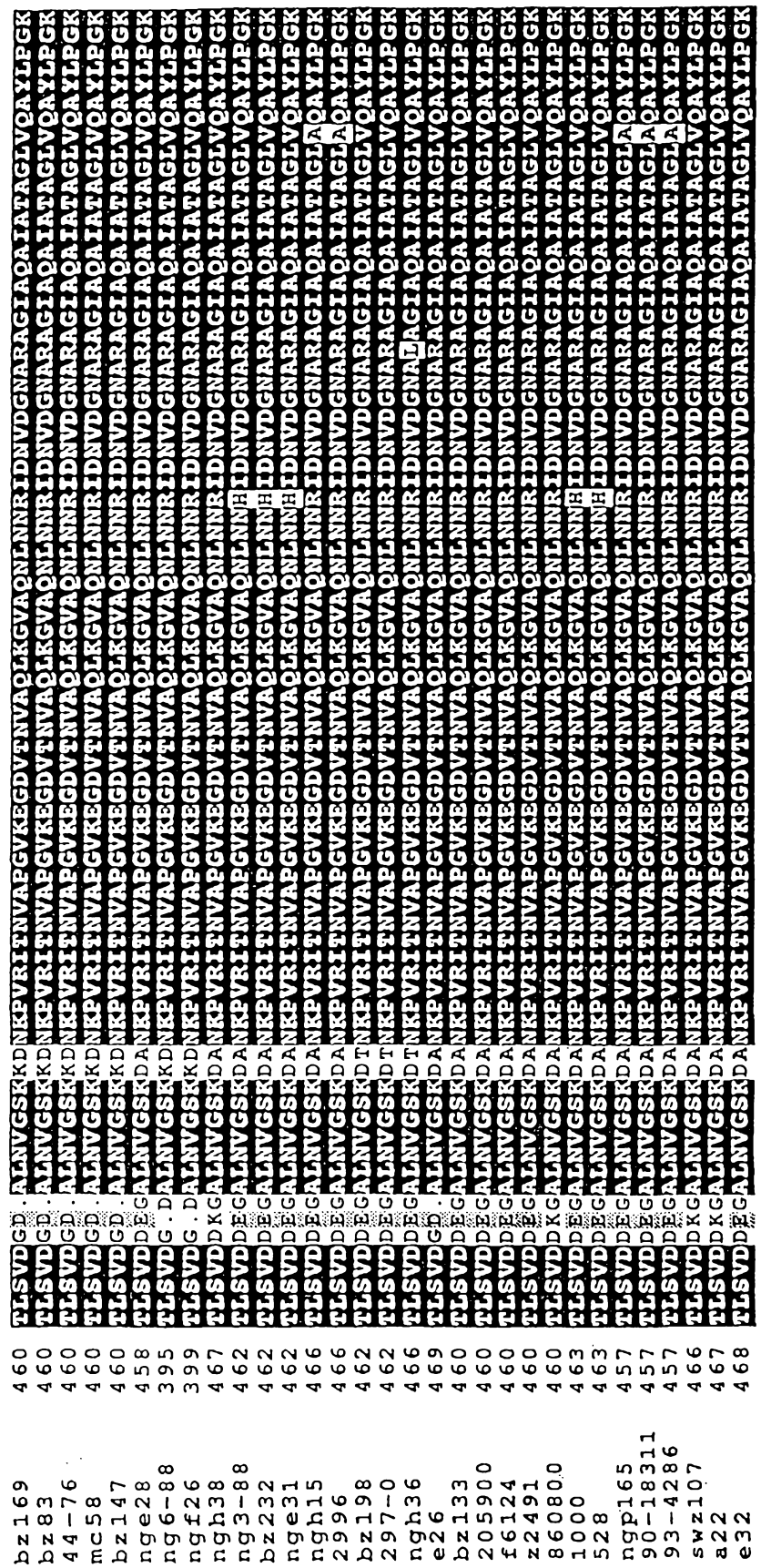


FIG. 11D



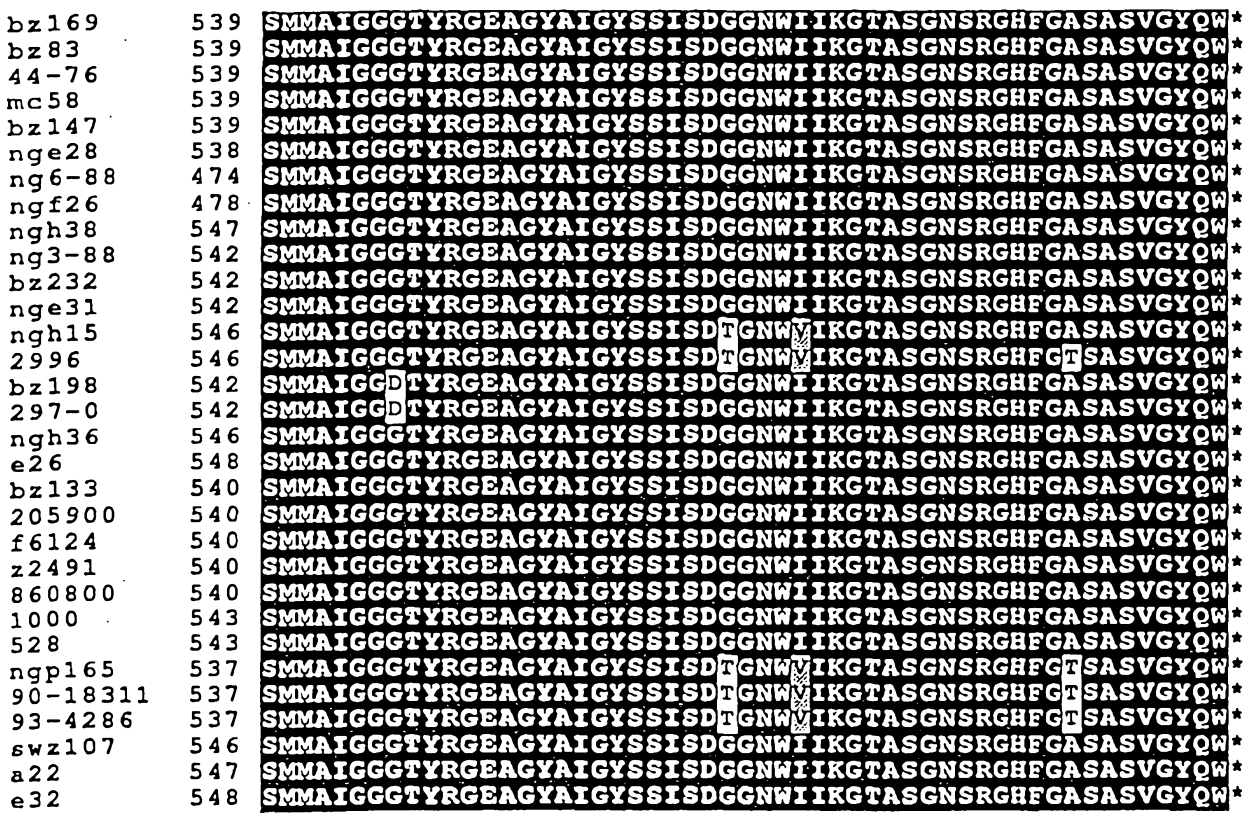


FIG. 11D(CONTD.)

ORF46

FIG. 12A

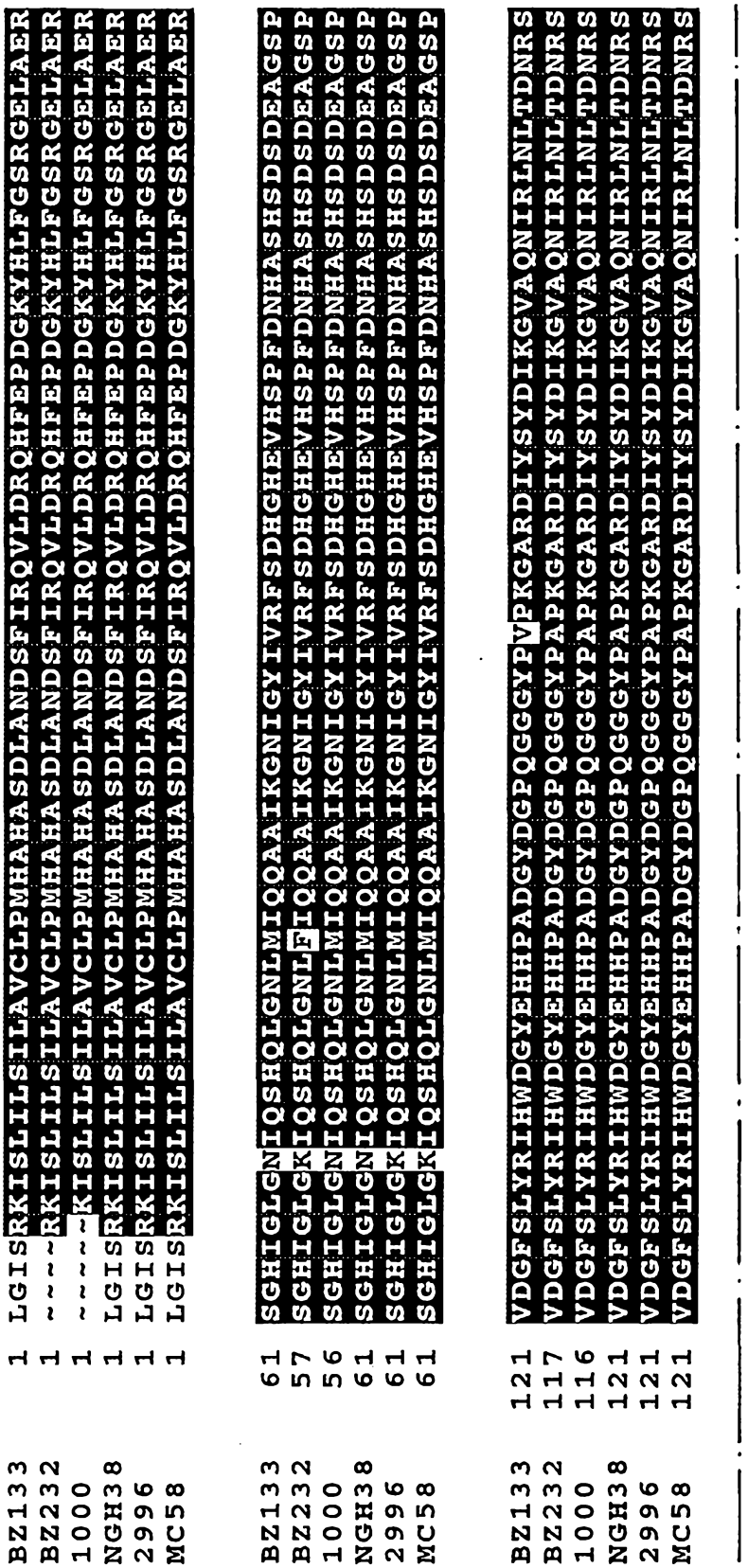


FIG. 12A(CONTD.)

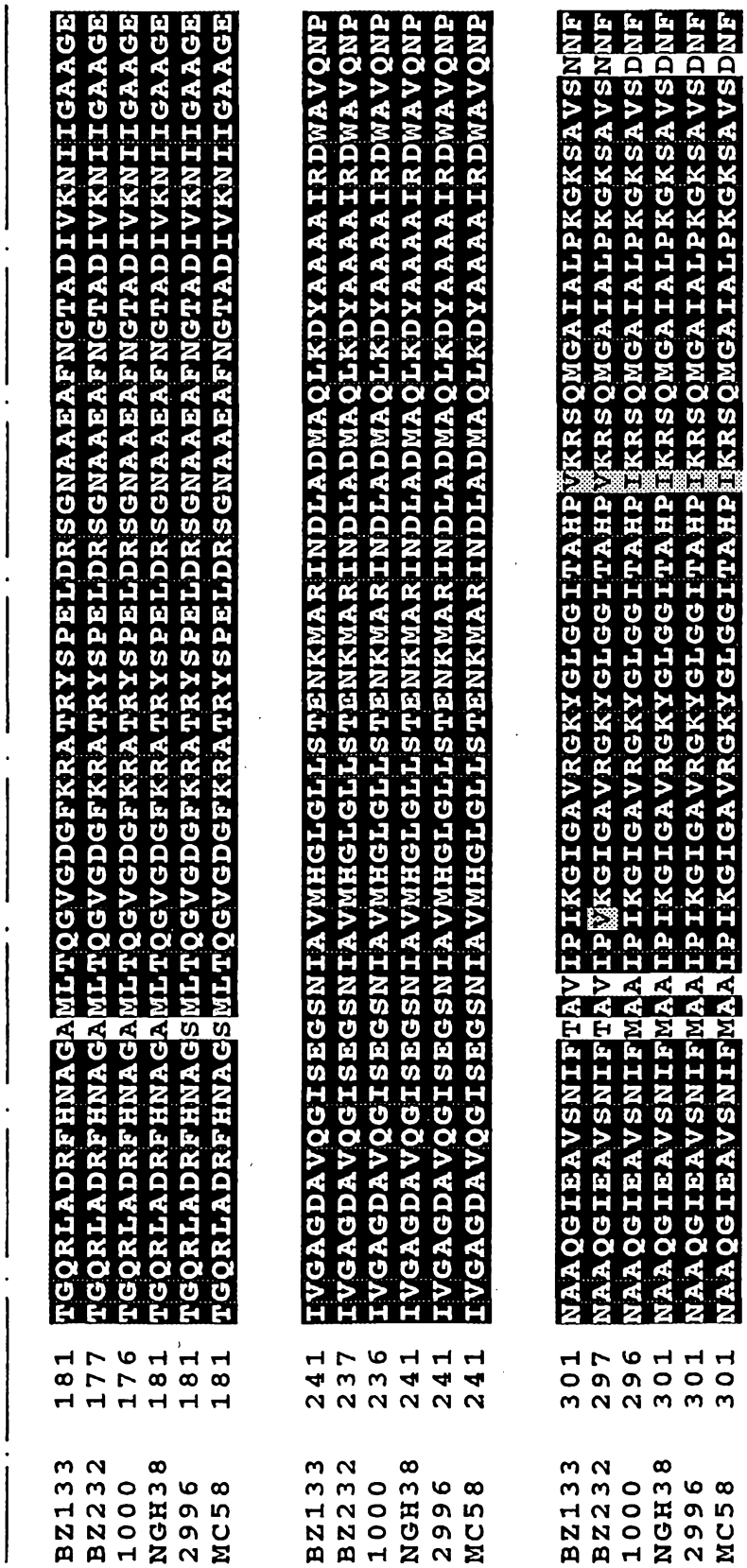


FIG. 12A(CONTD.)

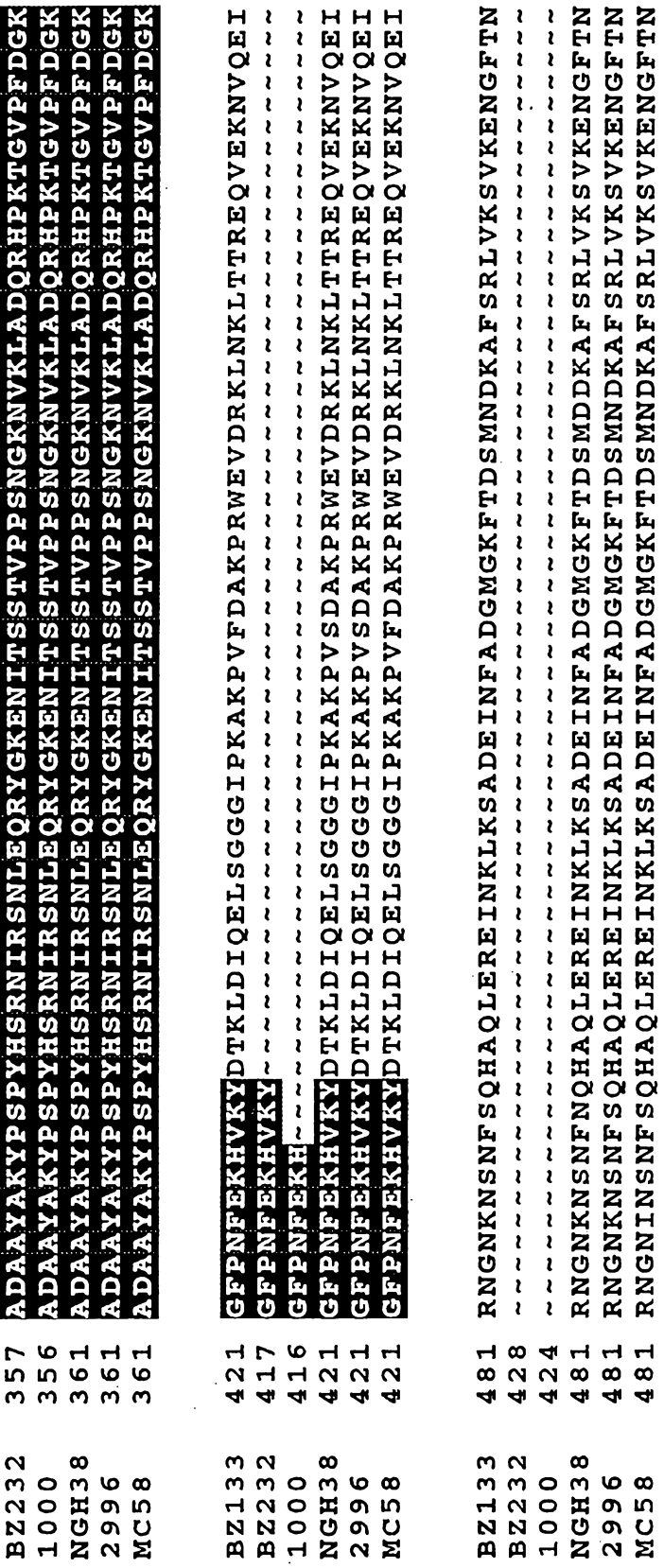


FIG. 12B

```
BZ133   541 PVVEYVEINGKAYIVRGNNRVFAAEYLGRIHELKFKKVDFPVPNTSWKNPTDVLNESGNV
BZ232   428 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1000    424 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
NGH38   541 PVVEYVEINGKAYIVRGNNRVFAAEYLGRIHELKFKKVDFPVPNTSWKNPTDVLNESGNV
2996    541 PVVEYVEINGKAYIVRGNNRVFAAEYLGRIHELKFKKVDFPVPNTSWKNPTDVLNESGNV
MC58    541 PVVEYVEINGKAYIVRGNNRVFAAEYLGRIHELKFKKVDFPVPNTSWKNPTDVLNESGNV

BZ133   601 KRPRYRSK
BZ232   428 ~~~~~~~~
1000    424 ~~~~~~~~
NGH38   601 KRPRYRSK
2996    601 KRPRYRSK
MC58    601 KRPRYRSK
```

FIG. 13A

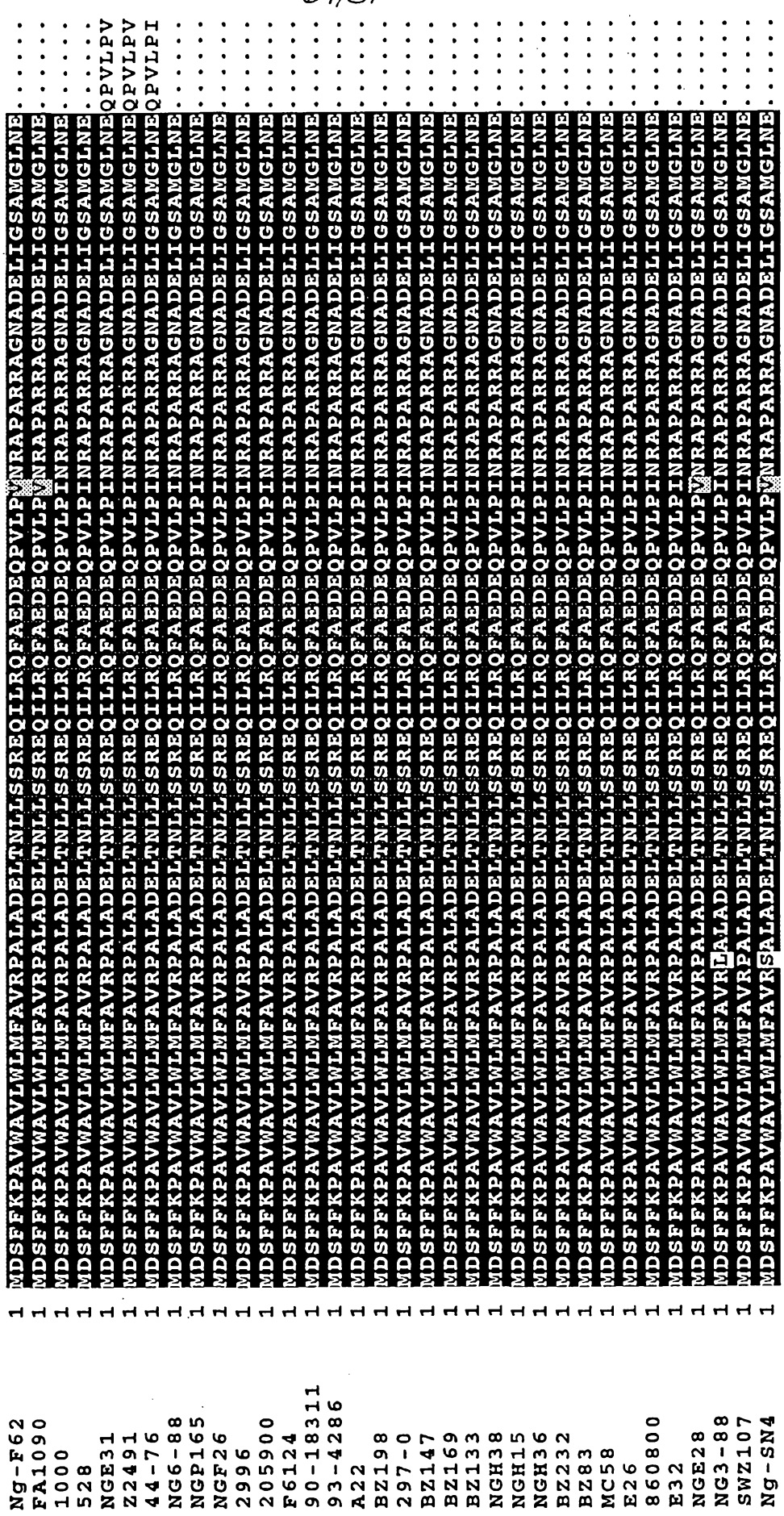

225
Ng-F62
FA1090
1000
528
NGE31
Z2491
44-76
NG6-88
NGP165
NGF26
2996
205900
F6124
90-18311
93-4286
A22
BZ198
297-0
BZ147
BZ169
BZ133
NGH38
NGH15
NGH36
BZ232
BZ83
MC58
E26
860800
E32
NGE28
NG3-88
SWZ107
Ng-SN4

FIG. 13A(CONTD.)

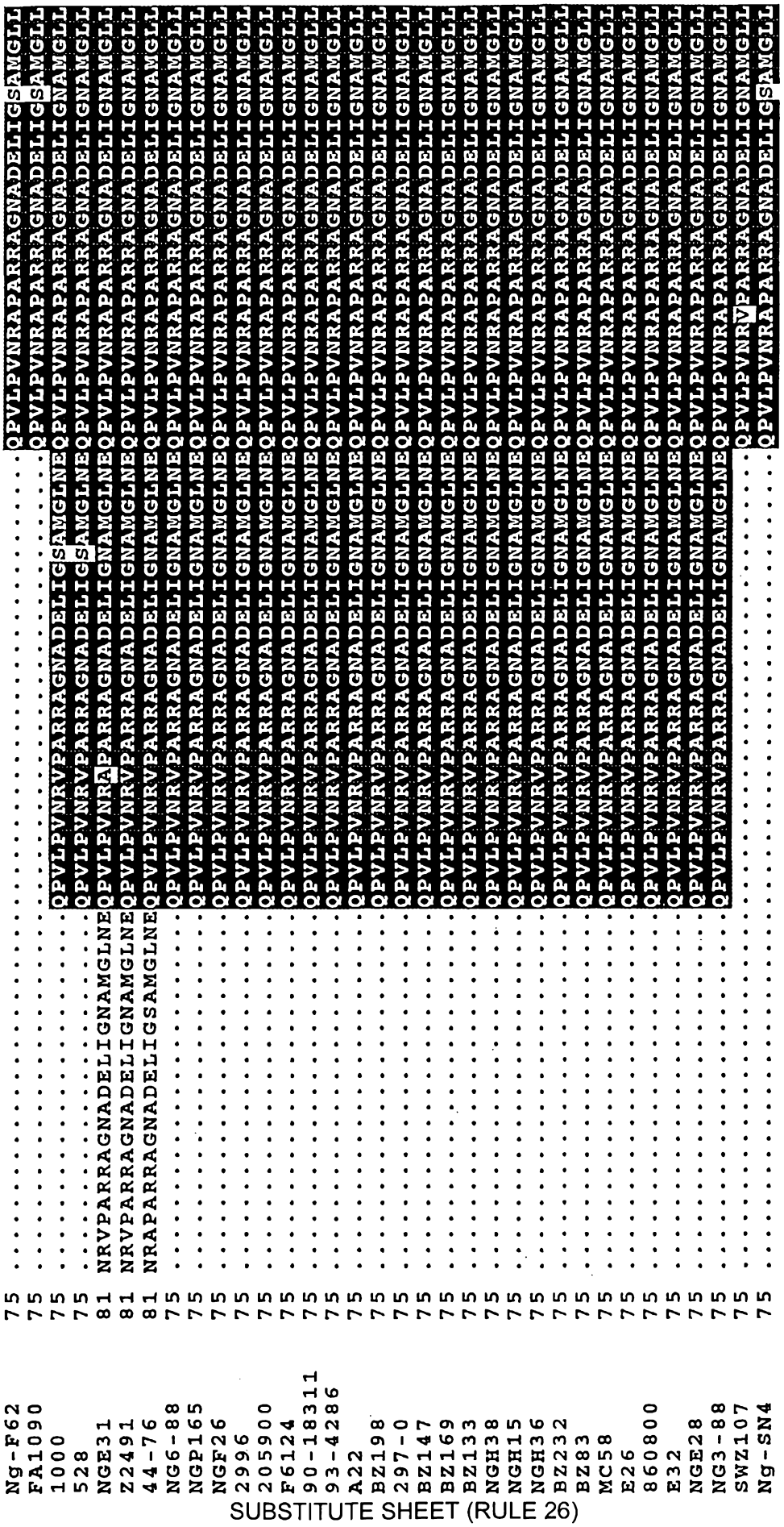
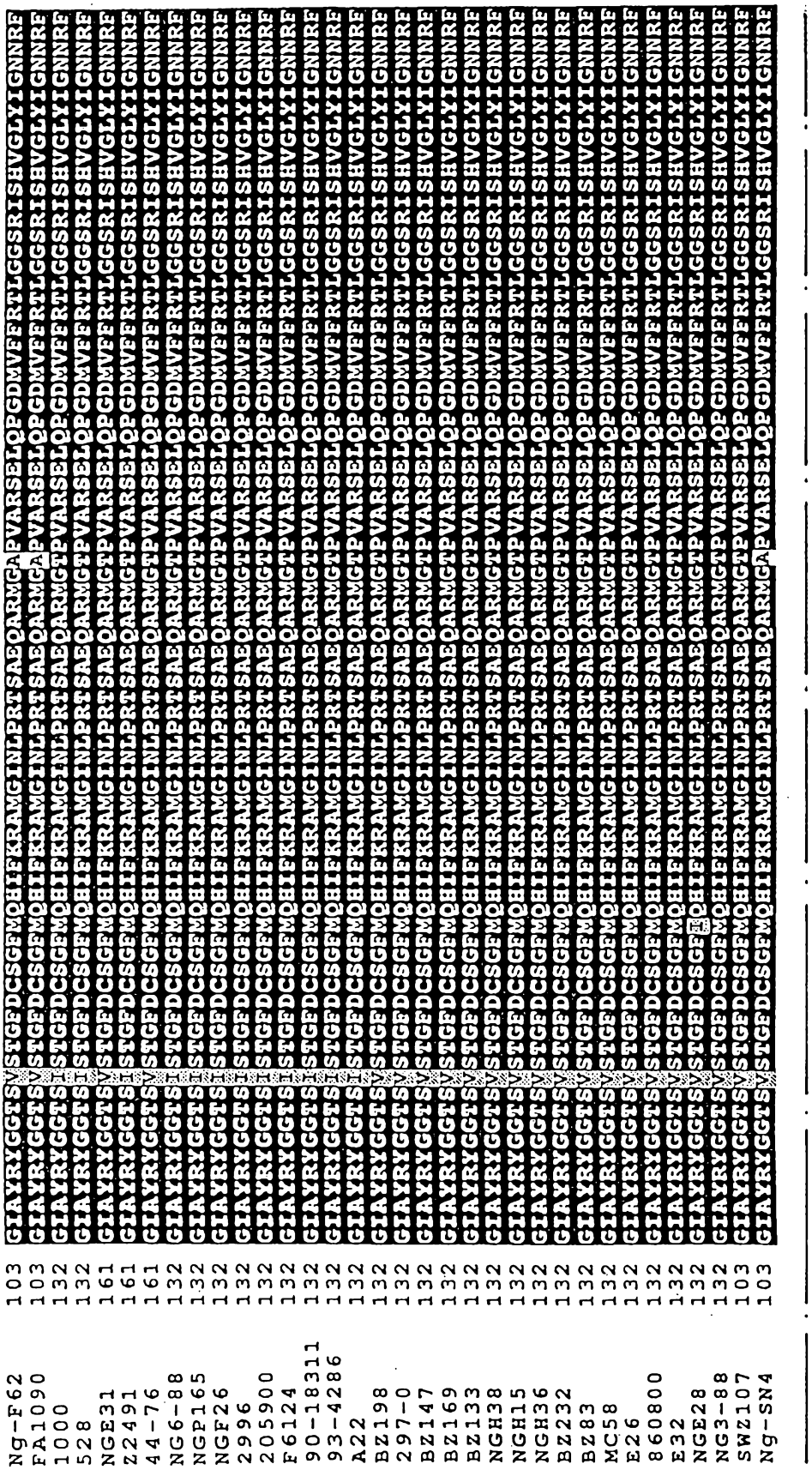


FIG. 13B


Ng-F62 103 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGAPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
FA1090 103 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGAPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
1000 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
528 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NGE31 161 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
Z2491 161 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
44-76 161 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NG6-88 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NGP165 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NGF26 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
2996 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
205900 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
F6124 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
90-18311 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
93-4286 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
A22 132 GIAYRYGGTSISTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
BZ198 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
297-0 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
BZ147 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
BZ169 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
BZ133 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NGH38 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NGH15 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NGH36 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
BZ232 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
BZ83 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
MC58 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
E26 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
860800 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
E32 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NGE28 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
NG3-88 132 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
SWZ107 103 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGTPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF
Ng-SN4 103 GIAYRYGGTSVSTGFDCSGFMQHIFKRAMGINLPRTSAEQARMGAPVARSELQPGDMVFFRTLGGSRISHVGLYIGNNRF

Ng-F62	183	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
FA1090	183	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
1000	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
528	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NGE31	241	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
Z2491	241	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
44-76	241	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NG6-88	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NGP165	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NGF26	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
2996	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
205900	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
F6124	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
90-18311	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
93-4286	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
A22	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
BZ198	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
297-0	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
BZ147	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
BZ169	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
BZ133	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NGH38	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NGH15	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NGH36	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
BZ232	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
BZ83	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
MC58	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
E26	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
860800	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
E32	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NGE28	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
NG3-88	212	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
SWZ107	183	IHAPRTGKNIEITSLSHKYWSGKYAFARRVKKNDPSRFLN*
Ng-SN4	183	IHAPRTGKNIEITSLSHKYWSGKYAFARR[TS]KKNDPSRFLN*

FIG. 13B(CONTD.)

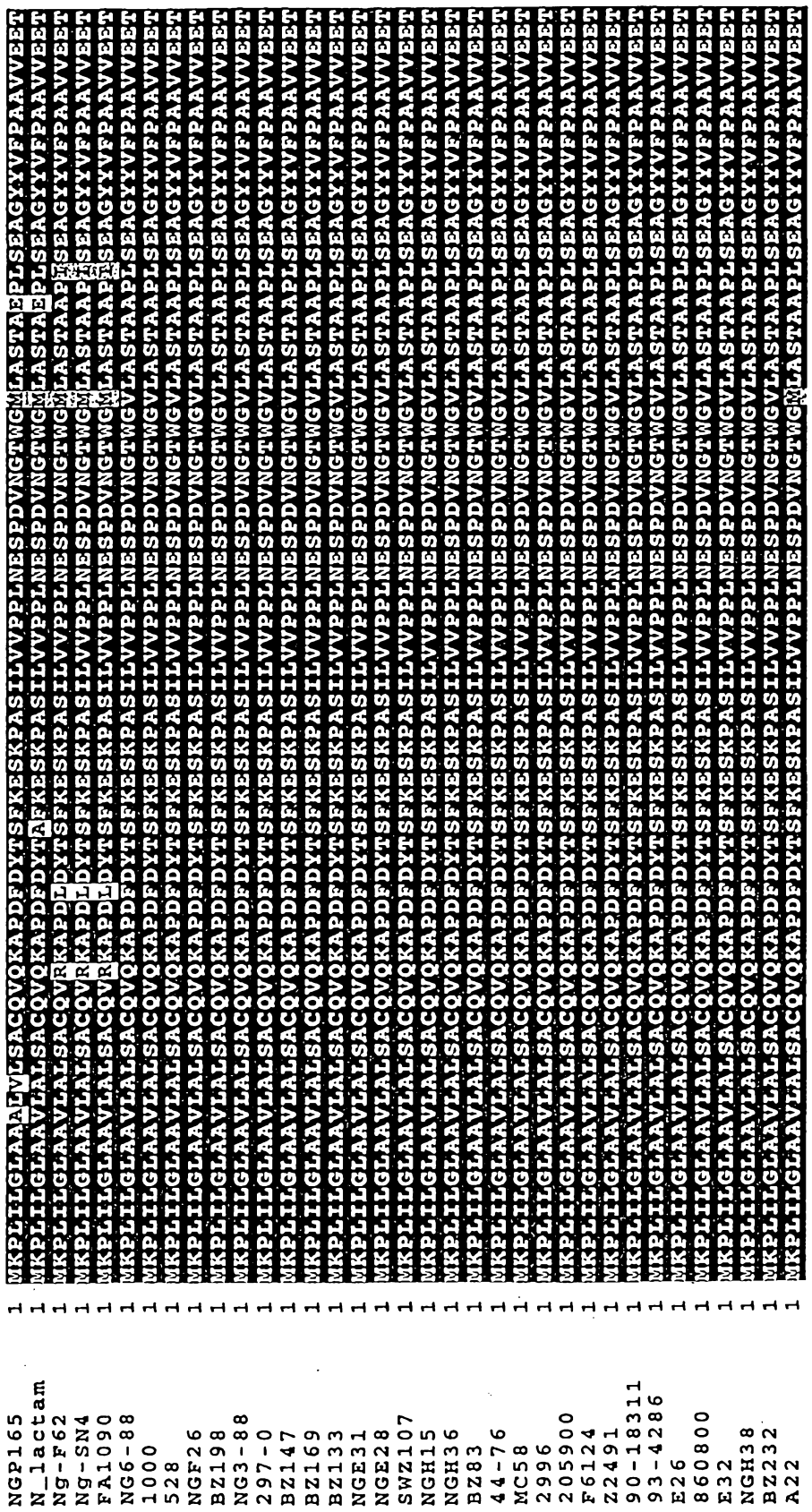

235
NGP165
N_lactam
Ng-F62
Ng-SN4
FA1090
NG6-88
1000
528
NGF26
BZ198
NG3-88
297-0
BZ147
BZ169
BZ133
NGE31
NGE28
SWZ107
NGH15
NGH36
BZ83
44-76
MC58
2996
205900
F6124
Z2491
90-18311
93-4286
E26
860800
E32
NGH38
BZ232
A22

FIG. 14A

FIG. 14A(CONTD.)

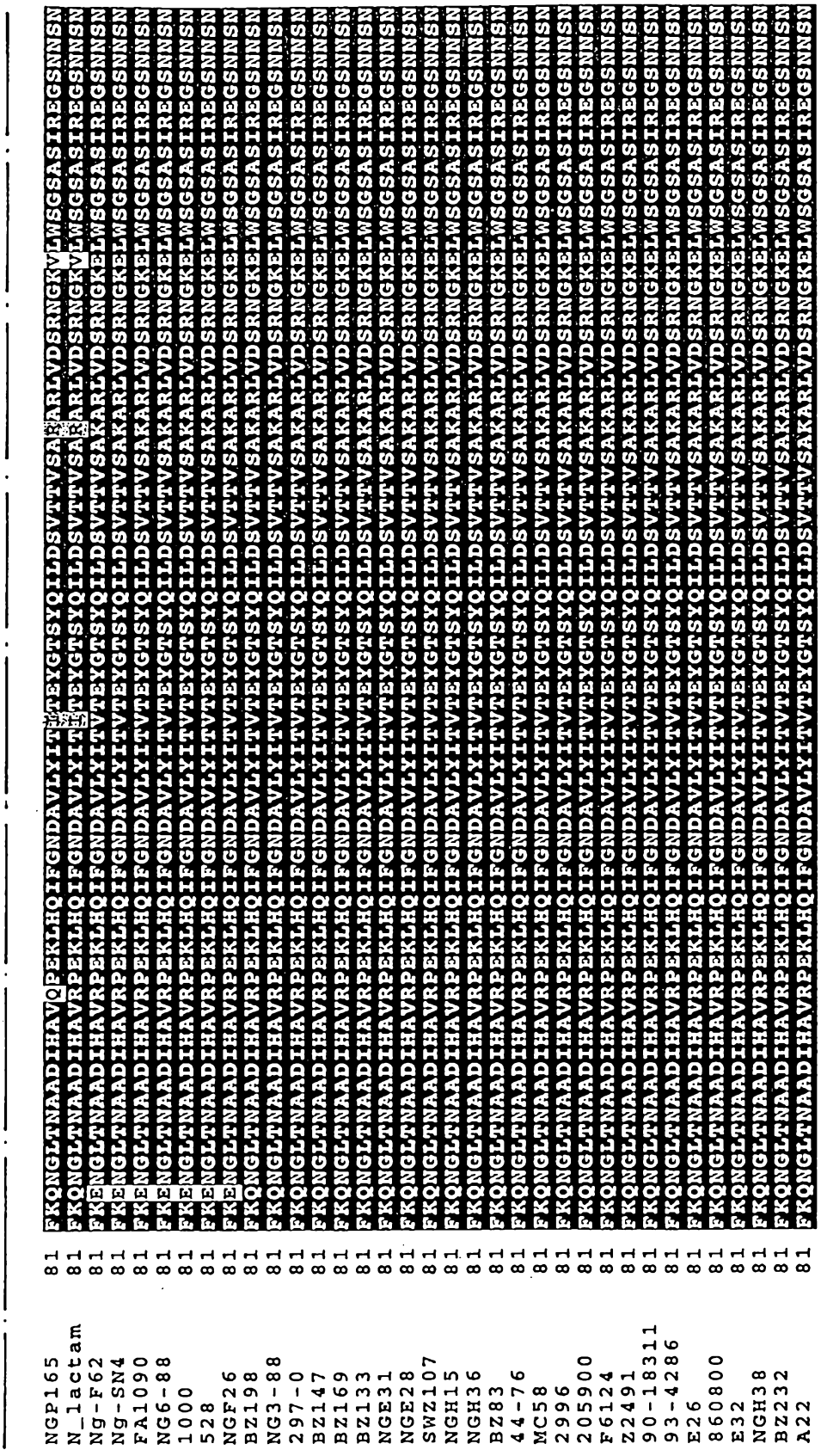


FIG. 14B

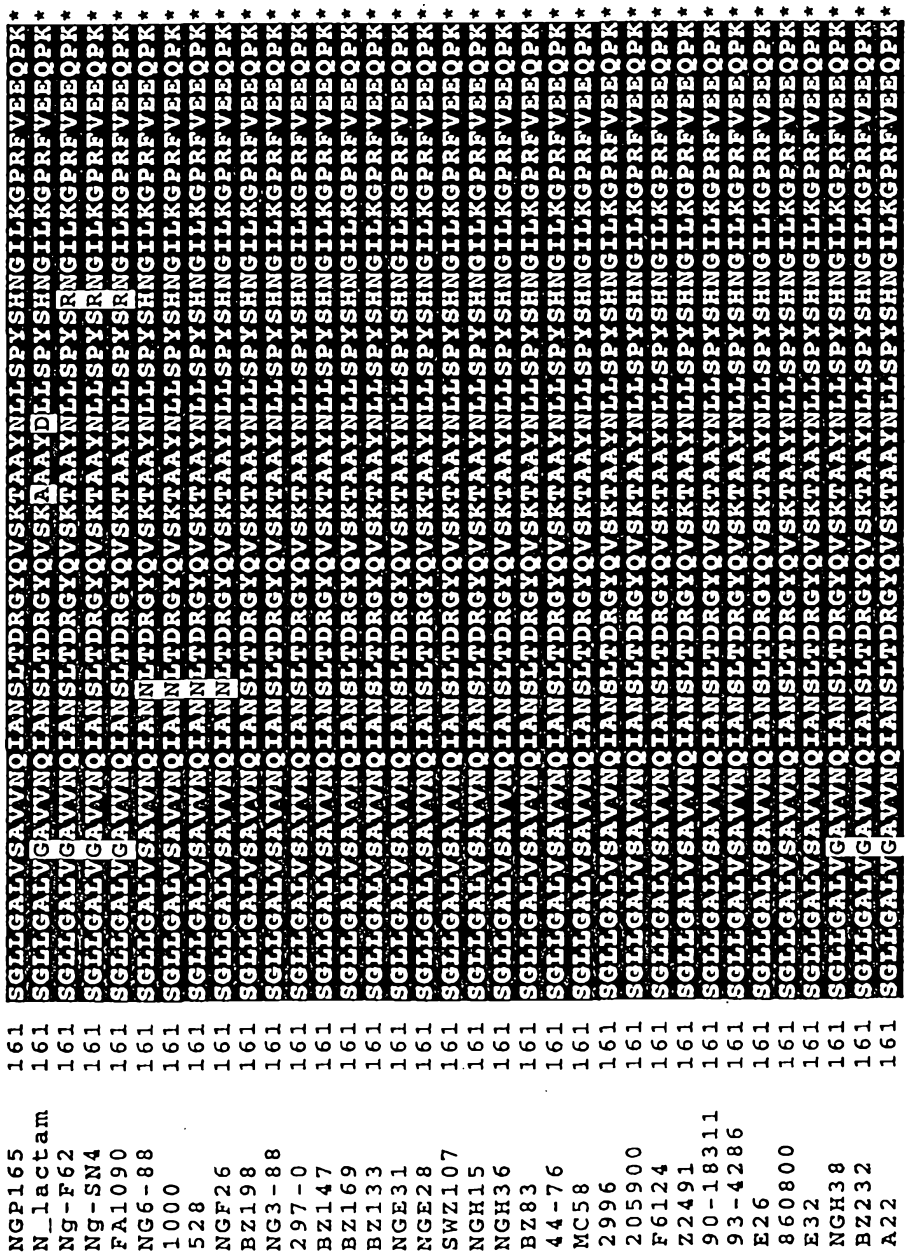

NGP165 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
N_lactam 161 SGLLGALVGAVVNQIANSLTDRGYQVSKAAAYDLLSPYSHNGILKGPRFVEEQPK*
Ng-F62 161 SGLLGALVGAVVNQIANSLTDRGYQVSKTAAYNLLSPYSRNGILKGPRFVEEQPK*
Ng-SN4 161 SGLLGALVGAVVNQIANSLTDRGYQVSKTAAYNLLSPYSRNGILKGPRFVEEQPK*
FA1090 161 SGLLGALVGAVVNQIANSLTDRGYQVSKTAAYNLLSPYSRNGILKGPRFVEEQPK*
NG6-88 161 SGLLGALVSAVVNQIANNLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
1000 161 SGLLGALVSAVVNQIANNLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
528 161 SGLLGALVSAVVNQIANNLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
NGF26 161 SGLLGALVSAVVNQIANNLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
BZ198 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
NG3-88 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
297-0 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
BZ147 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
BZ169 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
BZ133 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
NGE31 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
NGE28 161 SGLLGALVSAVVNQIANSLTDRGIQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
SWZ107 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
NGH15 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
NGH36 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
BZ83 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
44-76 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
MC58 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
2996 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
205900 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
F6124 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
Z2491 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
90-18311 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
93-4286 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
E26 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
860800 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
E32 161 SGLLGALVSAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
NGH38 161 SGLLGALVGAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
BZ232 161 SGLLGALVGAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*
A22 161 SGLLGALVGAVVNQIANSLTDRGYQVSKTAAYNLLSPYSHNGILKGPRFVEEQPK*

FIG. 15A

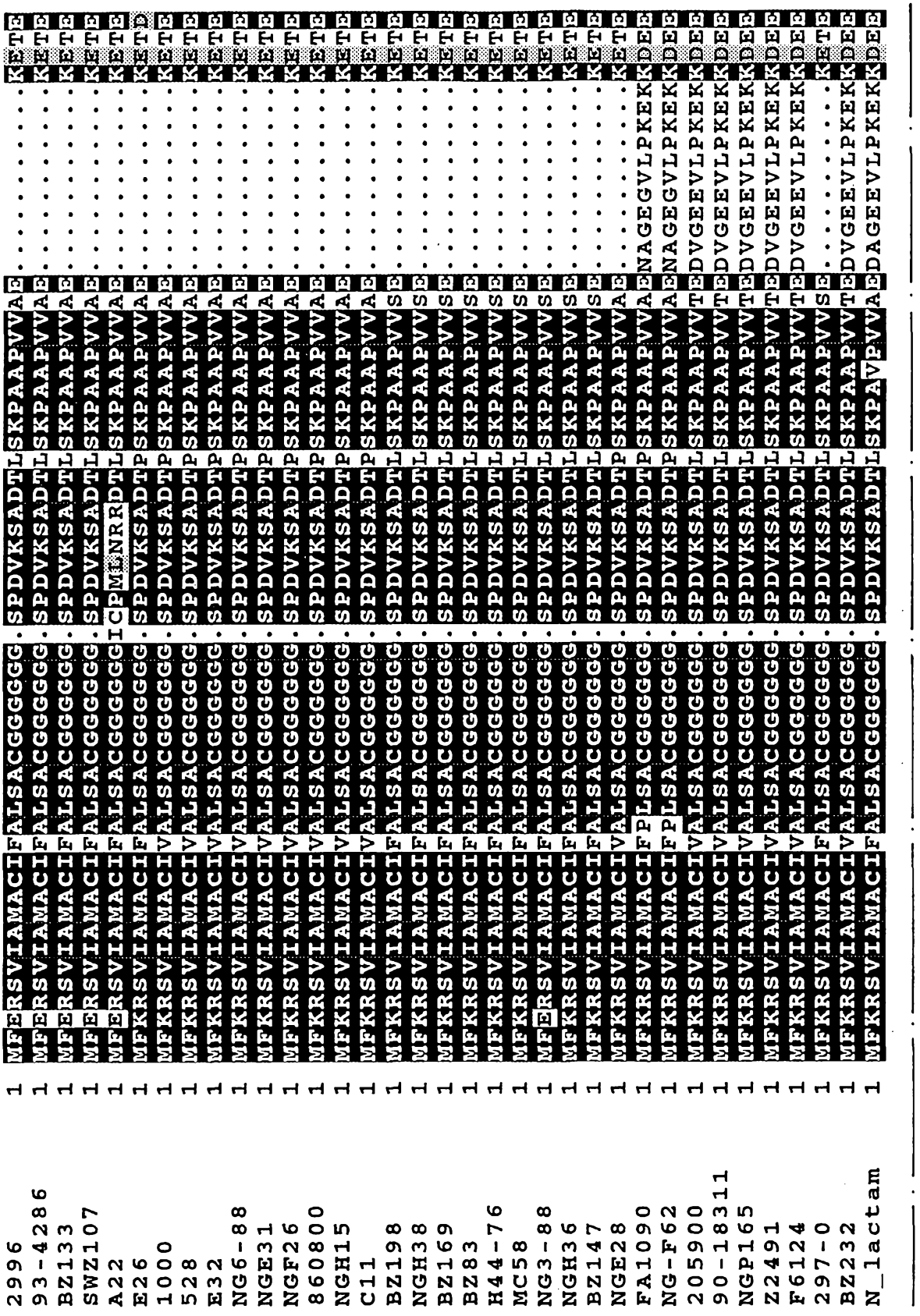

2996
93-4286
BZ133
SWZ107
A22
E26
1000
528
E32
NG6-88
NGE31
NGF26
860800
NGH15
C11
BZ198
NGH38
BZ169
BZ83
H44-76
MC58
NG3-88
NGH36
BZ147
NGE28
FA1090
NG-F62
205900
90-18311
NGP165
Z2491
F6124
297-0
BZ232
N_lactam

FIG. 15A(CONTD.)

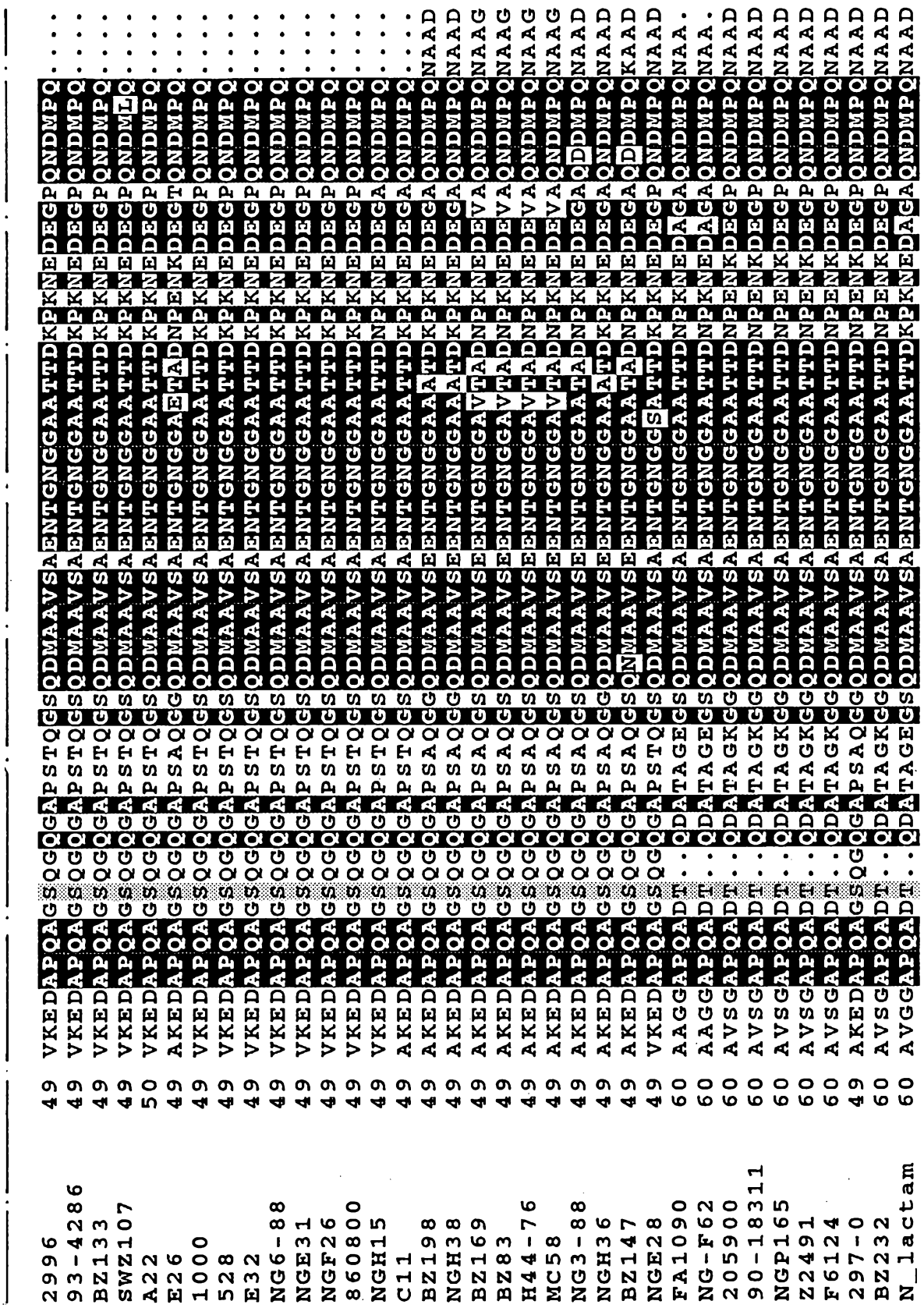


FIG. 15B

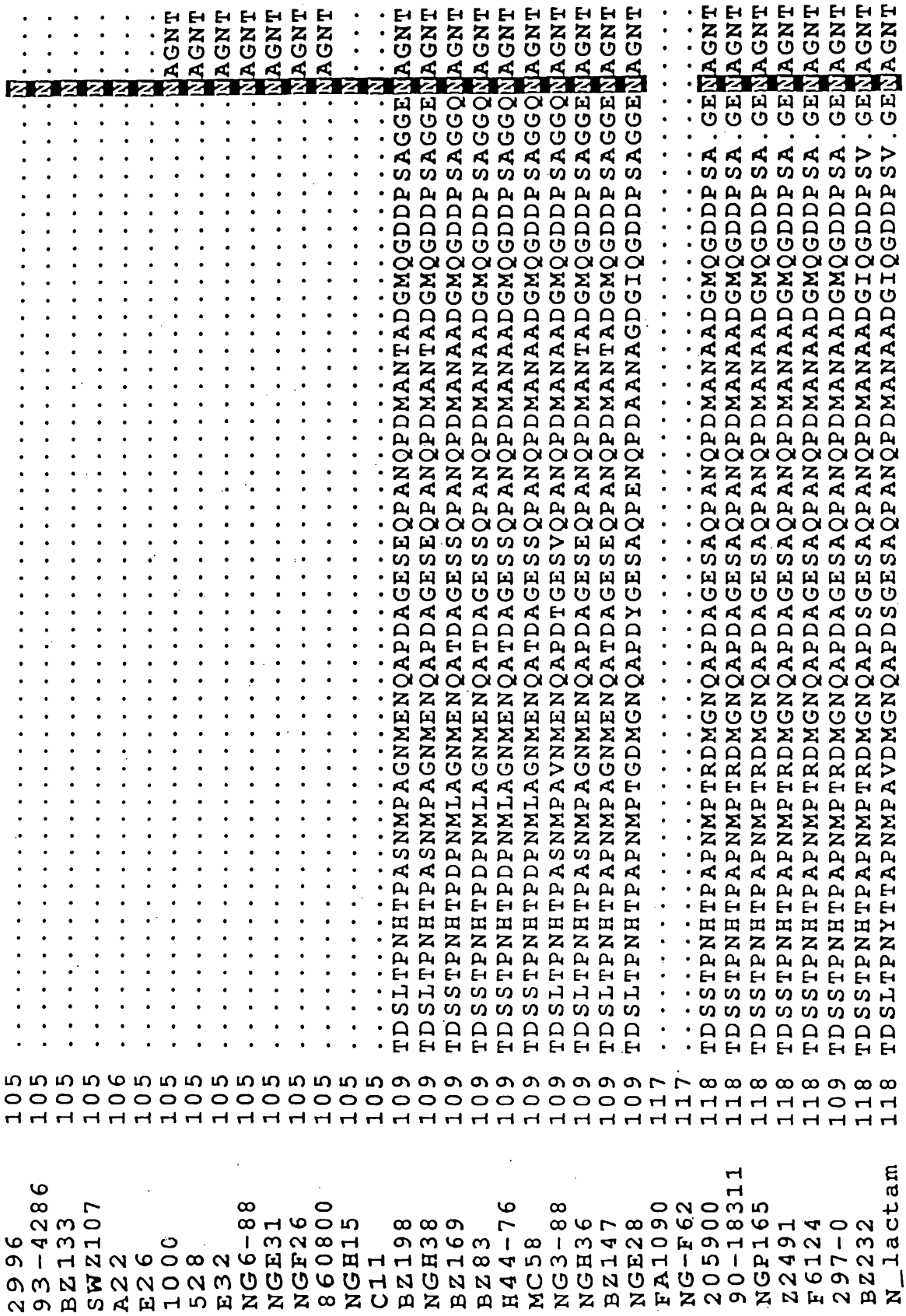

2996
93-4286
BZ133
SWZ107
A22
E26
1000
528
E32
NG6-88
NGE31
NGF26
860800
NGH15
C11
BZ198
NGH38
BZ169
BZ83
H44-76
MC58
NG3-88
NGH36
BZ147
NGE28
FA1090
NG-F62
205900
90-18311
NGP165
Z2491
F6124
297-0
BZ232
N_lactam

FIG. 15B(CONTD.)

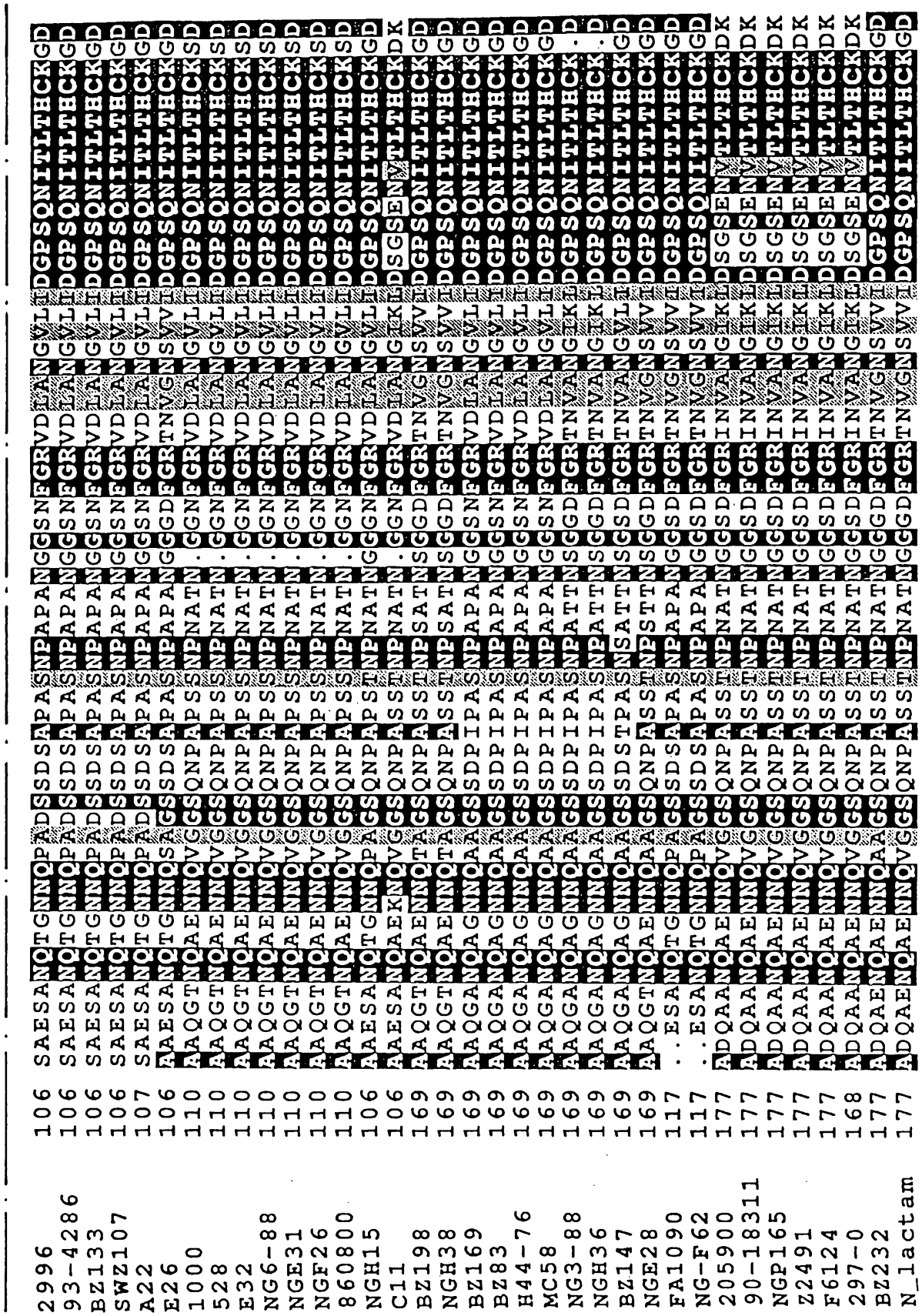


FIG. 15C

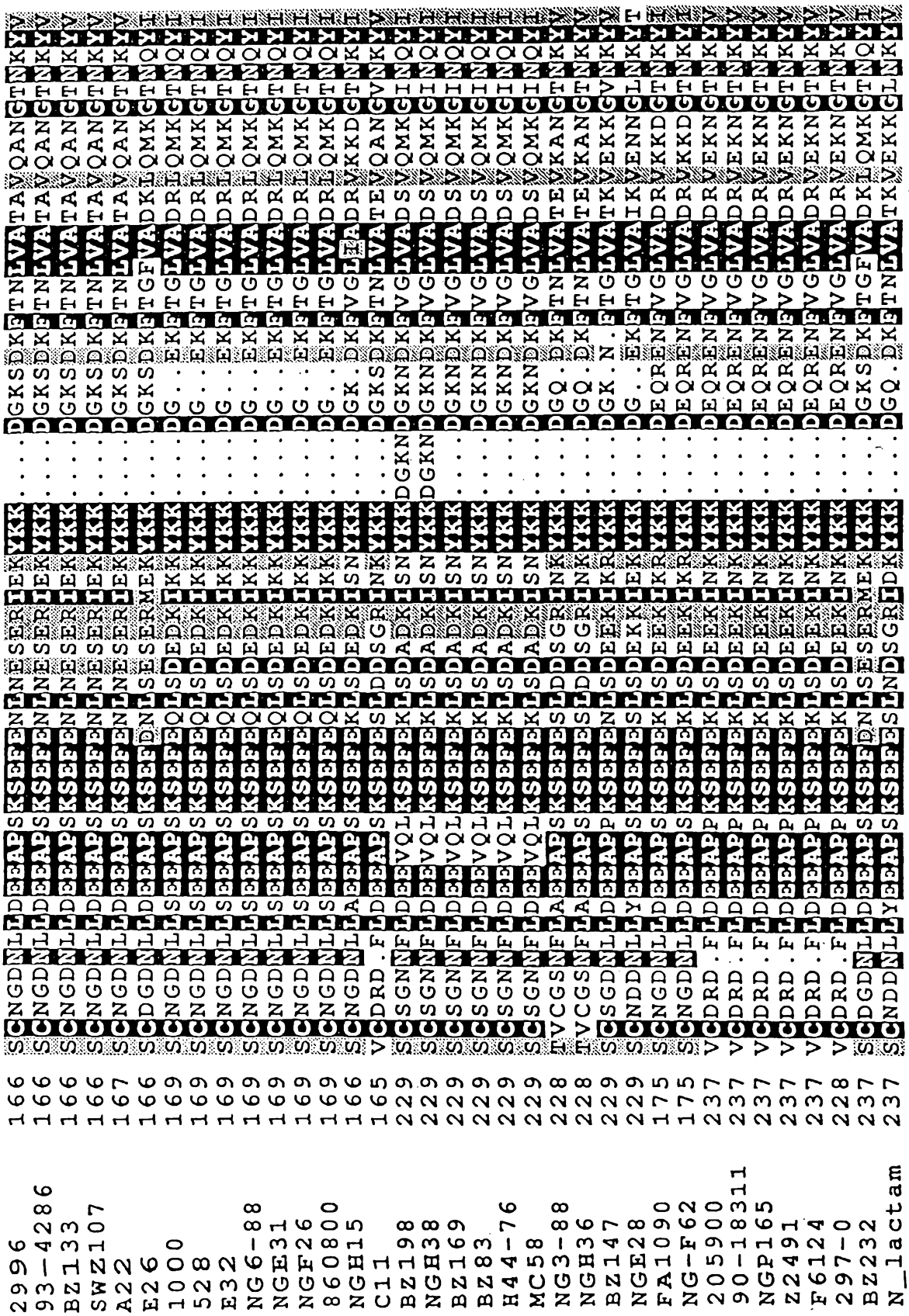


FIG. 15C(CONTD.)

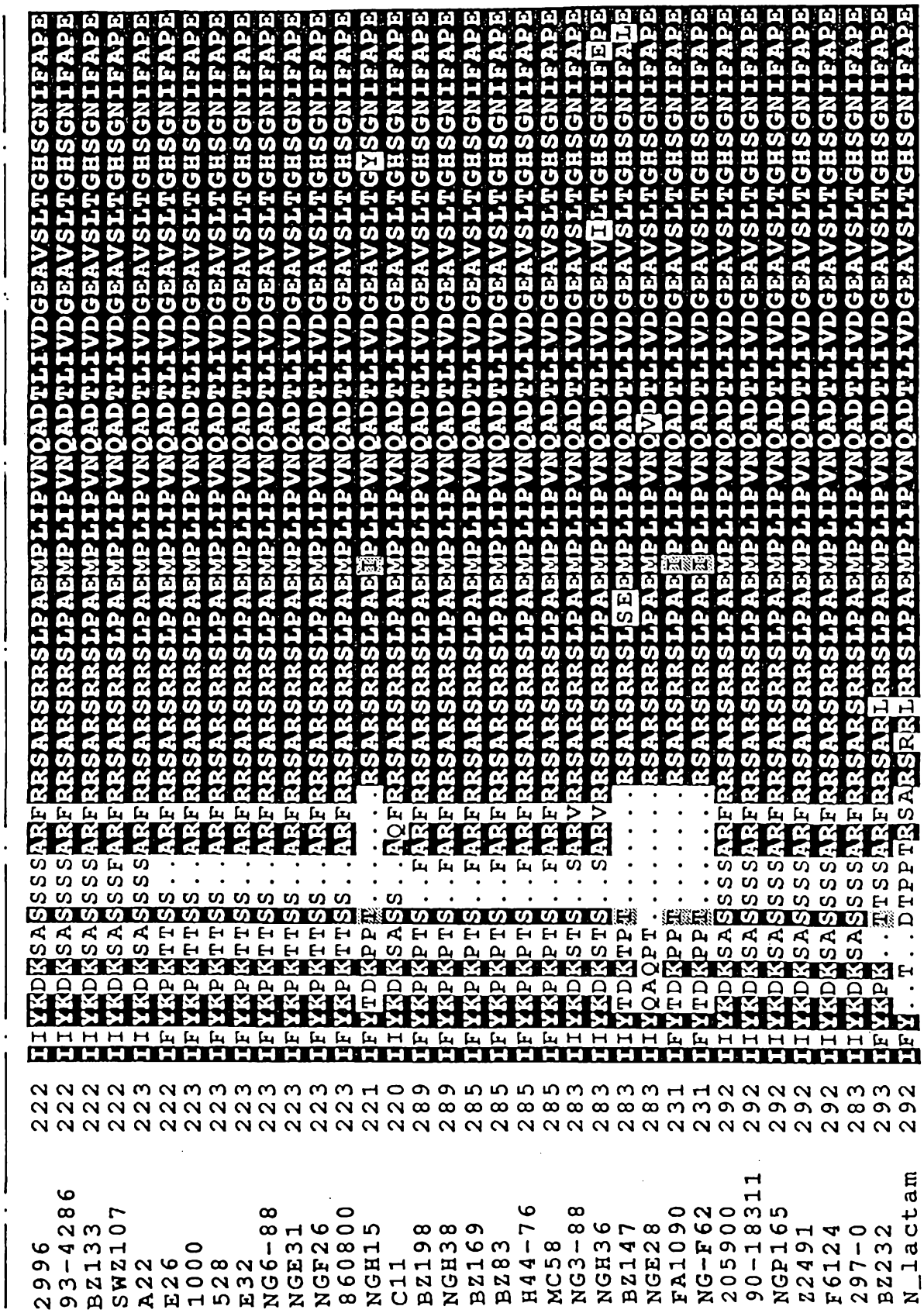


FIG. 15D

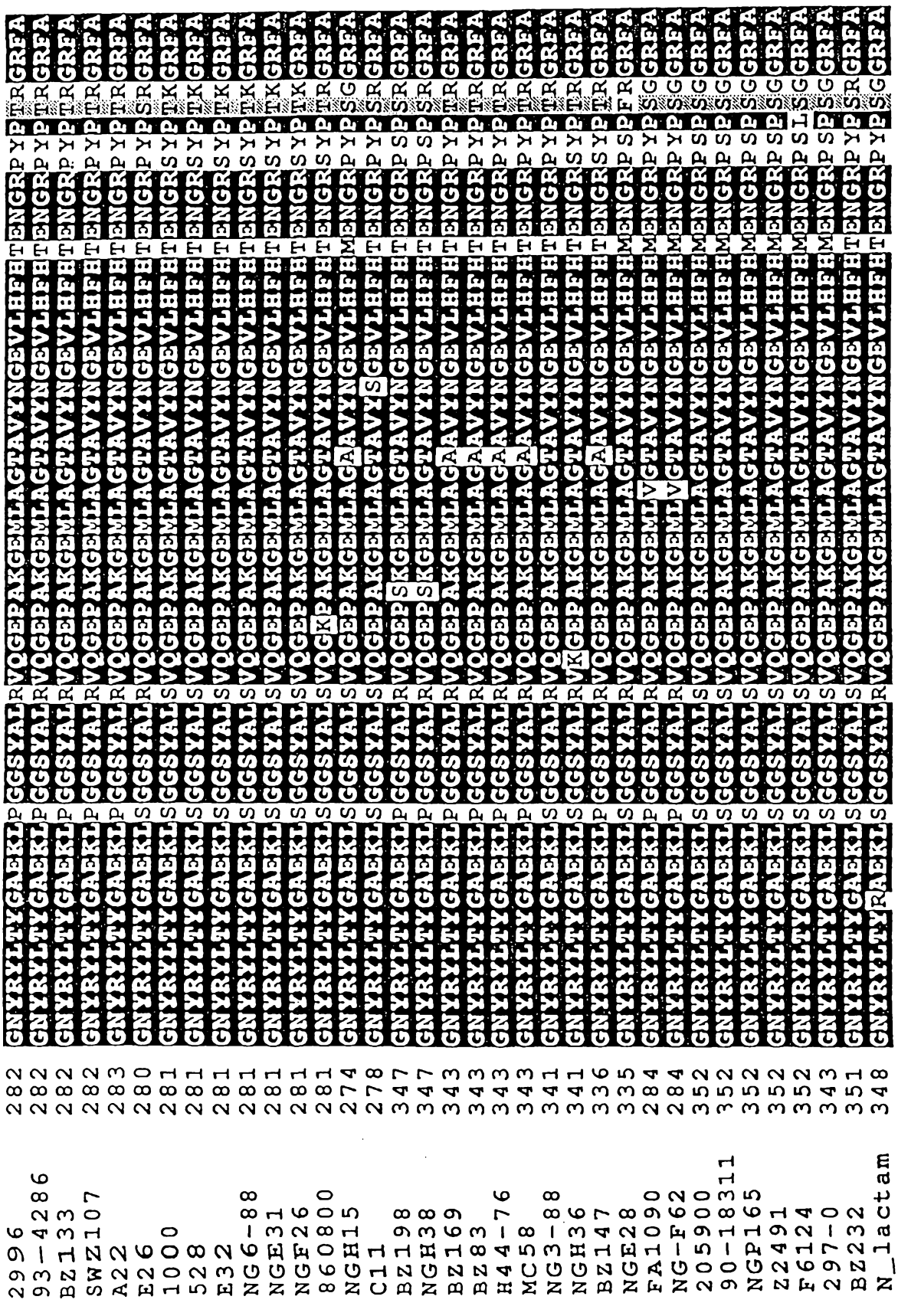

2996
93-4286
BZ133
SWZ107
A22
E26
1000
528
E32
NG6-88
NGE31
NGF26
860800
NGH15
C11
BZ198
NGH38
BZ169
BZ83
H44-76
MC58
NG3-88
NGH36
BZ147
NGE28
FA1090
NG-F62
205900
90-18311
NGP165
Z2491
F6124
297-0
BZ232
N_lactam

FIG. 15D(CONTD.)

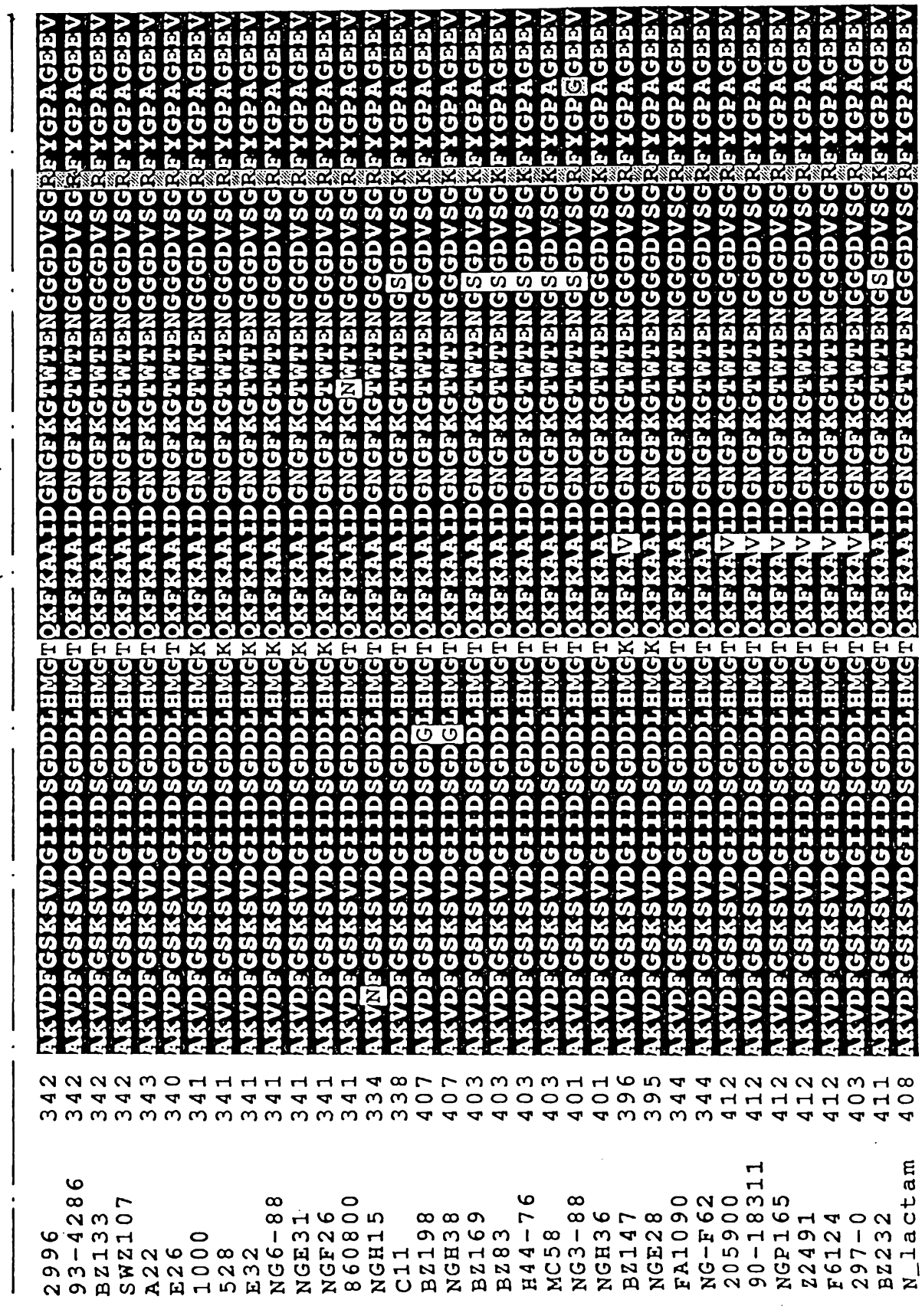


FIG. 15E

2996	402	AGKYSYRPTDAEKGGFGVFAGKKEQD
93-4286	402	AGKYSYRPTDAEKGGFGVFAGKKEQD
BZ133	402	AGKYSYRPTDAEKGGFGVFAGKKEQD
SWZ107	402	AGKYSYRPTDAEKGGFGVFAGKKEQD
A22	403	AGKYSYRPTDAEKGGFGVFAGKKEQD
E26	400	AGKYSYRPTDAEKGGFGVFAGKKEQD
1000	401	AGKYSYRPTDAEKGGFGVFAGKKEQD
528	401	AGKYSYRPTDAEKGGFGVFAGKKEQD
E32	401	AGKYSYRPTDAEKGGFGVFAGKKEQD
NG6-88	401	AGKYSYRPTDAEKGGFGVFAGKKEQD
NGE31	401	AGKYSYRPTDAEKGGFGVFAGKKEQD
NGF26	401	AGKYSYRPTDAEKGGFGVFAGKKEQD
860800	401	AGKYSYRPTDAEKGGFGVFAGKKEQD
NGH15	394	AGKYSYRPTDAEKGGFGVFAGKKEQD
C11	398	AGKYSYRPTDAEKGGFGVFAGKKEQD
BZ198	467	AGKYSYRPTDAEKGGFGVFAGKKEQD
NGH38	467	AGKYSYRPTDAEKGGFGVFAGKKEQD
BZ169	463	AGKYSYRPTDAEKGGFGVFAGKKEQD
BZ83	463	AGKYSYRPTDAEKGGFGVFAGKKEQD
H44-76	463	AGKYSYRPTDAEKGGFGVFAGKKEQD
MC58	463	AGKYSYRPTDAEKGGFGVFAGKKEQD
NG3-88	461	AGKYSYRPTDAEKGGFGVFAGKKEQD
NGH36	461	AGKYSYRPTDAEKGGFGVFAGKKEQD
BZ147	456	AGKYSYRPTDAEKGGFGVFAGKKEQD
NGE28	455	AGKYSYRPTDAEKGGFGVFAGKKEQD
FA1090	404	AGKYSYRPTDAEKGGFGVFAGKKDRD
NG-F62	404	AGKYSYRPTDAEKGGFGVFAGKKDRD
205900	472	AGKYSYRPTDAEKGGFGVFAGKKEQD
90-18311	472	AGKYSYRPTDAEKGGFGVFAGKKEQD
NGP165	472	AGKYSYRPTDAEKGGFGVFAGKKEQD
Z2491	472	AGKYSYRPTDAEKGGFGVFAGKKEQD
F6124	472	AGKYSYRPTDAEKGGFGVFAGKKEQD
297-0	463	AGKYSYRPTDAEKGGFGVFAGKKEQD
BZ232	471	AGKYSYRPTDAEKGGFGVFAGKKEQD
N_lactam	468	AGKYSYRPTDAEKGGFGVFAGKKEQD

FIG. 16A

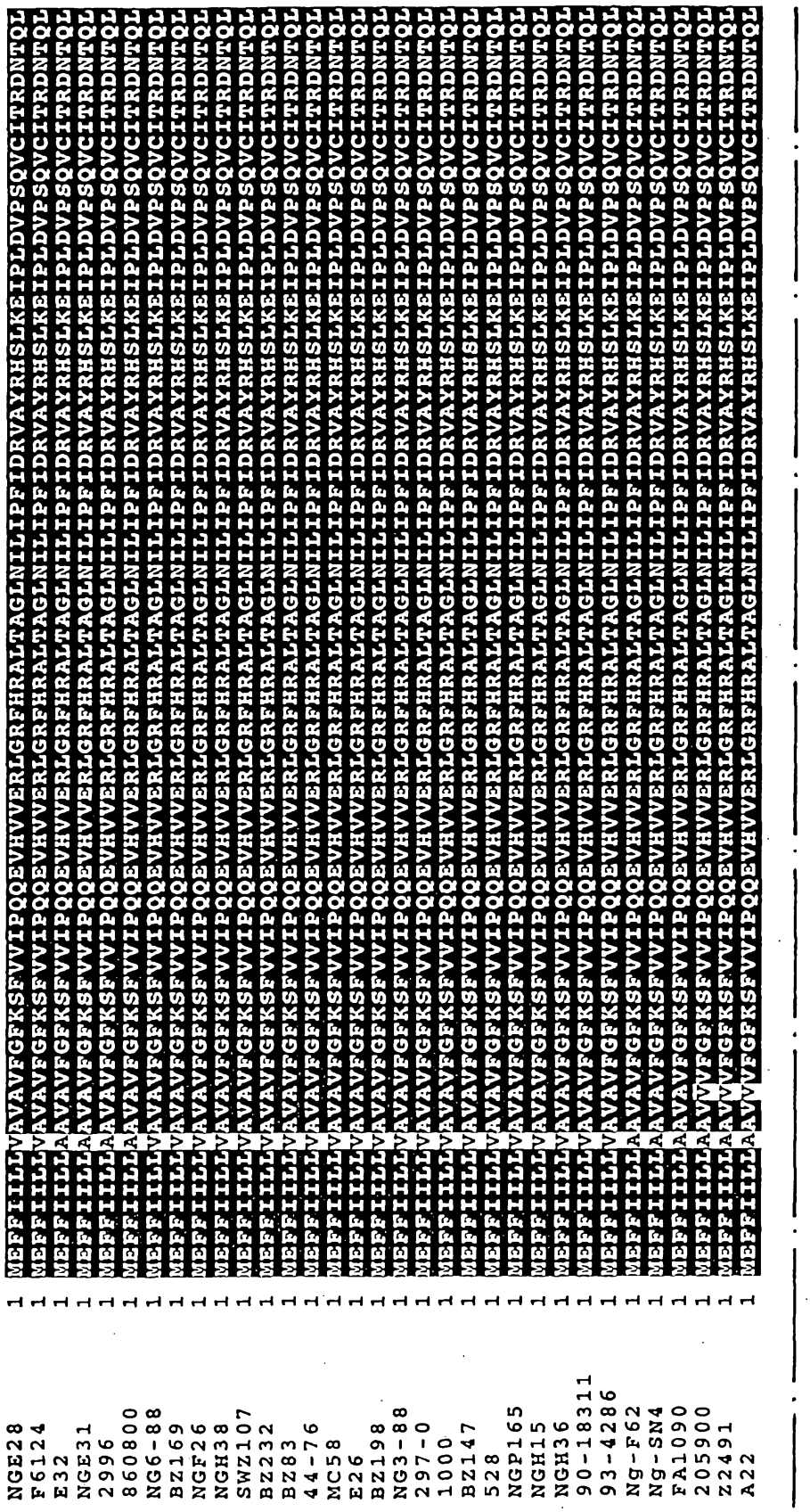

519
NGE28
F6124
E32
NGE31
2996
860800
NG6-88
BZ169
NGF26
NGH38
SWZ107
BZ232
BZ83
44-76
MC58
E26
BZ198
NG3-88
297-0
1000
BZ147
528
NGP165
NGH15
NGH36
90-18311
93-4286
Ng-F62
Ng-SN4
FA1090
205900
Z2491
A22

FIG. 16A(CONTD.)

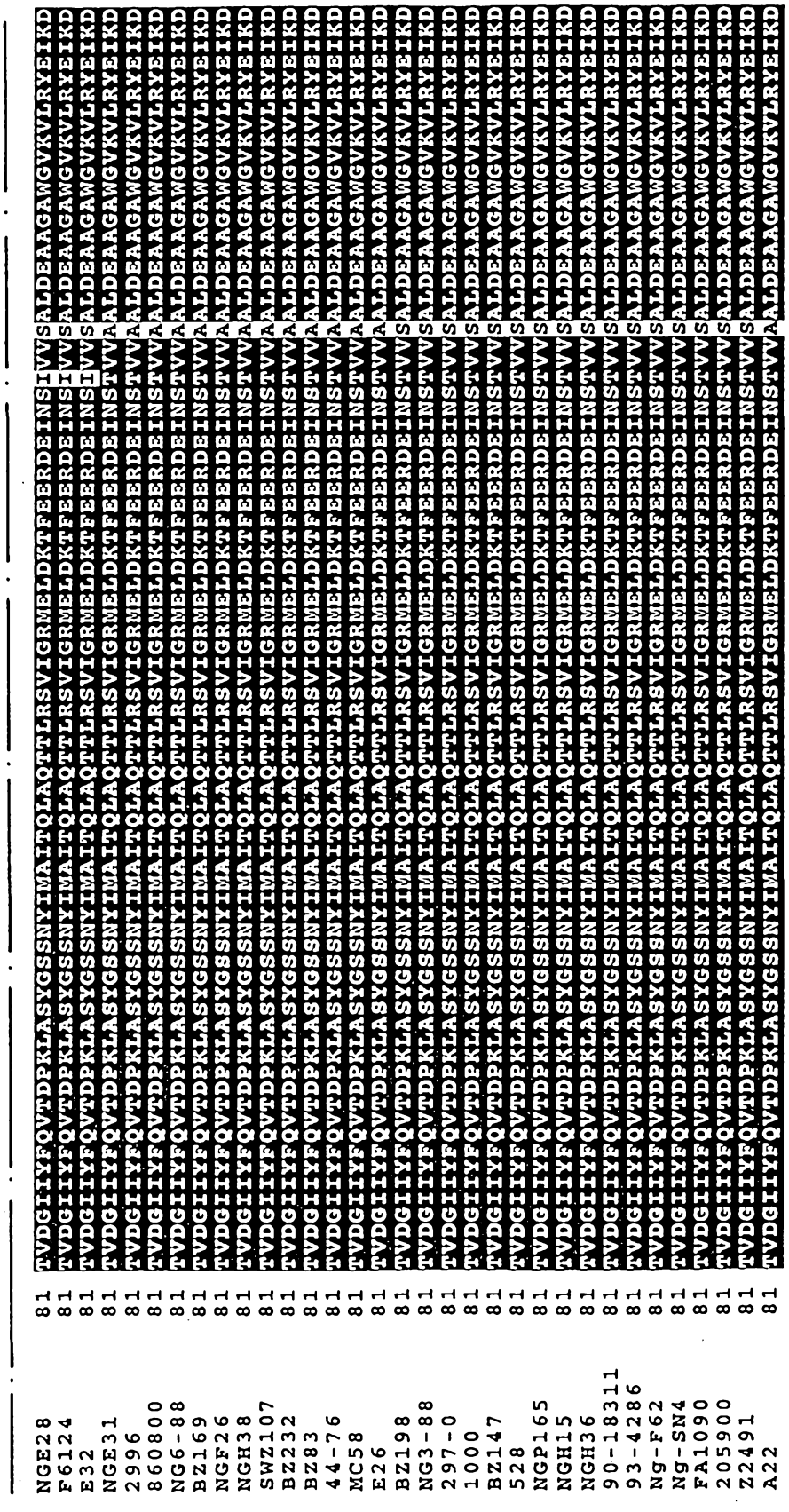


FIG. 16B

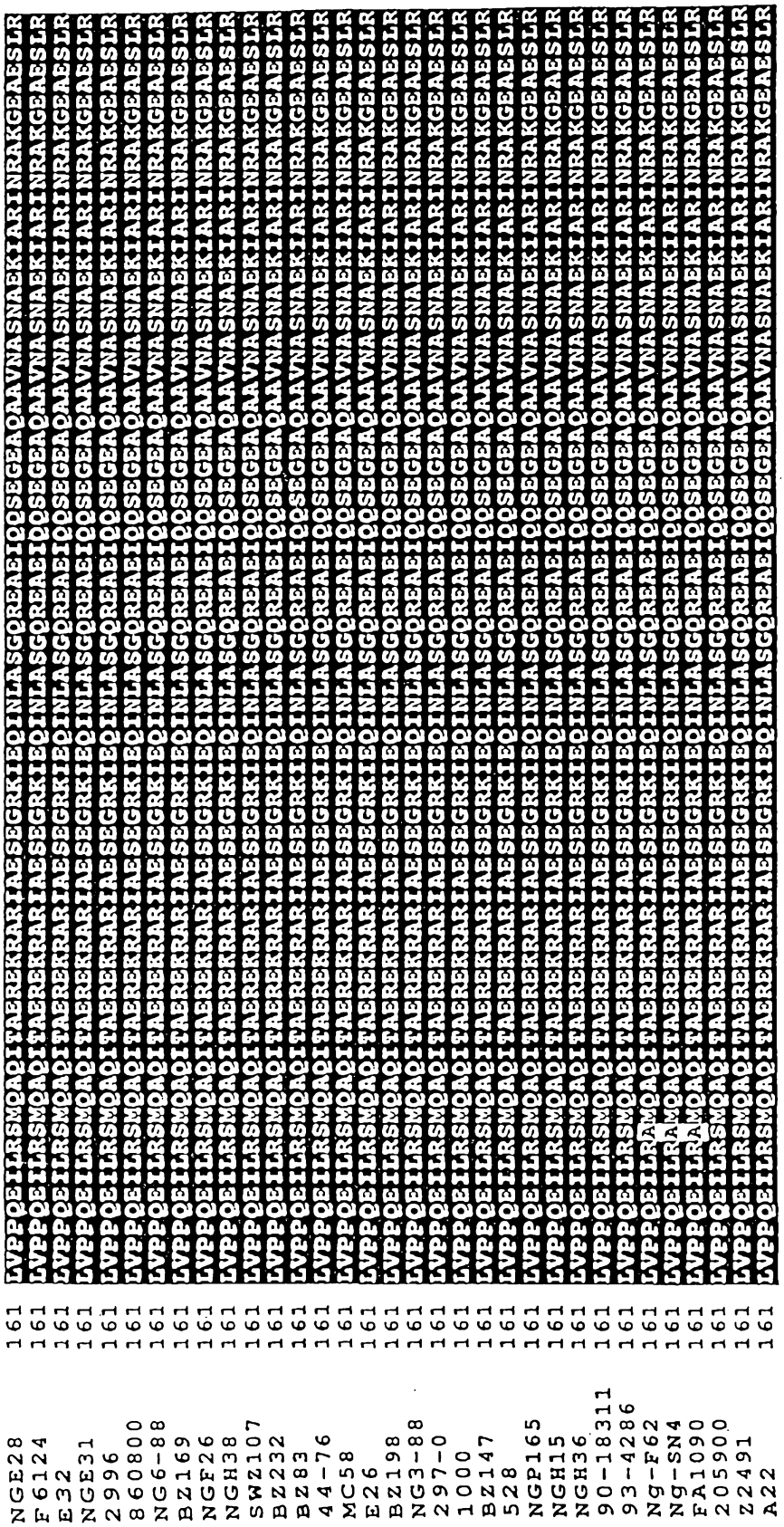

NGE28 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
F6124 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
E32 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NGE31 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
2996 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
860800 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NG6-88 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
BZ169 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NGF26 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NGH38 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
SW2107 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
BZ232 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
BZ83 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
44-76 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
MC58 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
E26 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
BZ198 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NG3-88 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
297-0 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
1000 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
BZ147 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
528 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NGP165 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NGH15 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
NGH36 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
90-18311 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
93-4286 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
Ng-F62 161 LVPQEILRAMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
Ng-SN4 161 LVPQEILRAMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
FA1090 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
205900 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
Z2491 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR
A22 161 LVPQEILRSMQAQITAEREKRARIAESEGRKIEQINLASGQREAEIQQSEGEAQAAVNASNAEKIARINRAKGEAESLR

FIG. 16B(CONTD.)

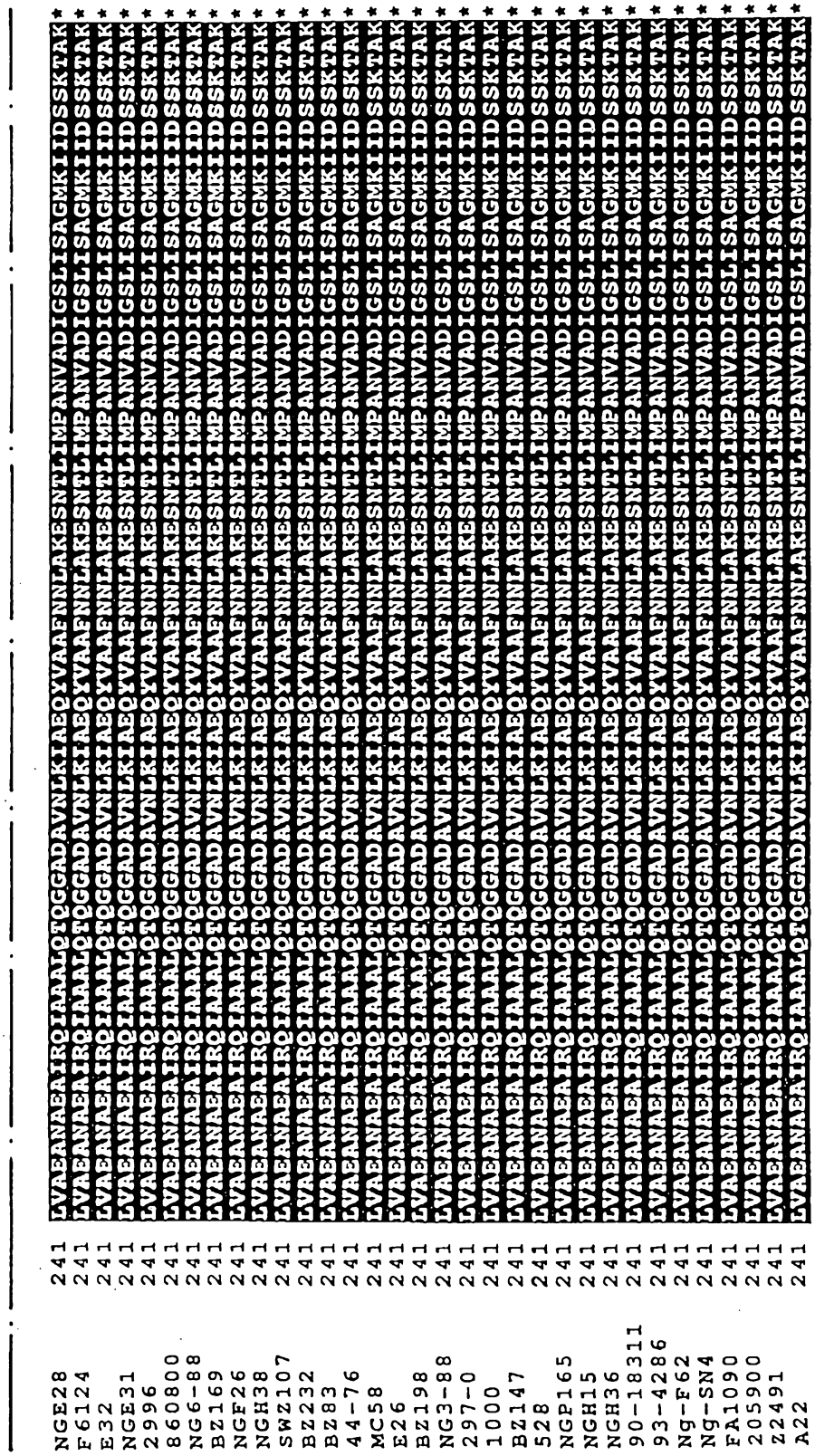


FIG. 17

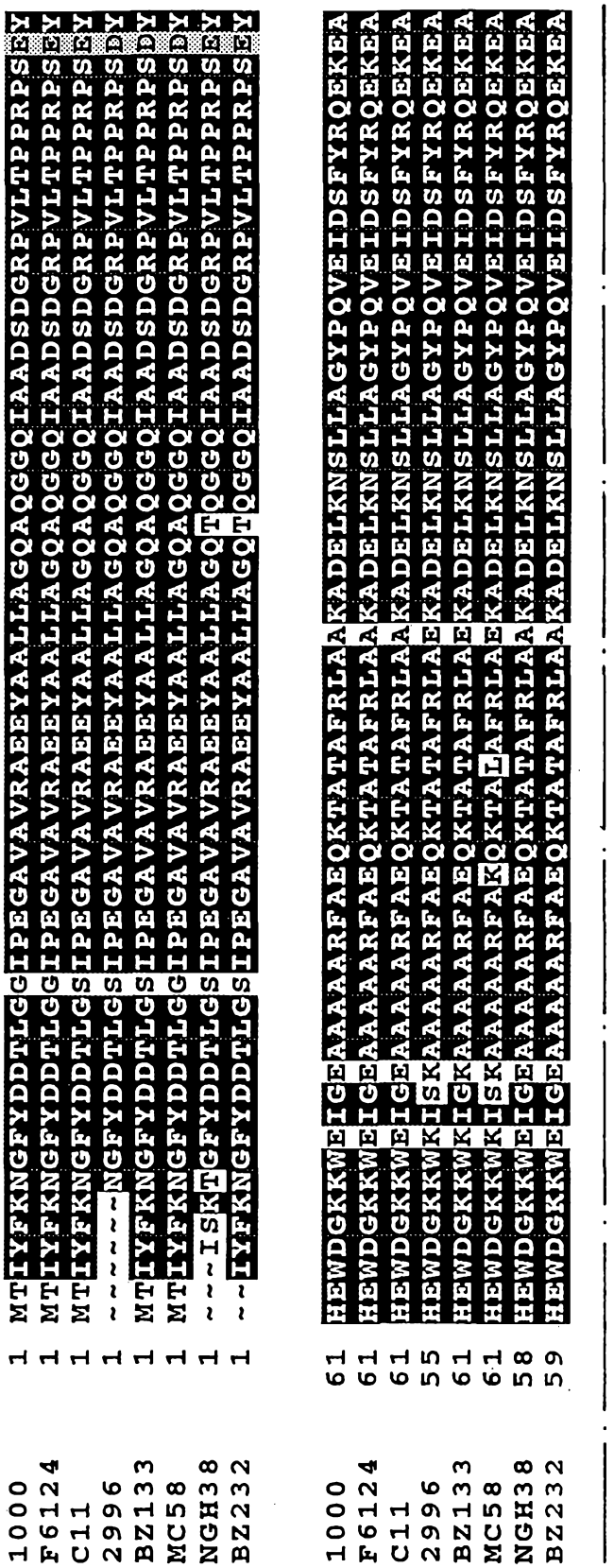


FIG. 17(CONTD.)

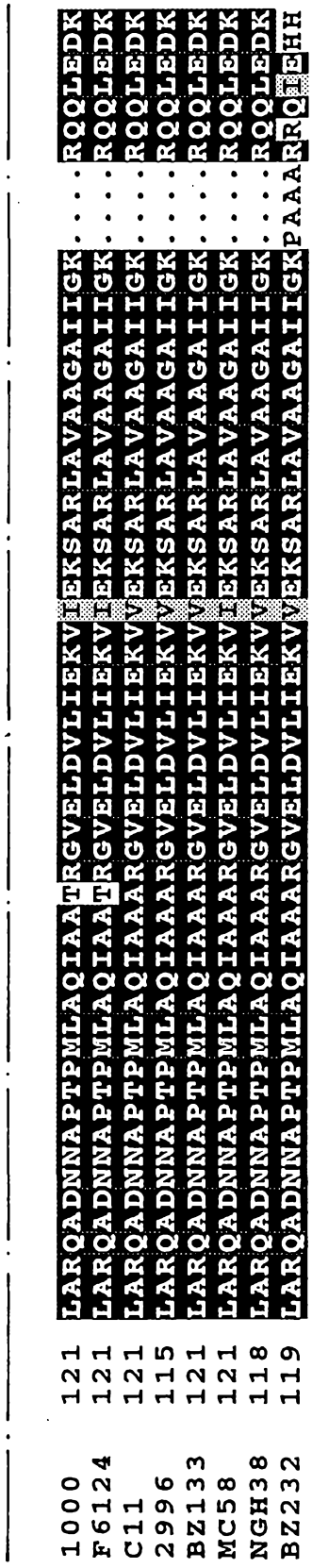

1000 121 LARQADNNAPTPMLAQIAATRGVELDVLIEKVIEKSARLAVAAGAIIGK....RQQLEDK
F6124 121 LARQADNNAPTPMLAQIAATRGVELDVLIEKVIEKSARLAVAAGAIIGK....RQQLEDK
C11 121 LARQADNNAPTPMLAQIAAARGVELDVLIEKVVEKSARLAVAAGAIIGK....RQQLEDK
2996 115 LARQADNNAPTPMLAQIAAARGVELDVLIEKVVEKSARLAVAAGAIIGK....RQQLEDK
BZ133 121 LARQADNNAPTPMLAQIAAARGVELDVLIEKVVEKSARLAVAAGAIIGK....RQQLEDK
MC58 121 LARQADNNAPTPMLAQIAAARGVELDVLIEKVIEKSARLAVAAGAIIGK....RQQLEDK
NGH38 118 LARQADNNAPTPMLAQIAAARGVELDVLIEKVVEKSARLAVAAGAIIGK....RQQLEDK
BZ232 119 LARQADNNAPTPMLAQIAAARGVELDVLIEKVVEKSARLAVAAGAIIGKPAAARRQIEHH
1000 177 LNTIETAPGLDALEKEIEEWTLNIG
F6124 177 LNTIETAPGLDALEKEIEEWTLNIG
C11 177 LNTIETAPGLDALEKEIEEWTLNIG
2996 171 LNAIETAPGLDALEKEIE~~~~~~~
BZ133 177 LNAIETAPGLDALEKEIEEWTLNIG
MC58 177 LNTIETAPGLDALEKEIEEWTLNIG
NGH38 174 LNAIETAPGLDALEKEIE~~~~~~~
BZ232 179 RN...PRPGLDALEKEIEEWTA~~~

FIG. 18A

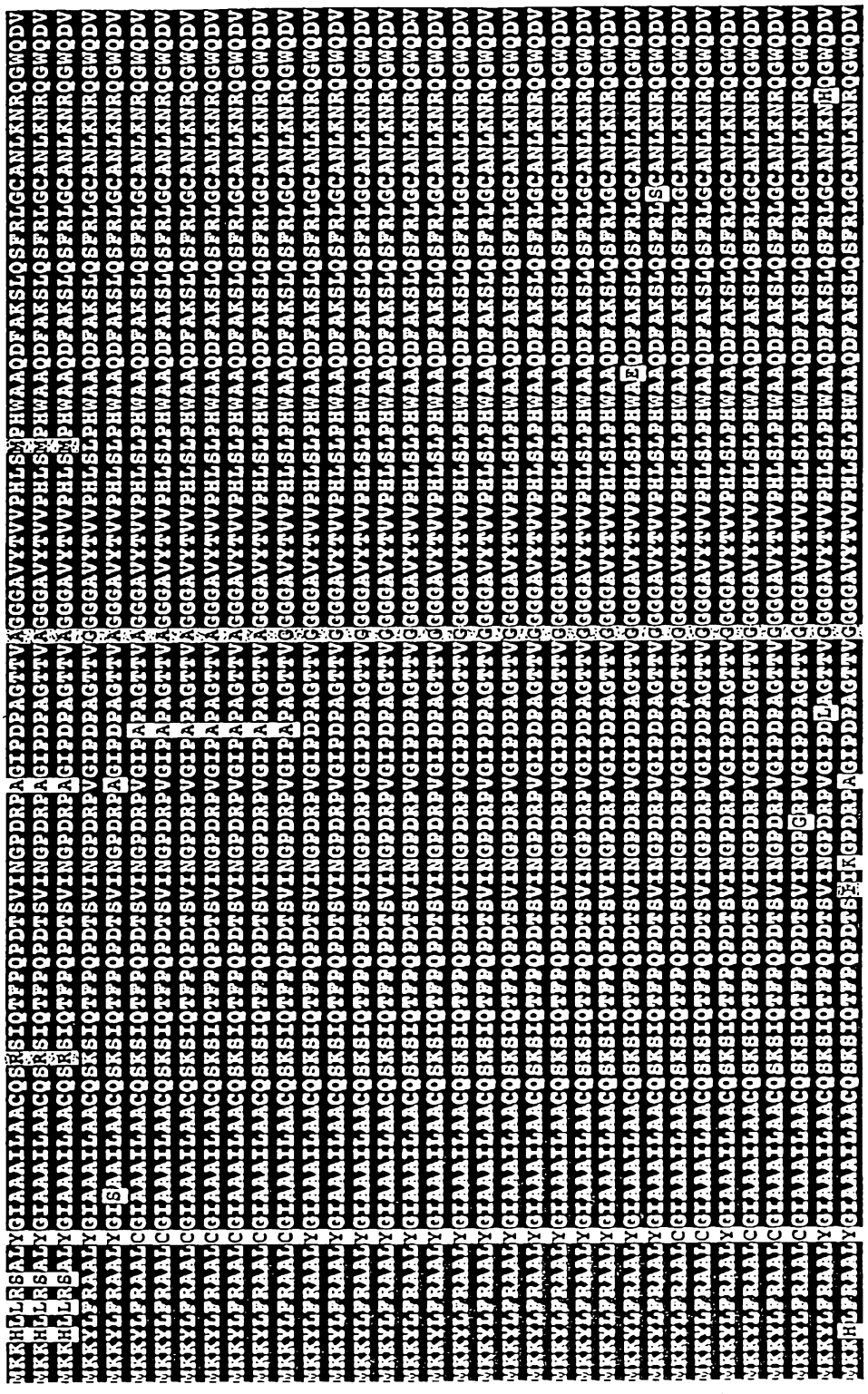


FIG. 18A(CONTD.)

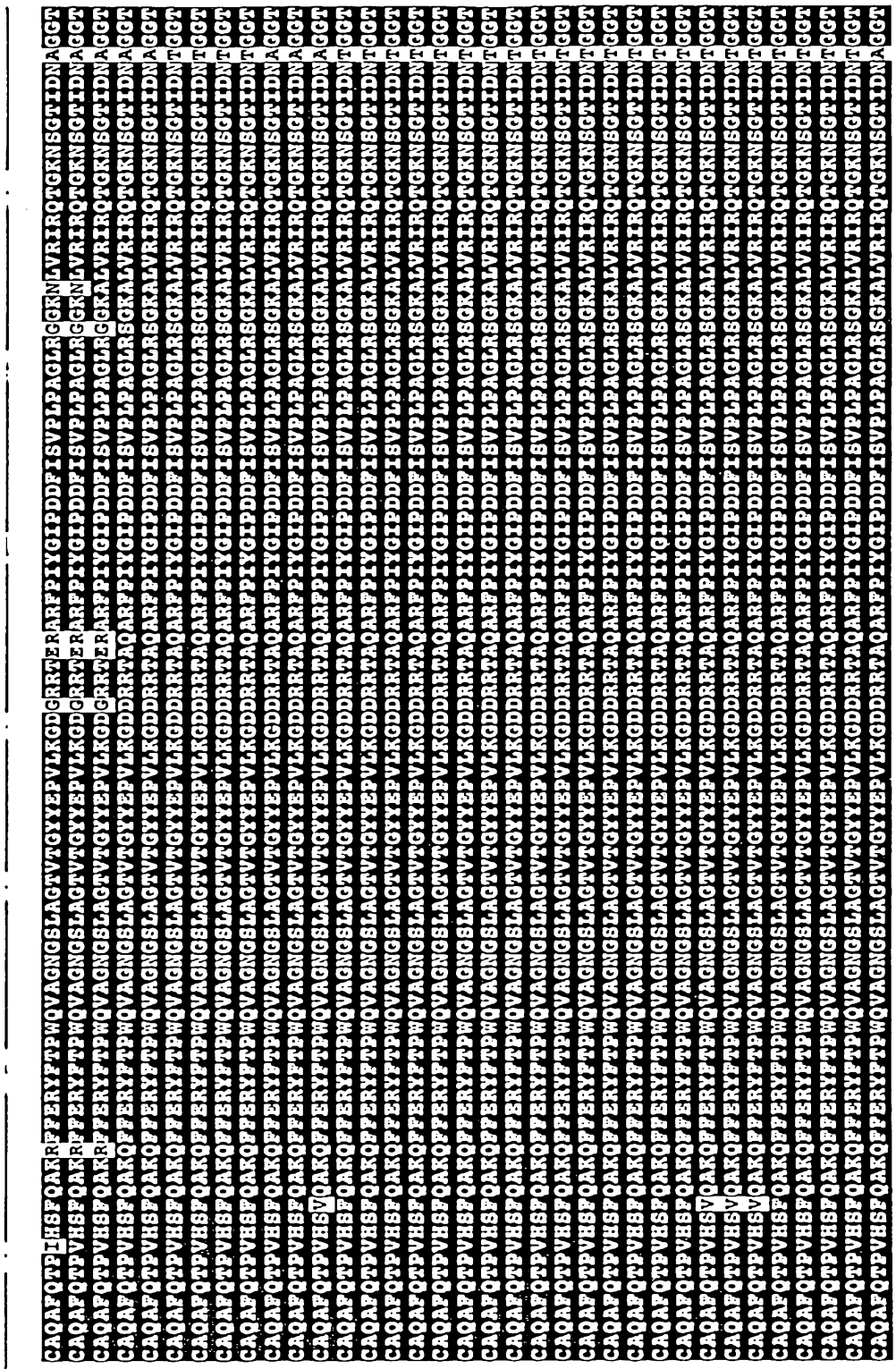


FIG. 18B

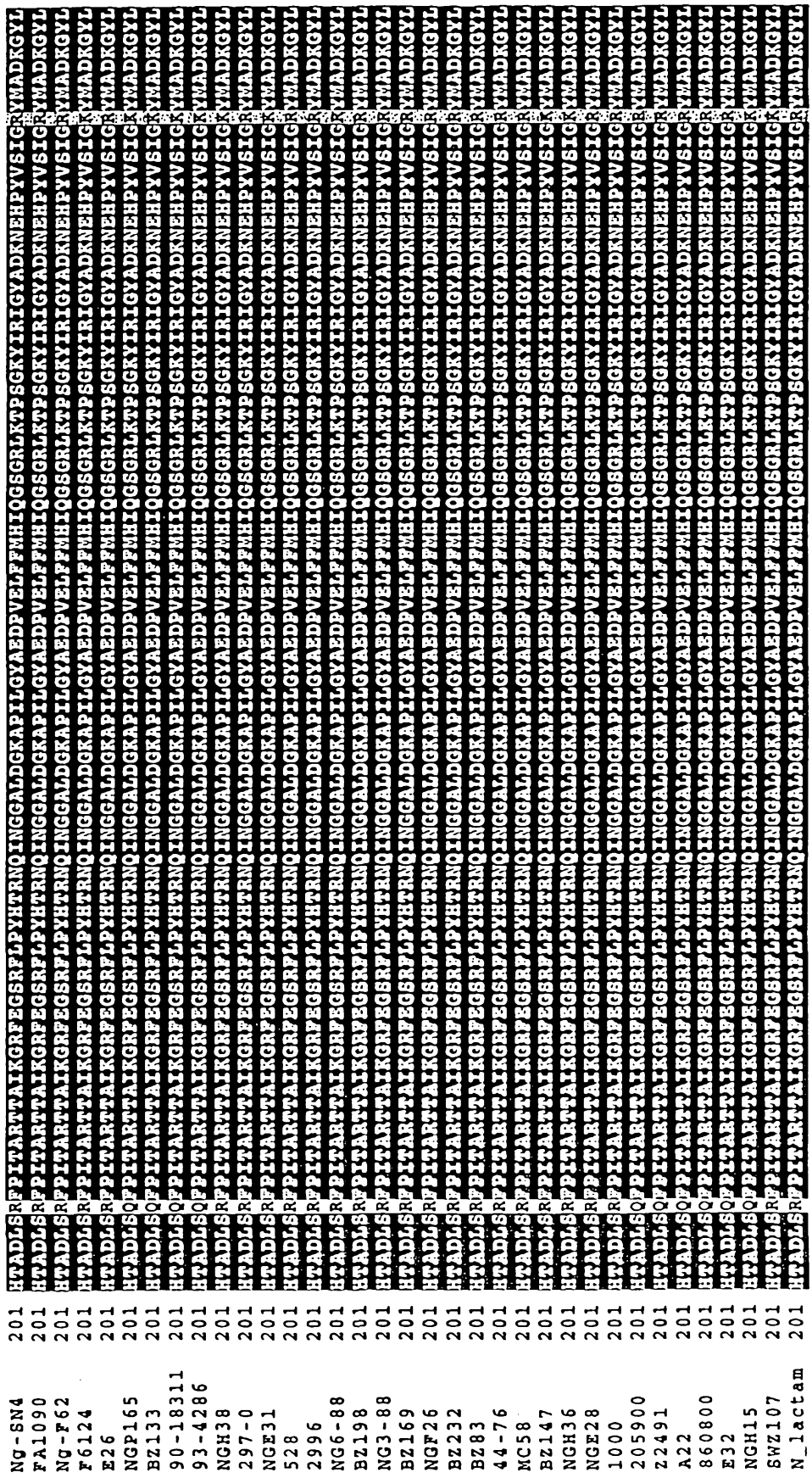


FIG. 18B(CONTD.)

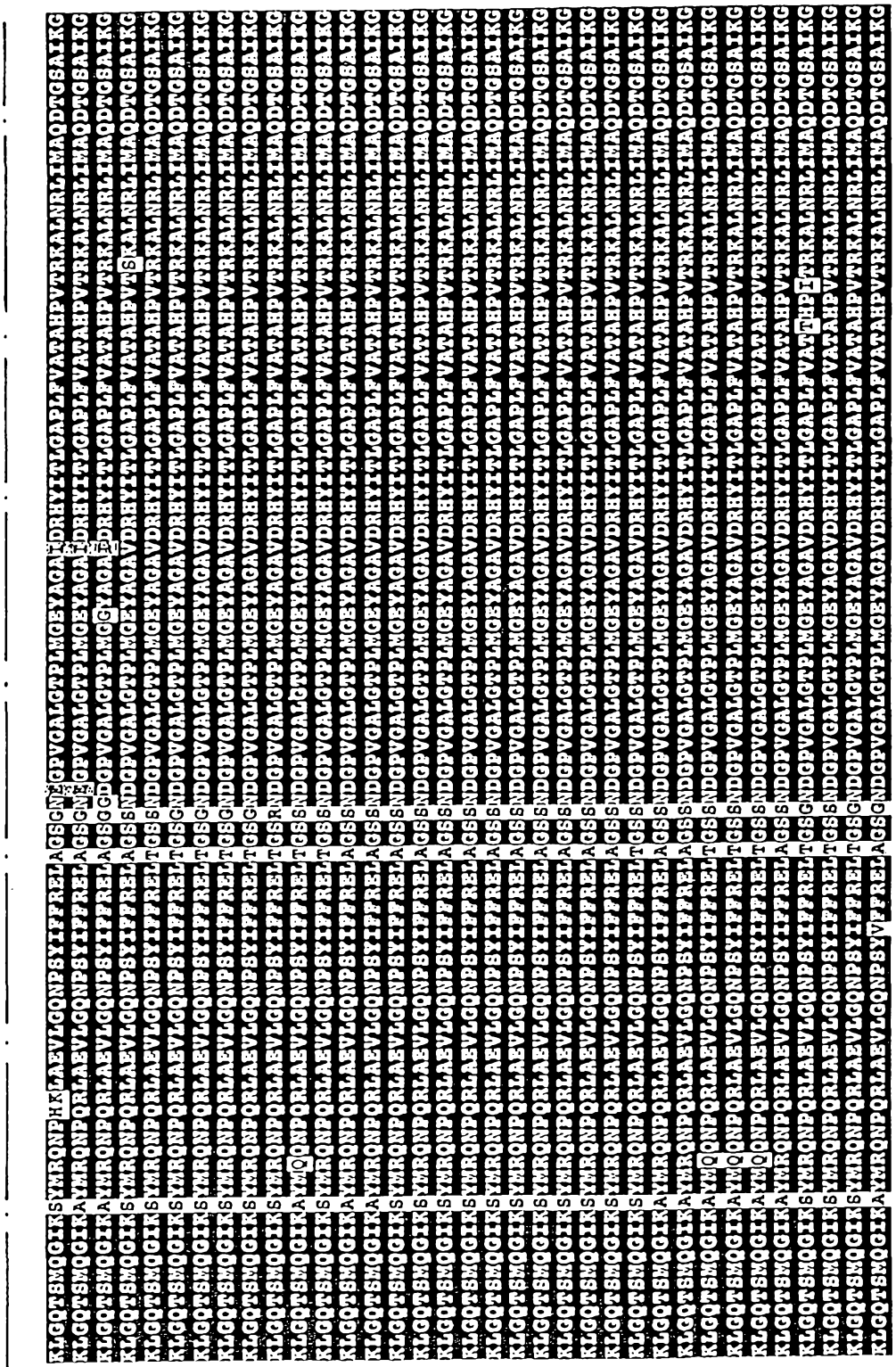


FIG. 18C

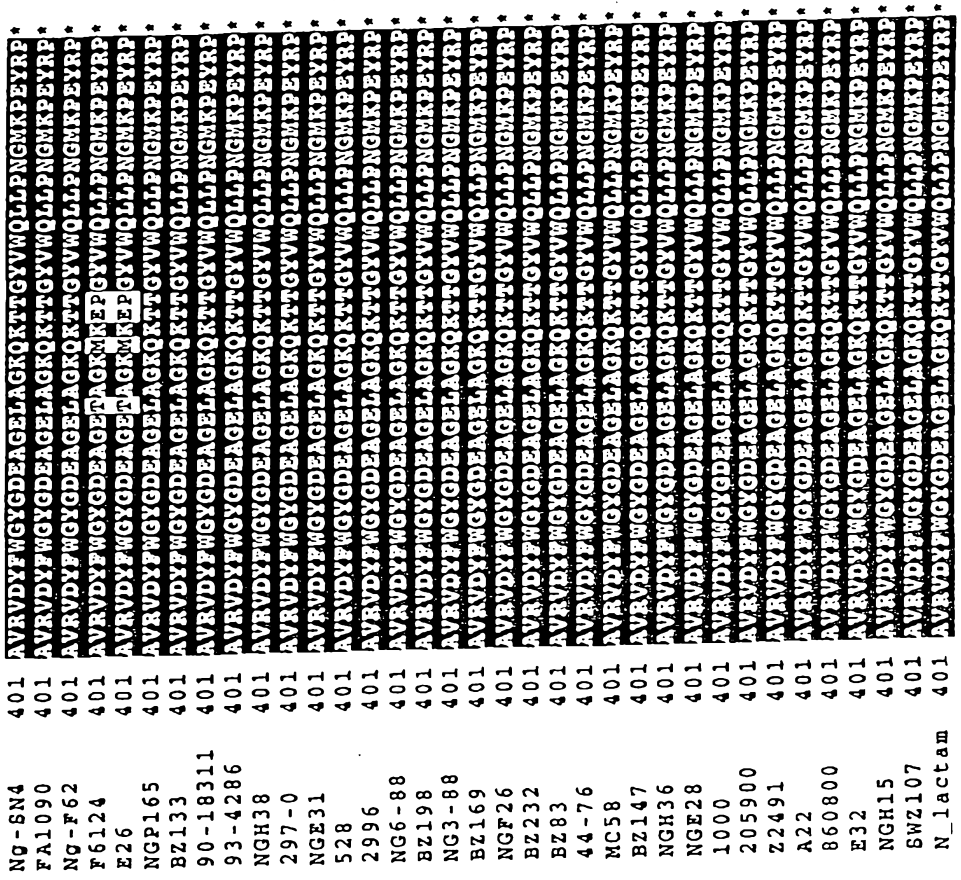

Ng-SN4 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
FA1090 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
Ng-F62 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
F6124 401 AVRVDYFWGYGDEAGETAGKMKEPGYVWQLLPNGMKPEYRP*
E26 401 AVRVDYFWGYGDEAGETAGKMKEPGYVWQLLPNGMKPEYRP*
NGP165 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
BZ133 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
90-18311 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
93-4286 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NGH38 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
297-0 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NGE31 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
528 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
2996 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NG6-88 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
BZ198 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NG3-88 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
BZ169 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NGF26 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
BZ232 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
BZ83 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
44-76 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
MC58 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
BZ147 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NGH36 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NGE28 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
1000 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
205900 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
Z2491 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
A22 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
860800 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
E32 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
NGH15 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
SWZ107 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*
N_lactam 401 AVRVDYFWGYGDEAGELAGKQKTTGYVWQLLPNGMKPEYRP*

FIG. 19

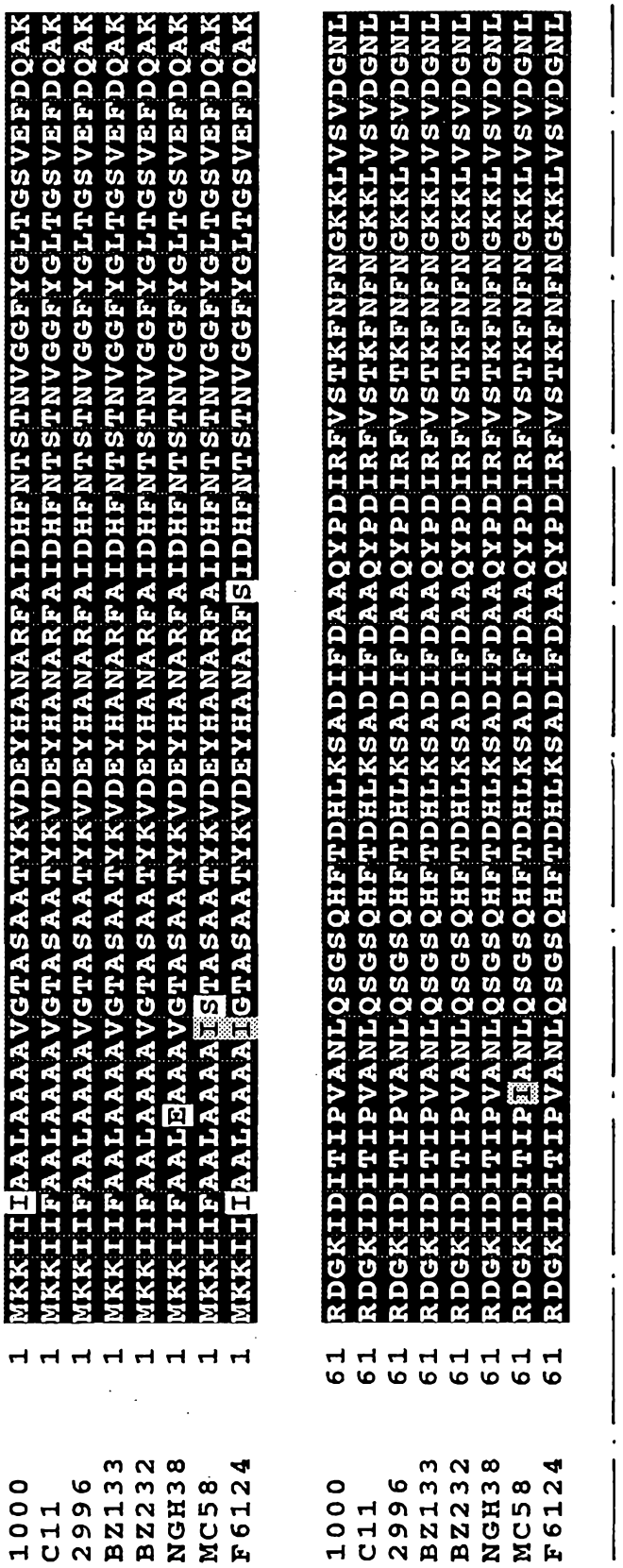


FIG. 19(CONTD.)

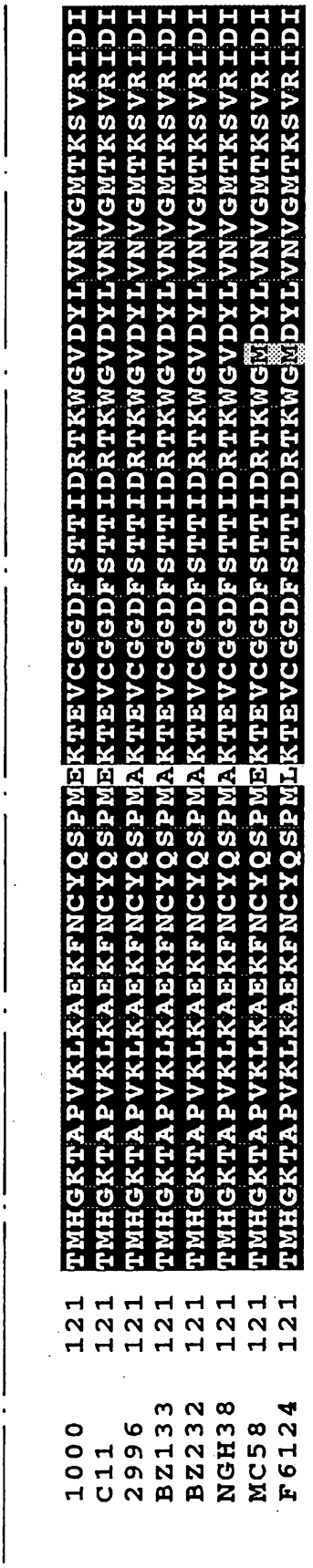

1000 121 TMHGKTAPVKLKAEKFNCYQSPMEKTEVCGGDFSTTIDRTKWGVDYLVNVGMTKSVRIDI
C11 121 TMHGKTAPVKLKAEKFNCYQSPMEKTEVCGGDFSTTIDRTKWGVDYLVNVGMTKSVRIDI
2996 121 TMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLVNVGMTKSVRIDI
BZ133 121 TMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLVNVGMTKSVRIDI
BZ232 121 TMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLVNVGMTKSVRIDI
NGH38 121 TMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLVNVGMTKSVRIDI
MC58 121 TMHGKTAPVKLKAEKFNCYQSPMEKTEVCGGDFSTTIDRTKWGMDYLVNVGMTKSVRIDI
F6124 121 TMHGKTAPVKLKAEKFNCYQSPMLKTEVCGGDFSTTIDRTKWGMDYLVNVGMTKSVRIDI
1000 181 QIEAAKQ~
C11 181 QIEAAKQ~
2996 181 QIEAAKQ~
BZ133 181 QIEAAKQ~
BZ232 181 QIEAAKQ~
NGH38 181 QIEAAKQ~
MC58 181 QIEAAKQ*
F6124 181 QIEAAKQ~

FIG. 20A Orf 4 (33 kD)

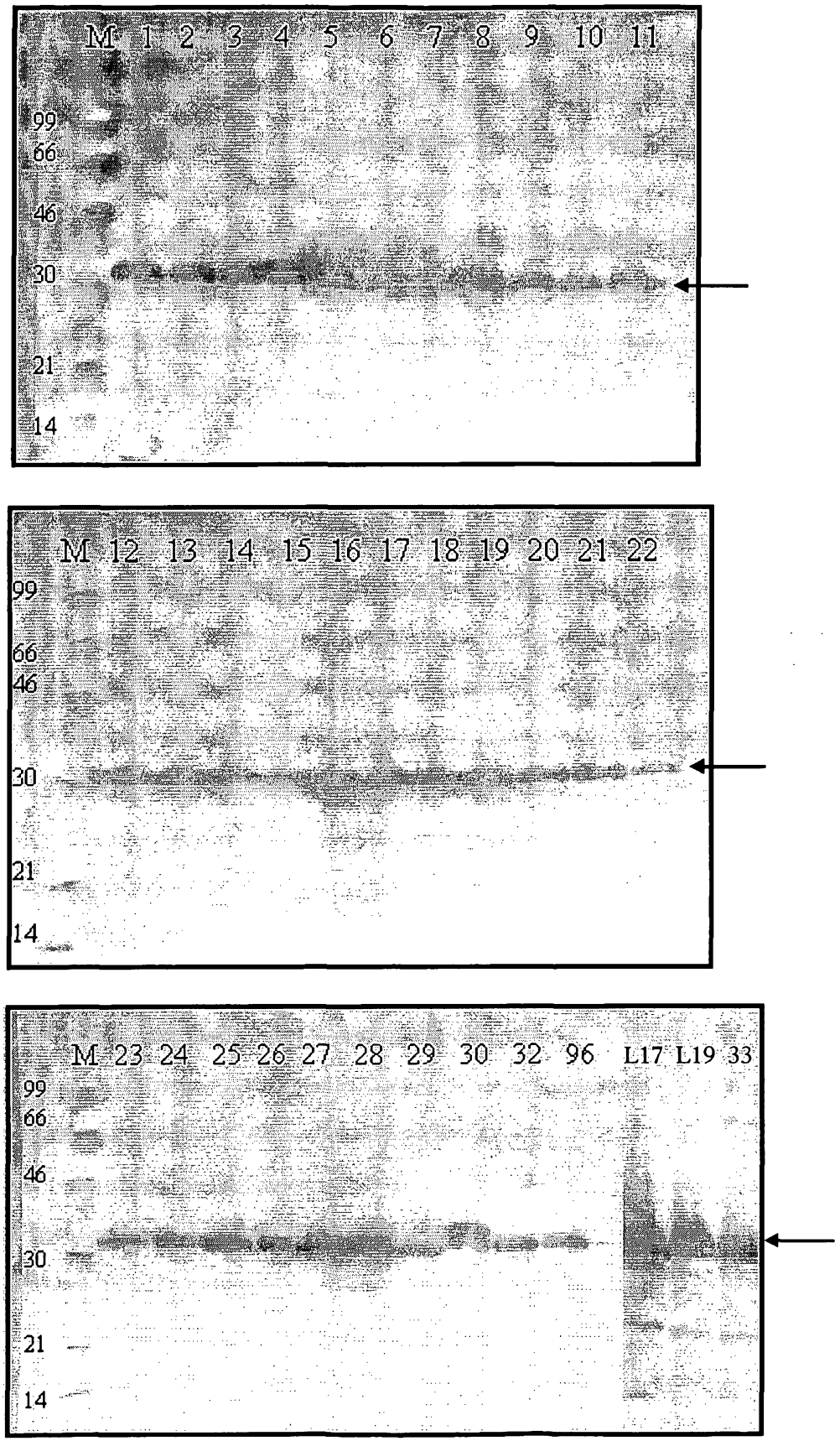
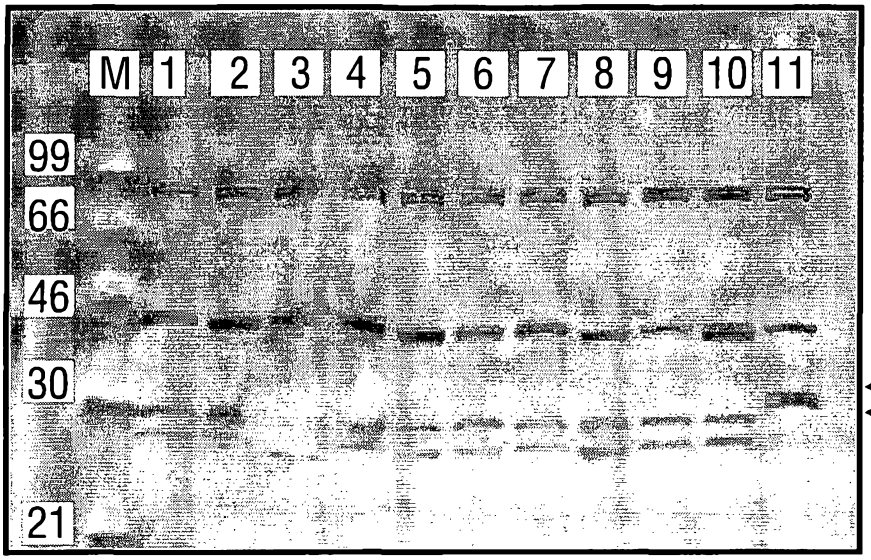
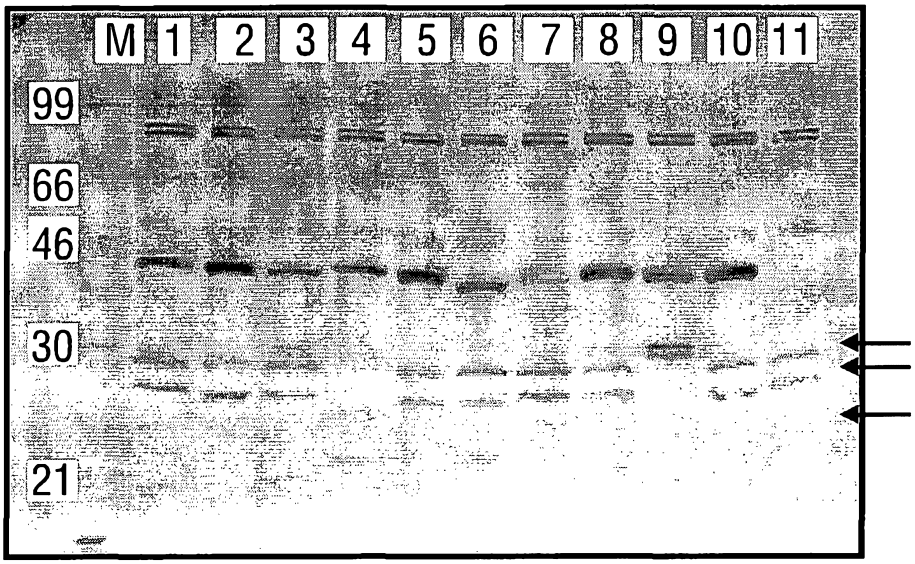
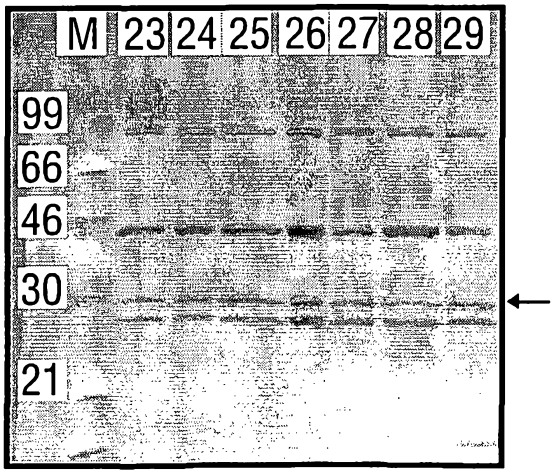


FIG. 20B 225 (27kD)*







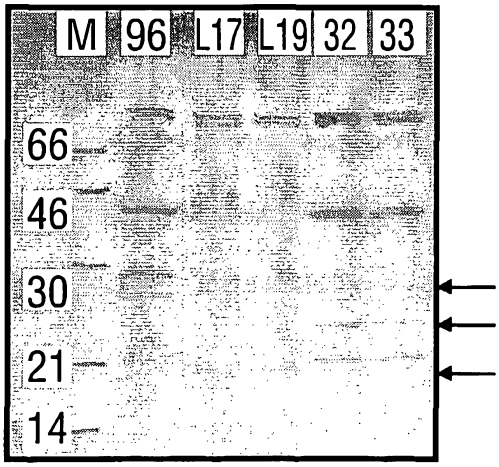
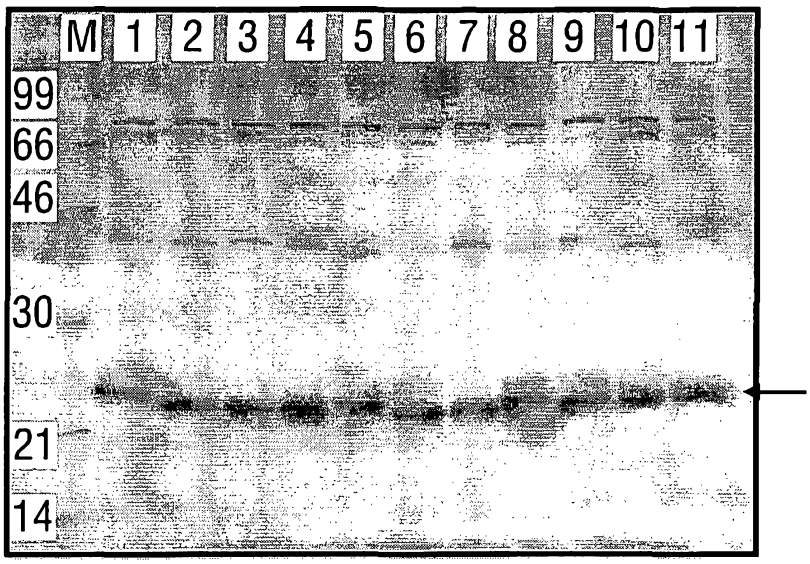
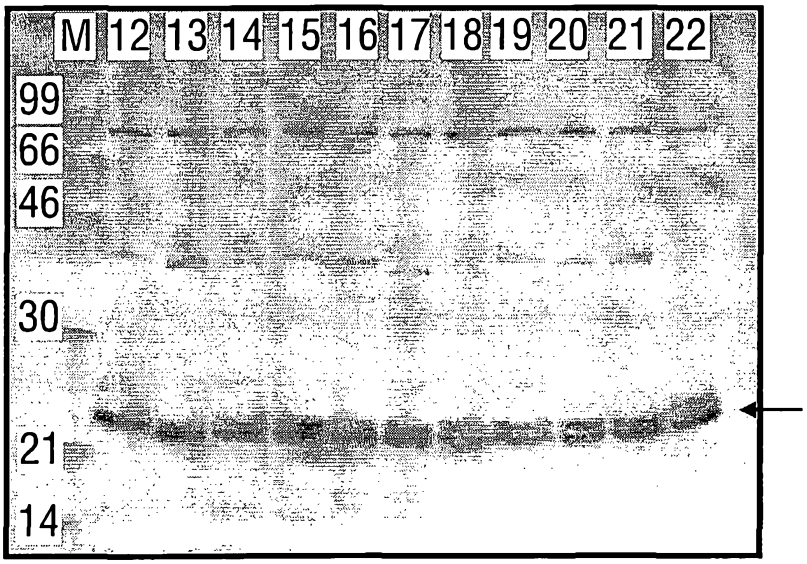


FIG. 20C 235 (23 kD)





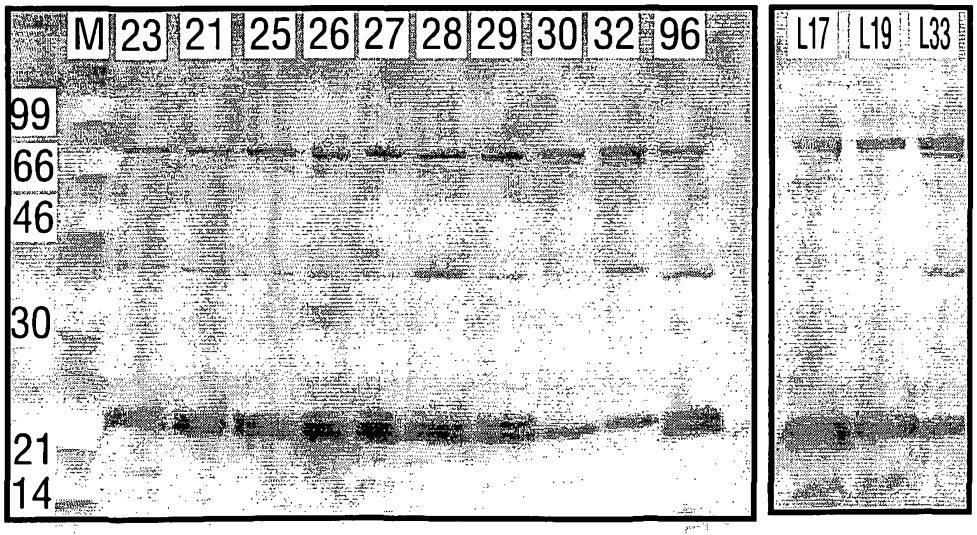
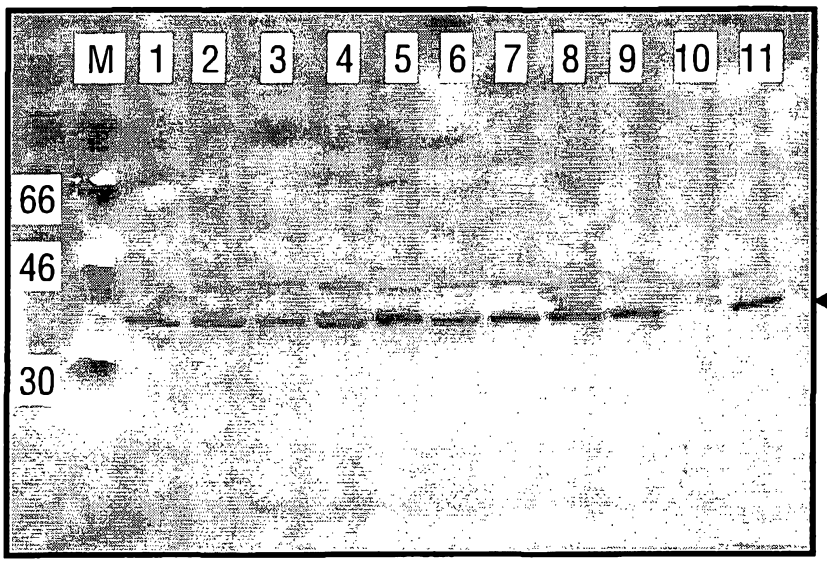
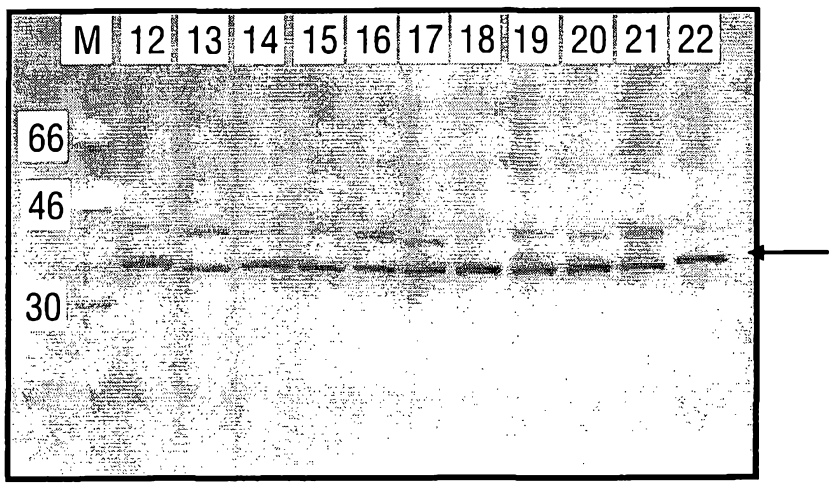
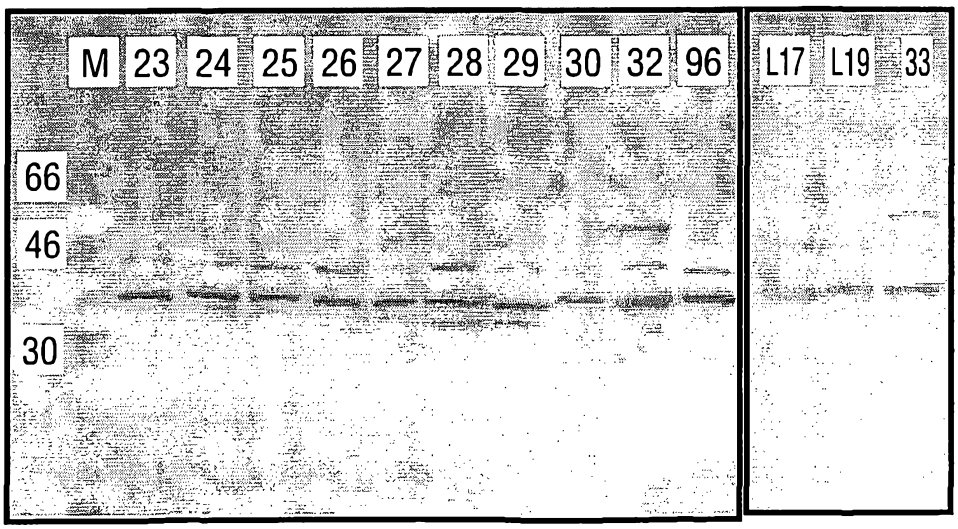


FIG. 20D 519 (35 kD)







97/97

FIG. 20E 919 (48kD)

