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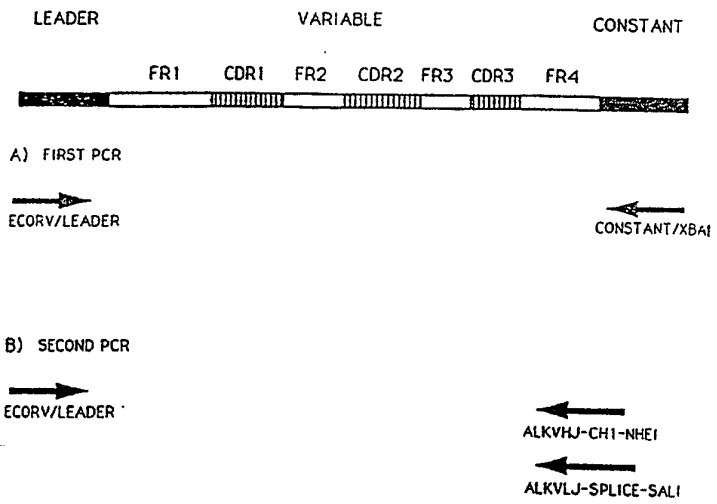

(57) Abstract

The present invention pertains to a method for delivering a neuropharmaceutical or diagnostic agent across the blood brain barrier to the brain of a host. The method comprises administering to the host a therapeutically effective amount of an antibody-neuropharmaceutical or diagnostic agent conjugate wherein the antibody is reactive with a transferrin receptor and the antibody is a chimera between the variable region from one animal source and the constant region from a different animal source. Other aspects of this invention include a delivery system comprising an antibody reactive with a transferrin receptor linked to a neuropharmaceutical or diganostic agent and methods for treating hosts afflicted with a disease associated with a neurological disorder. In embodiments of the present invention, the antibody that is reactive with a tranferrin receptor is a chimeric antibody. This antibody is composed of a variable region, immunologically reactive with the transferrin receptor, that is delivered from one animal source and a constant region that is derived from an animal source other than the one which provided the variable region. The chimeric antibodies of this invention can exist either as isolated entities or as conjugates with a neuropharmaceutical agent for transferal across the blood brain barrier. In the latter mode, the chimeric antibody-neuropharmaceutical agent conjugate forms a delivery system for delivering the neuropharmaceutical agent across the blood brain barrier.

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Process for the preparation of transferrin receptor specific antibody-neuropharmaceutical or diagnostic agent conjugates.

Description

Background

The capillaries that supply blood to the tissues of the brain constitute the blood brain barrier (Goldstein et al. (1986) Scientific American 255:74-83; Pardridge, W.M. (1986) Endocrin. Rev. 7:314-330). The endothelial cells which form the brain capillaries are different from those found in other tissues in the body. Brain capillary endothelial cells are joined together by tight intercellular junctions which form a continuous wall against the passive movement of substances from the blood to the brain. These cells are also different in that they have few pinocytic vesicles which in other tissues allow somewhat unselective transport across the capillary wall. Also lacking are continuous gaps or channels running through the cells which would allow unrestricted passage.

The blood-brain barrier functions to ensure that the environment of the brain is constantly controlled. The levels of various substances in the blood, such as hormones, amino acids and ions, undergo frequent small fluctuations which can be brought about by activities such as eating and exercise (Goldstein et al, cited supra). If the brain were not protected by the blood brain barrier from these variations in serum composition, the result could be uncontrolled neural activity.

The isolation of the brain from the bloodstream is not complete. If this were the case, the brain would be unable to function properly due to a lack of nutrients and because of the need to exchange chemicals with the rest of the body. The presence of specific transport systems within the capillary endothelial cells assures that the brain receives, in a controlled manner, all of the compounds required for normal growth and function. In many instances, these transport systems consist of membrane-associated receptors which, upon binding of their respective ligand, are internalized by the cell (Pardridge, W.M., cited supra). Vesicles containing the receptor-ligand complex then migrate to the abluminal surface of the endothelial cell where the ligand is released.

The problem posed by the blood-brain barrier is that, in the process of protecting the brain, it excludes many potentially useful therapeutic agents. Presently, only substances which are sufficiently lipophilic can penetrate the blood-brain barrier

(Goldstein et al, cited supra; Pardridge, W.M., cited supra). Some drugs can be modified to make them more lipophilic and thereby increase their ability to cross the blood brain barrier. However, each modification has to be tested individually on each drug and the modification can alter the activity of the drug. The modification can also have a very general effect in that it will increase the ability of the compound to cross all cellular membranes, not only those of brain capillary endothelial cells.

Summary of the Invention

The present invention pertains to a method for delivering a neuropharmaceutical or diagnostic agent across the blood brain barrier to the brain of a host. The method comprises administering to the host a therapeutically effective amount of an antibody-neuropharmaceutical or diagnostic agent conjugate wherein the antibody is reactive with a transferrin receptor and the antibody is a chimera between the variable region from one animal source and the constant region from a different animal source. The conjugate is administered under conditions whereby binding of the antibody to a transferrin receptor on a brain capillary endothelial cell occurs and the neuropharmaceutical agent is transferred across the blood brain barrier in a pharmaceutically active form. Other aspects of this invention include a delivery system comprising an antibody reactive with a transferrin receptor linked to a neuropharmaceutical agent and methods for treating hosts afflicted with a disease associated with a neurological disorder.

In embodiments of the present invention, the antibody that is reactive with a transferrin receptor is a chimeric antibody. This antibody is composed of a variable region, immunologically reactive with the transferrin receptor, that is derived from one animal source and a constant region that is derived from an animal source other than the one which provided the variable region. The chimeric antibodies of this invention can exist either as isolated entities or as conjugates with a neuropharmaceutical agent for transferal across the blood brain barrier. In the latter mode, the chimeric antibody-neuropharmaceutical agent conjugate forms a delivery system for delivering the neuropharmaceutical agent across the blood brain barrier.

Presently available means for delivering therapeutic agents to the brain are limited in that they are invasive. The delivery system of the present invention is non-invasive and can utilize readily available antibodies reactive with a transferrin receptor as carriers for neuropharmaceutical agents. The delivery system is advantageous in that the antibodies are capable of transporting neuropharmaceutical agents across the blood brain barrier without being susceptible to premature release of the neuropharmaceutical agent prior to reaching the brain-side of the blood brain barrier. Further, if the therapeutic activity of the agent to be delivered to the brain is not altered by the addition of a linker, a noncleavable linker can be

used to link the neuropharmaceutical agent to the antibody.

Description of the Drawings

Figure 1 is a graphic representation of rat brain uptake of ^{14}C-labelled murine monoclonal antibody (OX-26) to rat transferrin receptor in rats where the percent injected dose of radiolabelled antibody per brain and per 55 μl of blood is plotted versus time post-injection.

Figure 2 is a histogram illustrating time dependent changes in the disposition of radiolabelled OX-26 between brain parenchyma and vasculature.

Figure 3 is a histogram illustrating the enhanced delivery of methotrexate across the blood-brain barrier when administered as a conjugate with OX-26.

Figure 4 illustrates in three histograms (A,B and C) the distribution in the brain of both the antibody and the AZT components of an OX-26-AZT conjugate.

Figure 5 is a histogram illustrating the experimental results of delivery of a protein, horseradish peroxidase, across the blood-brain barrier in rat brains in the form of a conjugate with OX-26.

Figure 6 is a histogram illustrating the experimental results of delivering soluble CD4 to rat brain parenchyma using CD4 in the form of a conjugate with OX-26.

Figure 7 is a histogram illustrating the biodistribution of antibody 128.1 and control IgG in a cynomolgous monkey.

Figure 8 is a flow diagram of the general strategy for the expression of immunoglobulin variable region genes obtained by PCR.

Figure 9 illustrates the primers used for variable region amplification, both for first cloning and sequencing the V region and then for cloning into the final expression vector.

Figure 10 illustrates the cloning of the 128.1 heavy chain variable region.

Figure 11 is the antibody coding sequence of heavy chain expression vector pAH4602 containing the γ-1 isotype constant region.

Figure 12 illustrates the cloning of the 128.1 light chain variable region.

Figure 13 is the antibody coding sequence of light chain expression vector pAG4611.

Figure 14 illustrates the plasmid map of the heavy chain expression vector pAH4625 containing the γ-2 isotype.

Figure 15 illustrates the plasmid map of the heavy chain expression vector pAH4807 containing the γ-3 isotype.

Figure 16 illustrates the plasmid map of the heavy chain expression vector pAH4808 containing the γ-4 isotype.

Figure 17 is the antibody coding sequence of heavy chain expression vector pAH4625 containing the γ-2 isotype constant region.

Figure 18 is the antibody coding sequence of heavy chain expression vector pAH4807 containing the γ-3 isotype constant region.

Figure 19 is the antibody coding sequence of heavy chain expression vector pAH4808 containing the γ-4 isotype constant region.

Detailed Description

The method for delivering a neuropharmaceutical agent across the blood brain barrier to the brain of a host comprises administering to the host a therapeutically effective amount of an antibody-neuropharmaceutical agent conjugate wherein the antibody is reactive with a transferrin receptor present on a brain capillary endothelial cell. The method is conducted under conditions whereby the antibody binds to the transferrin receptor on the brain capillary endothelial cell and the neuropharmaceutical agent is transferred across the blood brain barrier in a pharmaceutically active form.

The host can be an animal susceptible to a neurological disorder (i.e., an animal having a brain). Examples of hosts include mammals such as humans, domestic animals (e.g., dog, cat, cow or horse), mice and rats.

The neuropharmaceutical agent can be an agent having a therapeutic or prophylactic effect on a neurological disorder or any condition which affects biological functioning of the central nervous system. Examples of neurological disorders include cancer

(e.g. brain tumors), Autoimmune Deficiency Syndrome (AIDS), stroke, epilepsy, Parkinson's disease, multiple sclerosis, neurodegenerative disease, trauma, depression, Alzheimer's disease, migraine, pain, or a seizure disorder. Classes of neuropharmaceutical agents which can be used in this invention include proteins, antibiotics, adrenergic agents, anticonvulsants, small molecules, nucleotide analogs, chemotherapeutic agents, anti-trauma agents, peptides and other classes of agents used to treat or prevent a neurological disorder. Examples of proteins include CD4 (including soluble portions thereof), growth factors (e.g. nerve growth factor and interferon), dopamine decarboxylase and tricosanthin. Examples of antibiotics include amphotericin B, gentamycin sulfate, and pyrimethamine. Examples of adrenergic agents (including blockers) include dopamine and atenolol. Examples of chemotherapeutic agents include adriamycin, methotrexate, cyclophosphamide, etoposide, and carboplatin. An example of an anticonvulsant which can be used is valproate and an anti-trauma agent which can be used is superoxide dismutase. Examples of peptides would be somatostatin analogues and enkephalinase inhibitors. Nucleotide analogs which can be used include azido thymidine (hereinafter AZT), dideoxy Inosine (ddI) and dideoxy cytodine (ddc).

The antibody, which is reactive with a transferrin receptor present on a brain capillary endothelial cell, may also be conjugated to a

diagnostic agent. In this method and delivery system, the neuropharmaceutical agent of the neuropharmaceutical agent - anti-transferrin receptor conjugate has been replaced with a diagnostic agent. The diagnostic agent is then delivered across the blood brain barrier to the brain of the host. The diagnostic agent is then detected as indicative of the presence of a physiological condition for which the diagnostic agent is intended. For example, the diagnostic agent may be an antibody to amyloid plaques. When conjugated to an antibody reactive with a transferrin receptor present on a brain capillary endothelial cell, this diagnostic agent antibody can be transferred across the blood brain barrier and can then subsequently immunoreact with amyloid plaques. Such an immunoreaction is indicative of Alzheimer's Disease.

Serum transferrin is a monomeric glycoprotein with a molecular weight of 80,000 daltons that binds iron in the circulation and transports it to the various tissues(Aisen et al. (1980) Ann. Rev. Biochem. 49:357-393; MacGillivray et al. (1981) J. Biol. Chem. 258:3543-3553). The uptake of iron by individual cells is mediated by the transferrin receptor, an integral membrane glycoprotein consisting of two identical 95,000 dalton subunits that are linked by a disulfide bond. The number of receptors on the surface of a cell appears to correlate with cellular proliferation, with the highest number being on actively growing cells and the lowest being on resting and terminally

differentiated cells. Jeffries et al (Nature Vol. 312 (November 1984) pp. 167-168) used monoclonal antibodies to show that brain capillary endothelial cells have a high density of transferrin receptors on their cell surface.

Antibodies which can be used within this invention are reactive with a transferrin receptor. The term antibody is intended to encompass both polyclonal and monoclonal antibodies. The preferred antibody is a monoclonal antibody reactive with a transferrin receptor. The term antibody is also intended to encompass mixtures of more than one antibody reactive with a transferrin receptor (e.g., a cocktail of different types of monoclonal antibodies reactive with a transferrin receptor). The term antibody is further intended to encompass whole antibodies, biologically functional fragments thereof, and chimeric antibodies comprising portions from more than one species, bifunctional antibodies, etc. Biologically functional antibody fragments which can be used are those fragments sufficient for binding of the antibody fragment to the transferrin receptor to occur.

The antibodies, chimeric or otherwise, are not to be considered as being restricted to a specific isotype. Any of the antibody isotypes are within the present invention. For example, antibodies with identical light chains but different heavy chains are intended. In addition, mutations of certain regions of the antibodies, e.g., in the γ chains, are also intended. These mutations, particularly point

mutations, may occur anywhere provided functionality of the antibodies as reactive with a transferrin receptor is still maintained.

The chimeric antibodies can comprise portions derived from two different species (e.g., human constant region and murine variable or binding region). The portions derived from two different species can be joined together chemically by conventional techniques or can be prepared as single contiguous proteins using genetic engineering techniques. DNA encoding the proteins of both the light chain and heavy chain portions of the chimeric antibody can be expressed as contiguous proteins.

One genetic engineering approach that can be used to produce or clone chimeric antibodies reactive with a transferrin receptor is to prime the DNAs encoding the variable region of functional antibodies for amplification by PCR using specific oligonucleotides. The variable region of functional antibodies is that portion of the antibody that immunologically reacts with the transferrin receptor antigen. Both the heavy chain and light chain of antibodies contribute to the variable region. Thus, the DNA encoding the variable region has two portions: a polynucleotide sequence encoding the variable region heavy chain and a polynucleotide sequence encoding the variable region light chain. The primed variable regions can then be cloned into vectors which contain the DNA encoding the constant region of antibodies. A particularly useful vector is one which contains DNA encoding the constant

region of human antibodies that has been designed to also express immunoglobulin variable regions from other sources. The DNA encoding the constant region is usually from a separate source than the one whose DNA encodes the variable region. Although different animals from the same species may be the sources of the DNA encoding the variable region and the constant region, the usual situation is where the animal species are different (e.g., human constant region and murine variable region). Following the cloning of the primed variable regions into vectors containing the constant region, chimeric antibodies can be expressed from such vectors.

A general strategy that can be used to amplify immunoglobulin variable regions has been previously described (Orlandi et al., Proc. Natl. Acad. Sci., 86: 3833-3837 (1989); Larrick et al., Bio/technology, 7: 934-938 (1989); Gavilondo et al., Hybridoma, 9(5): 407-417 (1990)). Two approaches have been used in the general strategy. In one approach, 5′ primers are designed to prime the first framework region of the variable region. The 3′ primers are designed to prime either the J region or the constant region. Priming in the frameworks (Orlandi) takes advantage of the conserved nature of these sequences. This makes it feasible to use relatively few degenerate primers to clone the majority of the variable regions. The disadvantage of this approach is that it may introduce amino acid substitutions into the framework regions which affect antibody affinity.

In the second approach (Larrick, Gavilondo), 5′ primers are designed to prime some portion of the leader sequence. The 3′ primers are designed to prime either the J region or the constant region, as in the first approach. The second approach takes advantage of the relatively conserved nature of the leader sequences and uses a set of redundant oligonucleotides to prime this site. Priming in the leader sequences is generally the more powerful approach since this (leader) peptide is removed from the mature antibody molecule and variations in its sequence will have no effect on antibody affinity. Many different leader peptide sequences are effective in targeting the immature antibody molecule to the endoplasmic reticulum. This second approach is the preferred embodiment in this disclosure.

The term transferrin receptor is intended to encompass the entire receptor or portions thereof. Portions of the transferrin receptor include those portions sufficient for binding of the receptor to an anti-transferrin receptor antibody to occur.

Monoclonal antibodies reactive with at least a portion of the transferrin receptor can be obtained (e.g., OX-26, B3/25 (Omary et al. (1980) Nature 286,888-891), T56/14 (Gatter et al. (1983) J. Clin. Path. 36 539-545; Jefferies et al. Immunology (1985) 54:333-341), OKT-9 (Sutherland et al. (1981) Proc. Natl. Acad. Sci. USA 78:4515-4519), L5.1 (Rovera, C. (1982) Blood 59:671-678), 5E-9 (Haynes et al.(1981) J. Immunol. 127:347-351), RI7 217 (Trowbridge et al. Proc. Natl. Acad. Sci. USA 78:3039 (1981) and T58/30

(Omary et al. cited supra) or can be produced using conventional somatic cell hybridization techniques (Kohler and Milstein (1975) Nature 256, 495-497). A crude or purified protein or peptide comprising at least a portion of the transferrin receptor can be used as the immunogen. An animal is vaccinated with the immunogen to obtain an anti-transferrin receptor antibody-producing spleen cells. The species of animal immunized will vary depending on the species of monoclonal antibody desired. The antibody producing cell is fused with an immortalizing cell (e.g. myeloma cell) to create a hybridoma capable of secreting anti-transferrin receptor antibodies. The unfused residual antibody-producing cells and immortalizing cells are eliminated. Hybridomas producing the anti-transferrin receptor antibodies are selected using conventional techniques and the selected anti-tranferrin receptor antibody producing hybridomas are cloned and cultured.

Polyclonal antibodies can be prepared by immunizing an animal with a crude or purified protein or peptide comprising at least a portion of a transferrin receptor. The animal is maintained under conditions whereby antibodies reactive with a transferrin receptor are produced. Blood is collected from the animal upon reaching a desired titer of antibodies. The serum containing the polyclonal antibodies (antisera) is separated from the other blood components. The polyclonal antibody-containing serum can optionally be further

separated into fractions of particular types of antibodies (e.g. IgG, IgM).

The neuropharmaceutical agent can be linked to the antibody using standard chemical conjugation techniques. Generally, the link is made via an amine or a sulfhydryl group. The link can be a cleavable link or non-cleavable link depending upon whether the neuropharmaceutical agent is more effective when released in its native form or whether the pharmaceutical activity of the agent can be maintained while linked to the antibody. The determination of whether to use a cleavable or non-cleavable linker can be made without undue experimentation by measuring the activity of the drug in both native and linked forms or for some drugs can be determined based on known activities of the drug in both the native and linked form.

For some cases involving the delivery of proteins or peptides to the brain, release of the free protein or peptide may not be necessary if the biologically active portion of the protein or peptide is uneffected by the link. As a result, antibody-protein or antibody peptide conjugates can be constructed using noncleavable linkers. Examples of such proteins or peptides include CD4, superoxide dismutase, interferon, nerve growth factor, tricosanthin, dopamine decarboxylase, somatostatin analogues and enkephalinase inhibitors. Terms such as "CD4" are used herein to include modified versions of the natural molecule, such as soluble CD4, truncated CD4, etc. Examples of non-cleavable linker

systems which can be used in this invention include the carbodiimide (EDC), the sulfhydryl-maleimide, the N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP; Pharmacia), and the periodate systems. In the carbodiimide system, a water soluble carbodiimide reacts with carboxylic acid groups on proteins and activates the carboxyl group. The carboxyl group is coupled to an amino group of the second protein. The result of this reaction is a noncleavable amide bond between two proteins.

In the sulfhydryl-maleimide system, a sulfhydryl group is introduced onto an amine group of one of the proteins using a compound such as Traut's reagent. The other protein is reacted with an NHS ester (such as gamma-maleimidobutyric acid NHS ester (GMBS)) to form a maleimide derivative that is reactive with sulfhydryl groups. The two modified proteins are then reacted to form a covalent linkage that is noncleavable.

SPDP is a heterobifunctional crosslinking reagent that introduces thiol-reactive groups into either the monoclonal antibody or the neuropharmaceutical agent. The thiol-reactive group reacts with a free sulfhydryl group forming a disulfide bond.

Periodate coupling requires the presence of oligosaccharide groups on either the carrier or the protein to be delivered. If these groups are available on the protein to be delivered (as in the case of horseradish peroxidase (HRP)), an active aldehyde is formed on the protein to be delivered which can react with an amino group on the carrier.

It is also possible to form active aldehyde groups from the carbohydrate groups present on antibody molecules. These groups can then be reacted with amino groups on the protein to be delivered generating a stable conjugate. Alternatively, the periodate oxidized antibody can be reacted with a hydrazide derivative of a protein to be delivered which will also yield a stable conjugate.

Cleavable linkers can be used to link neuropharmaceutical agents which are to be deposited in the brain or when a non-cleavable linker alters the activity of a neuropharmaceutical agent. Examples of cleavable linkers include the acid labile linkers described in copending patent application Serial No. 07/308,960 filed February 6, 1989, the contents of which are hereby incorporated by reference. Acid labile linkers include cis-aconitic acid, cis-carboxylic alkadienes, cis-carboxylic alkatrienes, and poly-maleic anhydrides. Other cleavable linkers are linkers capable of attaching to primary alcohol groups. Examples of neuropharmaceutical agents which can be linked via a cleavable link include AZT, ddI, ddc, adriamycin, amphotericin B, pyrimethamine, valproate, methotrexate, cyclophosphamide, carboplatin and superoxide dimutase. The noncleavable linkers used generally to link proteins to the antibody can also be used to link other neuropharmaceutical agents to the antibody.

The antibody-neuropharmaceutical agent conjugates can be administered orally, by

subcutaneous or other injection, intravenously, intramuscularly, parenternally, transdermally, nasally or rectally. The form in which the conjugate is administered (e.g., capsule, tablet, solution, emulsion) will depend at least in part on the route by which it is administered.

A therapeutically effective amount of an antibody-neuropharmaceutical agent conjugate is that amount necessary to significantly reduce or eliminate symptoms associated with a particular neurological disorder. The therapeutically effective amount will be determined on an individual basis and will be based, at least in part, on consideration of the individuals's size, the severity of symptoms to be treated, the result sought, the specific antibody, etc. Thus, the therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

Although the description above focuses on antibodies, any protein which interacts with the extracellular domain of the transferrin receptor, including the ligand binding site, could potentially serve as a vehicle for the delivery of drugs across the blood-brain barrier. In addition to anti-transferrin receptor antibodies, this would include transferrin, the ligand which binds to the receptor, and any transferrin derivatives which retain receptor-binding activity. In fact, any ligand which binds to the transferrin receptors could potentially be employed.

A procedure for producing chimeric antibodies reactive with a transferrin receptor may be performed as follows: cDNA is synthesized from mRNA purified from a small number of cells producing the antibody of interest. A PCR reaction is performed in order to obtain the antibody heavy and light chain variable regions which are then cloned and sequenced. After a second PCR reaction to modify the ends of these regions to make them compatible with the expression cassettes, they are cloned into novel expression vectors which contain human constant regions, immunoglobulin promoter and enhancers, and selection markers. In these vectors, a murine heavy chain promoter has been provided with restriction sites so that the leader sequences primed and expanded can be directly cloned into a functional promoter. Restriction sites have also been provided for the direct cloning of the 3′ end of the variable region into a constant region. In the heavy chain vector, a novel restriction site has been engineered into the CH1 domain of the human $\gamma 1$ heavy chain gene. VH can then be joined at this site to provide a complete heavy chain protein. For VL, a restriction site has been engineered just 3′ of the splice site so that the cloned VL will then splice the kappa to produce a complete κ light chain protein. The final constructs are then transfected into non-producer hybridoma cell lines as SP2/0 or P3.X63.Ag8653 and the supernatants tested for antibody production (Figure 8).

Further procedures and materials, such as expression cassettes, for producing chimeric

antibodies reactive with a transferrin receptor can be found in the patent application: Attorney Docket No. 38996, filed on the same date as the present application. Such teachings of this co-filed application are herein incorporated by reference.

The present invention will be illustrated by the following examples.

EXAMPLE 1- In Vitro Binding of Murine Monoclonal Antibodies to Human Brain Endothelial Cells

Two murine monoclonal antibodies, B3/25 and T58/30, described by Trowbridge (U.S. Patent 4,434,156 issued February 28, 1984, and Nature Vol. 294, pp. 171-173 (1981)), the contents of both are hereby incorporated by reference, which recognize the human transferrin receptor were tested for their ability to bind to human brain capillary endothelial cells. Hybridoma cell lines which produce B3/25 and T58/30 antibodies were obtained from the American Type Culture Collection (ATTC) in Rockville, Maryland, and grown in DMEM medium supplemented with 2.0 mM glutamine, 10.0 mM HEPES (pH 7.2), 100 μM non-essential amino acids and 10% heat-inactivated fetal calf serum. The hybridoma cultures were scaled-up in 225 cm^2 T-flasks for the production of milligram quantities of IgG antibody. The hybridoma supernatants were concentrated 50x using vacuum dialysis and applied to a protein-A sepharose column using the BioRad MAPS buffer system. Purified antibody was eluted from the column, dialyzed against 0.1 M sodium

phosphate (pH 8.0), concentrated and stored in aliquots at -20°C.

Primary cultures of human brain endothelial cells were grown in flat-bottom 96-well plates until five days post-confluency. The cells were then fixed using 3.0% buffered formalin and the plate blocked with 1.0% bovine serum albumin (BSA) in Dulbecco's phosphate buffered saline (DPBS). Aliquots (100 μl) of the B3/25 or T58/30 antibodies, either in the form of culture supernatants or purified protein, were then added to the wells (antibody concentrations were in the range of 1-50 μg/ml). Antibody which had specifically bound to the fixed cells was detected using a biotin-labeled polyclonal goat-anti-mouse IgG antisera followed by a biotinylated horseradish peroxidase (HRP)/avidin mixture (Avidin Biotin Complex technique). Positive wells were determined using a Titertek Multiscan Enzyme Linked Immunosorbent Assay (ELISA) plate reader. The results showed that both antibodies bind to human brain capillary endothelial cells with the T58/30 antibody exhibiting a higher level of binding.

These same antibodies were also tested for binding to human brain capillaries using sections of human brain tissue that were fresh frozen (without fixation), sectioned on a cryostat (section thickness was 5-20 μm), placed on glass slides and fixed in acetone (10 minutes at room temperature). These sections were then stored at -20°C prior to use.

The slides containing the human brain sections were allowed to come to room temperature prior to

use. The sections were then rehydrated in DPBS and incubated in methanol containing 0.3% H_2O_2 to block endogenous peroxidate activity. The sections were blocked for fifteen minutes in a solution containing 0.2% non-fat dry milk and 0.2% methylmannopyranoside. B3/25 and T58/30 antibodies, purified as discussed above, were applied to the sections at a concentration of 5-50 μg/ml and incubated at room temperature for one to two hours. Antibody that specifically bound to the tissue was detected using the Avidin-Biotin Complex (ABC) technique as described above for the ELISA assay. Staining of capillaries in the human brain sections was observed with both the B3/25 and T58/30 antibodies. The T58/30 antibody also displayed some binding to the white matter of the brain cortex.

EXAMPLE 2- In-Vitro Binding of Murine Monoclonal Antibody OX-26 to Rat Transferrin Receptor

The OX-26 murine antibody, which recognizes the rat transferrin receptor, has been shown in vivo to bind to brain capillary endothelial cells (Jeffries et al., cited supra). The murine hybridoma line which produces the OX-26 murine antibody was obtained and the hybridoma cell line was grown in RPMI 1640 medium supplemented with 2.0 mM glutamine and 10% heat-inactivated fetal calf serum. The OX-26 antibody was purified using the affinity chromatography technique described in Example 1.

The purified antibody was tested in vitro as described for the anti-human transferrin receptor antibodies in Example 1 to determine whether it would bind to brain capillaries in fresh frozen, acetone-fixed rat brain sections. The results showed that the OX-26 anti-transferrin receptor antibody did bind to capillaries in rat brain sections in vitro.

EXAMPLE 3- In-Vivo Binding of OX-26 Murine Monoclonal Antibody to Rat Transferrin Receptor

Dose Range

The rat anti-transferrin receptor antibody OX-26 was tested in vivo by injecting purified antibody (purification as described in Example 1) into female Sprague-Dawley rats (100-150 gm) via the tail vein. Prior to injection, the rats were anesthetized with halothane. The samples, ranging from 2.0 mg to 0.05 mg of antibody/rat were injected into the tail vein in 400 μl aliquots. All doses were tested in duplicate animals. One hour post-injection, the animals were sacrificed and perfused through the heart with DPBS to clear the blood from the organs. Immediately after the perfusion was completed, the brain was removed and quick frozen in liquid nitrogen. The frozen brain was then sectioned (30-50 μm) on a cryostat and the sections placed on glass microscope slides. The brain sections were air dried at room temperature one to two hours before fixation in acetone (10 minutes at room temperature). After this treatment the sections could be stored at -20°C.

The OX-26 antibody was localized in the brain sections using immunohistochemistry as described above for the in vitro experiments in Example 1. The addition of the primary antibody was unnecessary in that it is present in the brain sections. The results indicated that the OX-26 antibody binds to rat brain capillary endothelial cells and that doses of as little as 50 μg result in detectable levels of antibody in the brain using the methods described herein. Doses above 0.5 mg did not appear to show significantly more antibody binding to the endothelial cells, suggesting that the sites for antibody binding may be saturated. No specific binding to capillary endothelium was detected in the liver, kidney, heart, spleen or lung.

A non-specific antibody of the same subclass as OX-26 (IgG 2a) was also tested in vivo to show that the binding of OX-26 to rat brain endothelial cells that has been observed is specific to the OX-26 antibody. 0.5 mg of the control antibody was injected per rat as described above. The results indicate that the staining pattern observed with the OX-26 antibody is specific to that antibody.

Time Course

After establishing that the OX-26 antibody is detectable in the rat brain capillaries after in vivo administration, the time frame in which this binding occurred was determined. Using 0.5 mg of purified OX-26 antibody as the standard dose, brain sections taken from animals sacrificed 5 minutes, 15 minutes,

1 hour, 2 hours, 4 hours, 8 hours and 24 hours post-injection were examined for the presence of OX-26 antibody. All doses were administered in 400 μl aliquots and each time point was tested in duplicate animals. Samples were injected and the rats were processed at the various times post-injection as described above in the dose range section.

The results showed that the OX-26 antibody can be detected in or on the rat brain capillary endothelial cells as early as five minutes and as late as 24 hours post-injection. At 4 and 8 hours post-injection, the staining pattern of the antibody is very punctate suggesting that the antibody has accumulated in vesicular compartments either in endothelial or perivascular cells.

EXAMPLE 4- The Use of a Conjugate of OX-26 Murine Monoclonal Antibody for Tranferring Horseradish Peroxidase Across the Blood Brain Barrier

Horseradish Peroxidase (HRP; 40 kD) was chosen as a compound to be delivered to the brain because it is similar in size to several therapeutic agents and it can be easily detected in the brain using an enzymatic assay. HRP was conjugated to the OX-26 antibody using a non-cleavable periodate linkage and the ability of the antibody to function as a carrier of compounds to the brain was examined. The antibody conjugate was tested in vivo to determine if the antibody could deliver HRP to the brain.

The antibody (10 mg) was first dialyzed overnight against 0.01 M sodium bicarbonate (pH 9.0). The HRP (10 mg) was dissolved in 2.5 ml deionized water, 0.1 M sodium periodate (160 μl) was added and the mixture was incubated for five minutes at room temperature. Ethylene glycol (250 μl) was added to the HRP solution followed by an additional five minute incubation. This solution was then dialyzed overnight against 1.0 mM sodium acetate buffer (pH 4.4). To the dialyzed OX-26 antibody (2.0 ml, 5.08 mg/ml) was added 200 μl of 1.0 M sodium bicarbonate buffer, pH 9.5 and 1.25 ml of the dialyzed HRP solution. This mixture was incubated in the dark for two hours followed by the addition of 100 μl of 10 mg/ml sodium borohydride. The resulting mixture was incubated two additional hours in the dark at 4°C. The protein was precipitated from the solution by the addition of an equal volume of saturated ammonium sulfate and resuspended in a minimal volume of water. Free antibody was removed from the mixture by chromatography on a concanavalin A-sepharose column (a column which binds HRP and the HRP-antibody conjugate and allows the free antibody to pass through). The free HRP was removed by chromatography on a protein A-sepharose column which retains the antibody-HRP conjugate. The final product had an HRP/antibody ratio of 4/1.

A time course experiment identical to that described in Example 3 was performed using the antibody-HRP conjugate. The antibody-HRP conjugate (0.5 mg) was injected in a 400 μl aliquot/rat. The

animals were sacrificed at the various times post-injection and the brains processed as described above in Example 3. The antibody HRP conjugate was localized in the brain either by staining for antibody immunohistochemically as described in Example 1 or by directly staining the brain sections for the presence of HRP. To detect HRP, the slides were first allowed to come to room temperature before incubating in methanol for thirty minutes. The brain sections were then washed in DPBS and reacted with 3,3'-diamino benzidine (DAB), the substrate for HRP. The results showed that the OX-26 antibody HRP conjugate binds to rat brain capillary endothelial cells in a manner identical to that of the unconjugated antibody. The punctate staining 4-8 hours after injection which was seen with the antibody alone is also seen with the antibody conjugate, suggesting that the conjugate can also be going into the pericytes on the abluminal side of the blood brain barrier. Taken together, these results indicate that the OX-26 antibody can deliver a protein molecule of at least 40 KD to the brain.

EXAMPLE 5- The In-Vivo Delivery of Adriamycin to the Brain by Murine Monoclonal Antibody OX-26

A non-cleavable linker system similar to that used in Example 4, was used to couple the chemotherapeutic drug adriamycin to the OX-26 antibody. The availability of antibodies that can detect adriamycin as well as the system previously

described in Example 1 for detecting the antibody carrier allowed the use of immunohistochemical techniques for monitoring the localization of the antibody carrier as well as the delivery of adriamycin to the brain.

To conjugate adriamycin to the antibody, the drug (10 mg in 0.5 ml DPBS) was oxidized by the addition of 200 μl of 0.1 M sodium periodate. This mixture was incubated for one hour at room temperature in the dark. The reaction was quenched by the addition of 200 μl of ethylene glycol followed by a five minute incubation. The OX-26 antibody (5.0 mg in 0.5 ml of carbonate buffer (pH 9.5)) was added to the oxidized adriamycin and incubated at room temperature for one hour. Sodium borohydride (100 μl of 10 mg/ml) was added and the mixture was incubated for an additional two hours at room temperature. The free adriamycin was separated from the OX-26 antibody-adriamycin conjugate by chromatography on a PD-10 column. The adriamycin/OX-26 antibody ratio within the conjugate was 2/1. for this particular batch of conjugate.

The effectiveness of the OX-26 antibody as a carrier for delivering adriamycin to the brain was determined by administering 0.5 mg of the antibody-adriamycin conjugate in a 400 μl aliquot per rat by injection via the tail vein. One hour post-injection, the rat was sacrificed and the brain processed as described in Example 1. All injections were performed in duplicate. As a control, 400 μg of free adriamycin in a 400 μl aliquot was also injected

into a rat. Immunohistochemistry was used to detect both the carrier OX-26 antibody and the adriamycin in the rat brain sections. In the case of adriamycin, polyclonal rabbit anti-adriamycin antisera was applied to the sections followed by a biotinylated goat anti-rabbit IgG antisera. This was then followed by the addition of a biotinylated HRP/avidin mixture and enzymatic detection of HRP.

The results indicate that both the OX-26 antibody and the conjugated adriamycin localized to the rat brain capillary endothelial cells after in vivo administration. There is no evidence that free adriamycin binds to brain capillary endothelial cells or enters the brain.

An adriamycin-OX-26 conjugate coupled via a carbodiimide linkage was also synthesized (drug/ antibody ratio of 10/1) and tested in vivo. The results of this experiment were essentially identical to that obtained with the periodate-linked antibody-drug conjugate. In both cases, staining for the antibody carrier was quite strong and was visualized in the capillaries in all areas of the brain. This staining was evenly distributed along the capillaries. Staining for adriamycin was less intense but again was seen in capillaries throughout the brain. Some punctate staining was observed which suggests accumulation in pericytes which lie on the brain side of the blood-brain barrier.

EXAMPLE 6- In Vivo Delivery of Methotrexate to the Brain by Murine Monoclonal Antibody OX-26.

A noncleavable carbodiimide linkage was used to couple methotrexate to the OX-26 murine monoclonal antibody. A system analogous to that described in Example 5 was used to monitor the delivery of both the methotrexate and the carrier antibody to the brain capillary endothelial cells.

Methotrexate was coupled to murine monoclonal antibody OX-26 via its active ester. Briefly, 81 mg (0.178 mM) of methotrexate (Aldrich) was stirred with 21 mg (0.182 mM) of N-hydroxysuccinimide (Aldrich) in 3 ml of dimethylformamide (DMF) at 4°C. Ethyl-3-dimethylaminopropyl-carbodiimide (180 mg;EDC;0.52mM) was added to this solution and the reaction mixture was stirred overnight. The crude ester was purified from the reaction by-products by flash chromatography over silica gel 60 (Merck) using a solution of 10% methanol in chloroform as an eluant. The purified active ester fractions were pooled and concentrated to dryness. The ester was dissolved in 1 ml of DMF and stored at -20°C until use. 50 mg (50%) of active ester was recovered as determined by $A_{372}(\epsilon_{372}=7200)$.

A solution of OX-26 containing 2.1 mg (14 nmoles) of antibody in 0.9 ml of 0.1 M phosphate (pH 8.0) was thawed to 4 °C. To this stirred antibody solution was added 1.4 μL (140 nmoles) of the active ester prepared as described above. After 16 hours at 4°C, the mixture was chromatographed over Sephadex PD-10 column (Pharmacia) using phosphate buffered saline (PBS) to separate conjugate from free drug. The fractions containing the antibody-methotrexate

conjugate were pooled. Antibody and drug concentration were determined spectrophotometrically as described by Endo et al. (Cancer Research (1988) 48:3330-3335). The final conjugate contained 7 methotrexates/antibody.

The ability of the OX-26 monoclonal antibody to deliver methotrexate to the rat brain capillary endothelial cells was tested in vivo by injecting 0.2 mg of conjugate (in 400 μl) into each of two rats via the tail vein. The animals were sacrificed one hour post-injection and the brains processed for immunohistochemistry as described in Example 1. To detect methotrexate in the brain, a rabbit antisera raised against methotrexate was used as the primary antibody. A biotinylated goat-anti-rabbit antisera in conjunction with a mixture of biotinylated HRP and avidin was then used to visualize methotrexate in the rat brain. The carrier antibody was detected as described previously.

The results of these experiments indicate that methotrexate in the form of a conjugate with OX-26 does accumulate along or in the capillary endothelial cells of the brain. The staining observed for methotrexate is comparable in intensity to that seen for the carrier. The staining appears to be in all areas of the brain and is evenly distributed along the capillaries.

EXAMPLE 7- Antibody Derivatives

The Fc portion of the OX-26 murine monoclonal antibody was removed to determine whether this would alter its localization to or uptake by the rat brain capillary endothelial cells. $F(ab)_2$ fragments of OX-26 were produced from intact IgG's via digestion with pepsin. A kit available from Pierce Chemical Co. contains the reagents and protocols for cleaving the antibody to obtain the fragments . The $F(ab')_2$ fragment (0.2 mg doses) in 400 μl aliquots were injected into rats via the tail vein. A time course experiment identical to that done with the intact antibody (Example 2) was then performed. $F(ab')_2$ fragment was detected immunohistochemically using a goat anti-mouse $F(ab')_2$ antisera followed by a biotinylated rabbit anti-goat IgG antisera. A biotinylated HRP/avidin mixture was added and the antibody complex was visualized using an HRP enzymatic assay. The results indicate that the $F(ab)_2$ fragment of the OX-26 antibody binds to the capillary endothelial cells of the rat brain.

EXAMPLE 8 - Measurement of OX-26 in Brain Tissue

To quantitate the amount of OX-26 which accumulates in the brain, radioactively-labelled antibody was injected into rats via the tail vein. Antibodies were labelled with either ^{14}C-acetic anhydride or ^{3}H-succinimidyl proprionate essentially as described in Kummer, U., Methods in Enzymology, 121: 670-678 (1986), Mondelaro, R.C., and Rueckert, R.R., J. of Biological Chemistry, 250: 1413-1421

(1975), hereby incorporated by reference. For all experiments, the radiolabelled compounds were injected as a 400 μl bolus into the tail vein of female Sprague-Dawley rats (100-125 gms) under Halothane anesthesia and the animals were sacrificed at the appropriate time post-injection using a lethal dose of anesthetic. A ^{3}H-labelled IgC2a control antibody was co-injected with the ^{14}C-labelled OX-26 to serve as a control for non-specific radioactivity in the brain due to residual blood. At the appropriate time post-injection, animals were sacrificed and the brains were removed immedicately and homogenized in 5 ml of 0.5% sodium dodecysulfate using an Omni-mixer. An aliquot of the homogenate was incubated overnight with 2 ml of Soluene 350 tissue solubilizer prior to liquid scintillation counting. All data were collected as disintegrations per minute (dpm). Blood samples were centrifuged to pellet red blood cells (which do not display significant binding of radiolabelled materials) and the radioactivity in an aliquot of serum determined using liquid scintillation counting.

The amount of antibody associated with the brain was determined at various times post-injection to examine the pharmacokinetics of brain uptake. In addition, the amount of labelled antibody in the blood was measured so that the rate of clearance from the bloodstream could be determined. This information was also used to calculate the amount of radioactivity in the brain due to blood contamination, which was then subtracted from the

total to give the amount of antibody that is specifically associated with the brain.

A peak level of ^{14}C-labelled OX-26 corresponding to approximately 0.9% of the injected dose was reached in the brain between 1 and 4 hours post-injection as illustrated in Figure 1 (with the values shown as means plus or minus standard error of measurement (SEM) and N=3 rats per time point). The amount of radioactivity associated with the brain decreased steadily from 4 to 48 hours post-injection, at which point it leveled off at approximately 0.3% of the injected dose. The accumulation of OX-26 in the brain was significantly reduced by the addition of unlabelled monoclonal antibody (0.5 or 2.0 mg in the bolus injection). As an additional control, a ^{3}H-IgG2a control antibody was co-injected with the ^{14}C-OX-26. The control antibody did not accumulate in the brain and represented the blood contamination of the brain.

In contrast to the levels in the brain, the blood level of OX-26 dropped quite dramatically immediately after injection such that by 1 hour post-injection, the percent of injected dose in 55 μl of blood (the volume of blood associated with the brain) was approximately 0.16% as illustrated in Figure 1. This corresponds to a value of approximately 20% of the injected dose in the total blood volume of the rat. Extraction of total IgG from serum followed by polyacrylamide gel electrophoresis (PAGE) and autoradiography did not reveal detectable levels of OX-26 degradation indicating

that the antibody remains intact in the blood as long as 48 hours after injection.

EXAMPLE 9 - Distribution of OX-26 in Brain Parenchyma and Capillaries

To demonstrate that anti-transferrin receptor antibody accumulates in the brain parenchyma, homogenates of brains taken from animals injected with labelled OX-26 were depleted of capillaries by centrifugation through dextran to yield a brain tissue supernatant and a capillary pellet. Capillary depletion experiments followed the procedure of Triguero, et al., J. of Neurochemistry, 54: 1882-1888 (1990), hereby incorporated by reference. As for the brain uptake experiments of Example 8, the radiolabelled compounds were injected as a 400 μl bolus into the tail vein of femals Sprague-Dawley rats (100-125 gm) under Halothane anesthesia and the animals were sacrificed at the appropriate time post-injection using a lethal dose of anesthetic. A ^{3}H-labelled IgG 2a control antibody was co-injected with the ^{14}C-labelled OX-26 to serve as a control for non-specific radioactivity in the brain due to residual blood. After sacrifice, the brains were removed and kept on ice. After an initial mincing, the brains were homogenized by hand (8-10 strokes) in 3.5 ml of ice cold physiologic buffer (100 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 14.5 mM HEPES, 10 mM D-glucose, pH 7.4). Four ml of 26% dextran solution in buffer was added and

homogenization was continued (3 strokes). After removing an aliquot of the homogenate, the remainder was spun at 7200 rpm in a swinging bucket rotor. The resulting supernatant was carefully removed from the capillary pellet. The entire capillary pellet and aliquots of of the homogenate and supernatant were incubated overnight with 2 ml of Soluene 350 prior to liquid scintillation counting. This method removes greater than 90% of the vasculature from the brain homogenate (Triguero et al., cited supra).

A comparison of the relative amounts of radioactivity in the different brain fractions as a function of time indicates whether transcytosis of the labelled antibody has occurred. The amount of OX-26 in total brain homogenate, the brain parenchyma fraction and the brain capillary fraction at an early time (30 minutes) and a later time (24 hours) post-injection is illustrated in Figure 2. The values in Figure 2 are shown as means±SEM with N=3 rats per time point. At the 30 minute time point, more of the radiolabelled antibody is associated with the capillary fraction than with the brain parenchyma fraction (0.36% of the injected dose (%ID) and 0.23% ID, respectively). By 24 hours post-injection, the distribution is reversed and the majority of the radioactivity (0.36% ID) is in the parenchymal fraction as compared to the capillary fraction (0.12% ID). The redistribution of the radiolabelled OX-26 from the capillary fraction to the parenchyma fraction is consistent with the time dependent

migration of the anti-transferrin receptor antibody across the blood-brain barrier.

EXAMPLE 10 - Distribution of an OX-26-methotrexate Conjugate in Brain Parenchyma and Capillaries

Capillary depletion studies following the procedures described in Example 9 were performed with an OX-26-methotrexate (MTX) conjugate linked via a gamma-hydrazid as described in Kralovec, et al., J. of Medicinal Chem., 32: 2426-2431 (1989), hereby incorporated by reference, in which the MTX moiety was labelled with ^{3}H. As with unconjugated antibody, the amount of label in the capillary fraction at 30 minutes post-injection is greater than the parenchyma fraction (approximately 2-fold as illustrated in Figure 3, with the data expressed as means±SEM and N=3 rats per time point). This distribution changes over time such that by 24 hours post-injection, approximately 4.5-fold more of the labelled MTX is in the brain parenchyma than in the capillaries. These results are consistent to those obtained with unconjugated antibody and, again, suggest that these compounds cross the blood-brain barrier.

To ensure that these results were not due to contaminating amounts of free ^{3}H-MTX or ^{3}H-MTX that had been cleaved from the conjugate after injection, a co-mix of labelled drug and antibody was injected into rats and a capillary depletion experiment performed. The amount of ^{3}H-MTX in the different brain fraction is significantly lower for the co-mix

as compared to the conjugate (as much as 47 fold in the case of the capillary fraction at 30 minutes post-injection as illustrated in Figure 3). The ^{3}H-MTX and the co-mix also does not show the change in distribution of the label between the different brain fractions over time as was seen with the antibody-MTX conjugate or antibody alone. These results demonstrate that delivery of ^{3}H-MTX across the blood-brain barrier to the brain parenchyma is greatly enhanced by the conjugation of the drug to the anti-transferrin receptor antibody OX-26.

EXAMPLE 11 - Distribution of OX-26-AZT in Brain Parenchyma and Capillaries

Capillary depletion studies following the procedures of Example 9 were performed with an OX-26-AZT conjugate using a pH-sensitive succinate linker. These studies employed a dual-labelled conjugate in which the AZT was ^{14}C-labelled and the antibody carrier was ^{3}H-labelled. The use of such a conjugate allowed independent monitoring of the disposition of both the antibody and AZT within the brain.

The linker was synthesized as follows. Succinic anhydride was used to acylate the AZT by reacting equimolar amounts of these two compounds for 3 hours at room temperature under argon in the presence of dimethylaminopyridine and sodium bisulfate in freshly distilled pyridine. The product was isolated by chromatography on a DEAE sephadex A50 column run with

a triethylammonium bicarbonate buffer. The succinate derivative of AZT was activated at the carboxyl group as the NHS ester by reaction with equimolar amounts of N-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in freshly distilled THF at 4°C for 2 hours. The product was purified by flash charomatography on silica gel. The resulting NHS-ester of AZT-succinate was used to acylate amine groups on OX-26, resulting in an AZT-OX-26 conjugate. A 15-fold molar excess of AZT-NHS ester was reacted with OX-26 in HEPES buffer overnight at 4°C. The antibody-drug conjugate was isolated from free drug on a PD-10 column. The molar ratio of drug to antibody was 7:1. These studies employed a dual-labelled conjugate in which the AZT was ^{14}C-labelled and the antibody carrier was ^{3}H-labelled.

Similar levels of OX-26 and AZT are seen in the capillary fraction of the brain and these levels decrease with time, suggesting that the materials are not being retained by the capillary endothelial cells as illustrated in Figure 4c. As the levels of OX-26 in the capillary fraction decrease, the levels in the parenchyma fraction increase, indicating that the antibody is migrating from the capillaries to the parenchyma in a time-dependent manner as illustrated in Figure 4b. In contrast, the levels of AZT in the brain parenchyma do not rise significantly, suggesting that the majority of the drug is released in the endothelial cells and is not transported across the blood-brain barrier. The levels of OX-26 and AZT remained similar in unfractionated

homogenates over time as illustrated in Figure 4a. The data in Figure 4 are expressed as means±SEM with N=3 rats per time point. These results indicate that the linker is cleaved within the endothelial cells and may represent a method for delivering compounds to those cells.

EXAMPLE 12 - Distribution of OX-26-Horseradish Peroxidase (HRP) in Brain Perenchyma and Capillaries

Capillary depletion studies following the procedures described for OX-26 in Example 9 were performed with a ^{3}H-labelled OX-26-HRP conjugate that was prepared using a non-cleavable periodate linkage as described in Example 4. The tritium label was distributed between the antibody and the HRP portion of the conjugate. At 1 hour post-injection, the majority of the radioactivity associated with the brain is in the capillary fraction as illustrated in Figure 5. The data in Figure 5 are expressed as means±SEM with N=3 rats per time point. By 4 hours post-injection, the distribution of radioactivity associated with the brain changed such that the majority is in the fraction which represents the brain parenchyma. At 24 hours post-injection, essentially all of the ^{3}H-labelled OX-26-HRP conjugate is in the parenchyma fraction of the brain indicating that the material has crossed the blood-brain barrier. Similar results were obtained in experiments in which only the HRP portion of the conjugate was radiolabelled.

The percent of injected dose of the OX-26-HRP conjugate that reaches the brain is somewhat lower than that for antibody alone or the OX-26-HRP conjugate. This is most likely due to the presence of 2 to 3 40 kD HRP molecules attached to each carrier and that these "passenger" molecules are randomly attached to the carrier. Due to this, many of the HRP passengers may be attached to the antibody in such a way as to interfere with antigen recognition. This problem can be alleviated by directing the attachment of the passenger to regions of the carrier removed from critical functional domains.

EXAMPLE 13 - Distribution of OX-26-CD4 in Brain Parenchyma and Capillaries

A soluble form of CD4, consisting of amino acids 1-368, was conjugated to OX-26 using a linkage that directed the attachment of the CD4 to the carbohydrate groups located in the Fc portion of the antibody. By directing the site of attachment in this way, the chance that the passenger molecules will interfere with antibody-antigen recongition is lessened. The linkage between the proteins was achieved by first introducing a sulfhydryl group onto CD4 using SATA (N-Succinimidyl S-acetylthioacetate), a commerically available compound. A hydrizid derivative of SDPD, another commercial cross-linking agent, was attached to OX-26 via carbohydrate groups

on the antibody. Reaction of the two modified proteins gives rise to a disulfide-linked conjugate.

More specificallyk the linkage between the proteins was achieved by first introducing a sulfhydryl group onto CD4 using N-succinimidyl S-acetylthioacetate (SATA), a commercially available compound. A 4-fold molar excess of SATA was added to 5 mg of CD4 in 0.1 M sodium phosphate buffer containing 3 mM EDTA (pH 7.5). This mixture was reacted at room temperature in the dark for 30 minutes. Unreacted starting materials were removed by passage over a PD-10 column. A hydrizid derivative of SPDP, another commercially available cross-linking agent, was attached to OX-26 via carbohydrate groups on the antibody. Ten milligrams of OX-26 in 2.0 ml of 0.1 M sodium acetate, 0.15 M sodium chloride (pH 5.0) was reacted with a 1000-fold molar excess of sodium periodate for 1 hour at 4°C in the dark. Unreacted starting materials were removed by passage over a PD-10 column. The oxidized antibody was reacted with a 30-fold molar excess of hydrazido-SPDP overnight at 4°C with stirring. Reaction of the two modified proteins gives rise to a disulfide-linked conjugate. One tenth volume of 0.5 M hydroxylamine was added to the thioacetylated CD4 (CD4-DATA) and derivatized antibody was then added such that the ratio of CD4 to antibody was 7.5:1. This mixture was reacted at room temperature in the dark for 2 hours. Conjugate was purified by running the reaction mixture over a protein A column followed by a CD4 affinity column.

Capillary depletion experiments following the procedures described in Example 9 with OX-26 were performed with an OX-26-CD4 conjugate in which only the CD4 portion was ^{3}H-labelled. Time dependent changes in the distribution of the labelled conjugate between the capillary and parenchyma fractions of the brain which are consistent with transcytosis across the blood-brain barrier were observed as illustrated in Figure 6. The data in Figure 6 are expressed as means±SEM with N=3 rats per time point.

EXAMPLE 14 - Biodistribution and Brain Uptake of Anti-Human Transferrin Receptor Antibodies in Cynomolgous Monkeys

A collection of 32 murine monoclonal antibodies which recognize various epitopes on the human transferrin receptor were examined for reactivity with brain capillary endothelial cells in sections from human, monkey (cynomolgous), rat and rabbit brain samples by the immunohistochemical methods described in Example 1. These antibodies were obtained from Dr. Ian Trowbridge of the Salk Institute, LaJolla, CA. All 32 antibodies displayed some reactivity with human brain endothelial cells. Two antibodies reacted very weakly with rabbit brain capillaries and none reacted with rat. While 21 of the antibodies reacted with monkey brain capillaries, only 2 displayed strong reactivity comparable to that seen with human brain capillaries. These 2

antibodies are herewithin referred to as 128.1 and Z35.2.

These antibodies were used to determine the tissue distribution and blood clearance of the ^{14}C-labelled anti-human transferrin receptor antibodies 128.1 and Z35.2 in 2 male cynomolgous monkeys. 128.1 or Z35.2 was administered concurrently with a ^{3}H-labelled control IgG to one of the monkeys with an intravenous catheter. During the course of the study, blood samples were collected to determine the clearance of the antibodies from the circulation. At 24 hours post-injection, the animals were euthanized and selected organs and representative tissues were collected for the determination of isotope distribution and clearance by combustion. In addition, samples from different regions of the brain were processed as described for the capillary depletion experiments in Example 9 to determine whether the antibodies had crossed the blood-brain barrier. The results of the capillary depletion experiments were performed on samples from the cortex, frontal cortex, cerebellum and striatum. All samples had greater than 90% of the 128.1 or Z35.2 in the brain parenchyma, suggesting that the antibodies crossed the blood-brain barrier. The levels of the control antibody in the same samples were from 5 to 10-fold lower. Using the average brain homogenate value for dpm/G tissue, the percent injected dose of 128.1 in the whole brain is approximately 0.2-0.3%. This compares to a value of 0.3-0.5% for OX-26 in the rat at 24 hours post-injection. A comparison of the

ratios of 128.1 to the control antibody for various organs is illustrated in Figure 7. Similar results were obtained for Z35.2. These results suggest that 128.1 is preferentially taken up by the brain as compared to control antibody. For the majority of organs and tissues tested, the ratio of 128.1 to control is less than 2.

EXAMPLE 15 - Cloning and Expressing of ALK 128.1: An Anti-Human Transferrin Receptor Chimeric Antibody

RNA EXTRACTION:

RNA was extracted following the single step guanidinium/phenol method (P.Chomczynski and S. Sacchi. 1987, Anal. Bioch. 162:156-259). All the instruments and containers used were previously autoclaved and rinsed with diethyl pyrocarbonate (depc) treated water to avoid degradation due to RNAases. Several samples each containing $5x10^5$ cells from the 128.1 hybridoma which secretes a murine anti human transferrin receptor monoclonal antibody, were washed twice with PBS. The pellets were quick frozen in liquid nitrogen and either kept at -70°C for later use or extracted immediately.

For the extraction, in a RNase free microfuge tube, 1/2 ml of solution D (Solution D:36 μl 2-mercaptoethanol per 5 ml of 1X GITC [1x GITC: 250 g guanidinium thiocyanate, 17.6 ml 0.75 M Na citrate pH7, 26.4 ml 10% sarcosyl, 293 ml dH2O]), 50 μl of 2M Na acetate pH 4, 0.5 ml phenol (dH2O equilibrated)

and 100 μl of chloroform:isoamylalcohol (49:1) were added to the cell pellet mixing by inversion after each addition. The extraction was left on ice for 15 minutes and centrifuged at 13000g for 20 min at 4°C.

The upper aqueous phase containing the RNA was removed to a new tube and precipitated with 2 volumes of cold absolute ethanol for 2 hr. at -70°C. After two 70% depc-ethanol washes the RNA pellet was dried briefly and resuspended in dH2O 0.5% SDS.

FIRST STRAND cDNA SYNTHESIS

Total RNA from $5x10^5$ cells was resuspended in 18 μl of 0.5% SDS. 9μl of RNA were annealed with 2μl of 3′ primer (1mg/ml) at 60°C for 10 minutes. For light chain V region amplifications, an oligo dT primer was used, whereas for the amplification of heavy chain V regions a γ CH1 antisense primer, containing an XbaI site (underlined in Table 1), with degeneracies introduced so that it will prime all isotypes of murine heavy chains except γ3 was used (Table 1).

After annealing, the samples were cooled on ice, 4 μl of first strand cDNA buffer (50 mM Tris pH 8.3, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 0.5 mM spermidine), 1 μl of RNAse inhibitor (Promega), 2 μl of 10 mM dNTP's and 2 μl of prediluted 1:10 Promega AMV Reverse Transcriptase were added and the reaction incubated for 1 hour at 42°C. The cDNA was kept at -20°C until used for PCR.

Table 1: PRIMERS FOR cDNA SYNTHESIS

PRIMER FOR SYNTHESIS OF LIGHT CHAIN V REGION cDNA

OLIGO dT.R1.XBA.H3

5' GCCGGAATTCTAGAAGC$(T)_{17}$

PRIMER FOR SYNTHESIS OF HEAVY CHAIN V REGION cDNA

MγC.CHI AS (Degeneracies at a single position are shown in parenthesis.)

5' AGG TCTAGA A(CT)C TCC ACA CAC AGG (AG)(AG)C CAG TGG ATA GAC

PRIMERS AND PCR REACTION:

A first PCR reaction was performed in order to amplify the variable regions and determine their sequence. To achieve this the PCR primers were designed to hybridize to the leader sequence (5' primer) and to the constant region immediately downstream of the V-J region (3' primer).

The oligonucleotides were synthesized in an Applied Biosystem 391 DNA Synthesizer, eluted without purification, diluted to 20 μM and kept at 4°C.

All primers were designed with a restriction site with three additional bases upstream to protect the site and facilitate enzyme digestion. The sites were chosen to make possible the cloning of the PCR product into a subcloning vector and into the final expression cassett vectors.

For the leader region, the primers contain a ribosome recognition site (Kozak's sequence CACC; Kozak M. 1981, Nucl. Acid. Res., 9:20, 5233-5252) 5' of the start codon, and an EcoR V site (underlined in Tables 2 and 3) protected by three 5' G's. A set of 4 universal 5' sense primers was used simultaneously in the light variable region amplification, and a set of 3 universal 5' sense primers in the case of heavy variable regions (Coloma et al. 1991, Biotechniques 11,2,152-156). An equimolar amount of each primer was used in the PCR reaction. These primers contain degeneracies in order to hybridize with all the families of murine leader sequences reported in Kabat's database. (Kabat E. 1987, Sequences of

Proteins of Immunological Interest, NIH). The 3' primers were designed in the constant region 20 bases downstream of the V-J region and contain an XbaI site (underlined in Tables 2 and 3) for subcloning purposes (Tables 2 and 3).

Table 2: PRIMERS FOR MURINE HEAVY CHAIN VARIABLE REGION AMPLIFICATION. (Degeneracies at a single position are shown in parenthesis.)

LEADER REGION PRIMERS (5'SENSE)

MHALT1.RV #085
Leader Murine Heavy IgV

5' GGG GATATC CACC ATG G(AG)A TG(CG) AGC TG(TG) GT(CA) AT(CG) CTC TT

MHALT2.RV #086
Leader Murine Heavy IgV

5' GGG GATATC CACC ATG (AG)AC TTC GGG (TC)TG AGC T(TG)G GTT TT

MHALT3.RV #087
Leader Murine Heavy IgV

5' GGG GATATC CACC ATG GCT GTC TTG GGG CTG CTC TTC T

CONSTANT REGION PRIMER (3'ANTISENSE)

Primer designed to hybridize at aminoacids 130-120 in CH1 of Igγ. This primer is identical to the primer used for heavy chain first strand cDNA synthesis.

MCγ CH1AS.XBA #097
CH1 antisense primer for murine Igγ, except Igγ3

5' AGG TCTAGA A(CT)C TCC ACA CAC AGG (AG)(AG)C CAG TGG ATA GAC

Table 3: PRIMERS FOR MURINE LIGHT CHAIN VARIABLE REGION AMPLIFICATION. (Degeneracies at a single position are shown in parenthesis.)

LEADER REGION PRIMERS (5'SENSE)

MLALT1.RV #088
Leader Murine Light IgV

5' GGG GATATC CACC ATG GAG ACA GAC ACA CTC CTG CTA T

MLALT2.RV #089
Leader Murine Light IgV

5' GGG GATATC CACC ATG GAT TTT CAA GTG CAG ATT TTC AG

MLALT3.RV #090
Leader Murine Light IgV

5' GGG GATATC CACC ATG GAG (TA)CA CA(GT) (TA)CT CAG GTC TTT (GA)TA

MLALT4.RV #091
Leader Murine Light IgV

5' GGG GATATC CACC ATG (GT)CC CC(AT) (GA)CT CAG (CT)T(CT) CT(TG) GT

CONSTANT REGION PRIMER (3'ANTISENSE)

Primer designed to hybridize to amino acids 122-116 of kappa constant region.

MCκ AS.XBA #096
Constant Murine Light

5' GCG TCTAGA ACT GGA TGG TGG GAA GAT GGA

The primers for the second PCR reaction (Table 4) have the actual sequence of the V-J regions, determined by sequencing of the subcloned products (Figure 9). These primers have a Nhe I site in the case of the VH primer and Sal I for the VL primer, which permits the cloning into the expression vectors. (The restriction enzyme sites are underlined in Table 4). The Nhe I site in the 3′ primer for the VH allows the direct ligation of the VH-J region to the first two amino acids of the CH1 of the $\gamma 1$ constant region. The VL 3′ primer has a donor splice sequence before its Sal I site which is necessary to splice the VL to C κ in the expression vector.

Table 4: PRIMERS FOR 128.1 V-J REGION MODIFICATION BY SECOND PCR PRIOR TO THE CLONING INTO EXPRESSION VECTORS

HEAVY CHAIN PRIMER (3'ANTISENSE):

Primer designed to hybridize to amino acids 111-113 in J4 region of 128.1 heavy chain V region. It includes a Nhe I site for cloning into the expression vector (links J4 to CHI) and Sal I for subcloning (upstream Nhe I).

ALKJ4 AS.NHE.SAL1 #098
Antisense of VHJ4 + γ1 CH1

5' TGG GTCGAC AGA TGG GGG TGT TGT GCTAGC TGA GGA GAC

LIGHT CHAIN PRIMER (3'ANTISENSE):

Primer designed to hybridize to amino acids 101-107 in J4 region of 128.1 light chain V region. It includes a donor splicing sequence which is highlighted.

ALKκ-J4AS.SAL1 #101
Antisense of VL J4 + splicing donor

5' AGC GTCGAC TTACG TCT GAT TTC CAG CCT GGT CCCT

PCR reactions were performed in a volume of 100 μl with the following final conditions: 2μl of cDNA, 0.5 μl Taq polymerase (Cetus Corporation), 1X buffer (10 mM Tris pH8, 1.5 mM $MgCl_2$, 50 mM KCl, 100 μg BSA), 200μM each dNTP, 1μM of each primer and 50 μl of mineral oil. PCR was carried out for 30 cycles in a PTC 100 Thermal Controller (M.J. Research Inc.) with 1 min. denaturing (94°C), 1 min. annealing (55°C), 1.5 min. extension (72°C), and a final extension of 10 min.

The size of the PCR products was verified by agarose gel electrophoresis in a 2% TAE gel stained with ethidium bromide. The correct products were approximately 380 base pairs for the light chain and 420 base pairs for the heavy chain variable region.

SUBCLONING AND SEQUENCING:

After the PCR reaction the oil was removed by chloroform extraction and the samples kept at 4°C. For subcloning, the products were either directly cloned into Bluescript KS T-A (blunt ended by digestion at EcoR V site and tailed with dideoxythymidine triphosphate using terminal transferase) prepared following the procedure by Holton (T.A. Holton and M.W. Graham. 1990 Nucl. Acid. Res., 19:5, 1156), or gel isolated, cut with the appropriate restriction enzymes (EcoR V and Sal I) and cloned into Bluescript KS previously cut with the same enzymes.

For TA cloning 3 μl of the PCR product was directly ligated with 50 ng of T-A vector in a 15 μl reaction for 4-12 hours at 16°C. For sticky end ligations 200 ng of cut Bluescript was ligated with 200-400 ng of cut product in 20 μl ligation reactions. 5 μl of the ligation was used for transformation of E. Coli. XL1-blue (Stratagene) competent cells prepared by calcium chloride treatment. White colonies, containing inserts were picked above a blue colony background. Miniprep DNA was restriction digested, analyzed and the apparently correct clones sequenced.

Dideoxynucleotide chain termination sequencing was carried out using T7 DNA polymerase (Pharmacia, Uppsala, Sweden or Sequenase, US Biochemical Corp., Cleveland, Ohio) according to the manufacturer's protocol. Four independent clones from different PCR reactions were sequenced in both directions, to obtain the concensus sequence.

The obtained sequences were compared against other murine sequences in Genbank and aligned with reported V regions in Kabat's database to identify their family and conserved amino acids. (See Tables 5 and 6.)

Table 5: COMPLETE SEQUENCE OF CHIMERIC 128.1 (Anti-Human Transferrin Receptor) LIGHT CHAIN VARIABLE REGION, MOUSE KAPPA SUBGROUP VI

```
                                    -22          LEADER
                                    ATG GAT TTT CAA GTG CAG ATT
                                    Met Asp Phe Gln Val Gln Ile

TTC AGC TTC CTG CTA ATC AGT GCC TCA GTC ATA CTG TCC AGA
Phe Ser Phe Leu Leu Ile Ser Ala Ser Val Ile Leu Ser Arg

 -1       1                             FR1
GGA --- CAA ATT GTT CTC ACC CAG TCT CCA GCA ATC ATG TCT
Gly --- Gln Ile VAL LEU Thr GLN SER PRO ALA ILE Met Ser

                  FR1                                    24   CDR1
GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT GCC AGC
ALA SER Pro GLY Glu LYS VAL THR Met THR CYS Ser ALA SER

 27-29   *  CDR1                35              FR2
TCA AGT ATA GAT TAC ATT CAC TGG TAC CAG CAG AAG TCA GGC
SER SER Ile Asp TYR Ile His TRP Tyr GLN GLN LYS Ser Gly

            FR2                          50     CDR2
ACC TCC CCC AAA AGA TGG ATT TAT GAC ACA TCC AAA CTG GCT
Thr SER PRO LYS Arg Trp ILE TYR Asp Thr SER Lys LEU Ala

     57                        FR3
TCT GGA GTC CCT GCT CGC TTC AGT GGC AGT GGG TCT GGG ACC
SER GLY VAL PRO Ala ARG PHE SER GLY SER GLY SER GLY Thr

                              FR3
TCT TAT TCT CTC ACA ATC AGC AGC ATG GAG CCT GAA GAT GCT
Ser Tyr Ser LEU Thr ILE Ser Ser Met GLU Pro GLU ASP Ala

                       89           CDR3                  97
GCC ACT TAT TAC TGC CAT CAG CGG AAT AGT TAC CCA TGG ACG
ALA THR TYR TYR CYS His GLN Arg Lys Ser Tyr Pro Trp THR

 98              FR4  *                107      CONST.
TTC GGT GGA GGG ACC AGG CTG GAA ATC AGA --> CGG GCT
PHE GLY GLY GLY THR Arg LEU GLU Ile ARG  --> ARG ALA
```

Conserved amino acids are capitalized and bold.
* NOTE: Amino acid # 30 is a conserved Val and amino acid # 103 and #107 a conserved Lys in 98% of the sequences reported in Kabat's database for this family.

Table 6: COMPLETE SEQUENCE OF CHIMERIC 128.1 (Anti-Human Transferrin Receptor) HEAVY CHAIN VARIABLE REGION. MOUSE GAMMA SUBGROUP IIB.

```
                                        -19        LEADER
                                       ATG GAA TGG AGC TGG GTA
                                       Met Glu Trp Ser Trp Val

                          LEADER                                -1
ATG CTC TTC CTC CTG TCA GGA ACT GCA GGT GTC CGC TCT ---
Met Leu Phe LEU Leu Ser Gly Thr Ala Gly Val Arg Ser ---

1                              FR1
GAG GTC CAG CTG CAA CAG TCT GGA CCT GAA CTG GTG AAG CCT
Glu VAL GLN LEU Gln GLN Ser GLY Pro Glu LEU VAL Lys PRO

             *18               FR1
GGA GCT TCA ATG AAG ATT TCC TGC AAG GCT TCT GGT TAC TCA
GLY Ala SER Met LYS Ile SER CYS LYS ALA SER GLY TYR Ser

          31      CDR1          36              FR2
TTC ACT GGC TAC ACC ATG AAC TGG GTG AAG CAG AGC CAT GGA
Phe Thr Gly Tyr Thr Met Asn TRP VAL Lys GLN Ser His Gly

            FR2                 50       52--a-  53    CDR2
GAG AAC CTT GAG TGG ATT GGA CGT ATT AAT CCT CAC AAT GGT
Glu Asn Leu Glu Trp Ile Gly Arg Ile Asn PRO His Asn Gly

                  CDR2                              66      *68
GGT ACT GAC TAC AAC CAG AAG TTC AAG GAC AAG GCC CCT TTA
Gly Thr Asp TYR Asn Gln LYS PHE Lys Asp LYS Ala Pro LEU

                               FR3                          82--a-
ACT GTA GAC AAG TCA TCC AAC ACA GCC TAC ATG GAG CTC CTC
THR Val Asp Lys SER Ser Asn THR Ala TYR Met Glu LEU Leu

82b-c-   83                  FR3
AGT CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA
Ser Leu THR SER GLU ASP Ser ALA Val TYR Tyr CYS Ala Arg

 95           CDR3   100--a-          103          FR4
GGC TAC TAT TAC TAT TCT TTG GAC TAC TGG GGT CAA GGA ACC
Gly Tyr Tyr Tyr Tyr Ser Leu Asp Tyr TRP GLY Gln GLY THR

            FR4       113       CH1
TCA GTC ACC GTC TCC TCA --> GCC AAA
Ser Val THR VAL SER Ser --> Ala Lys
```

Conserved amino acids are capitalized and bold. Amino acid # 18 is a conserved Val and amino acid # 68 a conserved Thr in 98% of the sequences reported in Kabat's database for this family.

The final clones were named pBKS4600 for the VH region and pBKS4601 for the VL region.

CLONING INTO EXPRESSION VECTORS:

Plasmid pAH4274 is the vector for expression of heavy chain variable regions obtained by PCR with leader/J region priming. V region cloning into this cassette is performed by a complete digestion of vector and product with EcoR V and Nhe I. This vector has a human $\gamma 1$ constant region whose CH1 is directly linked with the 3′ end of the VH-J region by means of a Nhe I site. This 11 kb vector contains an ampicillin resistance gene for procaryotic selection, a heavy chain immunoglobulin enhancer and a histidine (histidinol) selection marker for selection of transfectants (Hartman, S., R. Mulligan, Proc. Natl. Acad. Sci. 85, 8047-8051); transcription is from the VH promoter of the murine 27.44 gene.

The 400 bp. EcoR V-Nhe I fragment (VH of 128.1) from pBKS4600 was used to replace the EcoR V-Nhe I fragment in plasmid pAH 4274. HB101 competent cells were transformed and plated on LB plates with 50 μg/ml of ampicillin. Colonies were screened by colony hybridization with a ^{32}P end labelled leader region oligonucleotide. Positive clones were restriction mapped and maxi plasmid preps prepared using the QIAGEN maxi prep kit (QIAGEN Inc., Studio City, California). The final expression vector with the VH of 128.1 joined to human $\gamma 1$ constant region

was named pAH4602 (Figure 10). The coding sequence for this expression vector is given in Figure 11.

Plasmid pAG4270 is the expression vector for light chain variable regions obtained by PCR with leader/J region priming. The 14 kb vector has an ampicillin resistance gene, a gpt (mycophenolic acid resistance) selected marker, an immunoglobulin H enhancer and an introl for V-Constant region splicing; transcription is from the murine VH promoter from the 27.44 gene.

Due to the presence of an EcoR V within the gpt gene in the vector, the cloning of the anti-transferrin receptor VL was performed in two steps to avoid inefficient partial digestions. The 380 bp EcoR V - Sal I fragment (VL) from pBKS4601 was cloned into pBR460x (6.9 kb), a subcloning vector with the VH promoter, previously cut with the same enzymes. The resulting construct (pBR4608) was then cut with Pvu I - Sal I and the 4 kb fragment containing the promoter, the V region and part of the ampicillin resistance gene was ligated to the 9.7 kb Pvu I - Sal I fragment of pSV4271 an intermediate vector which lacks the promoter. HB101 competent cells were transformed and positives screened by colony hybridization and restriction digestion. Maxipreps were prepared as described above. The final expression vector was named pAG4611 (Figure 12). The coding sequence of this expression vector is shown in Figure 13.

TRANSFECTION AND SELECTION:

Ten μg of maxiprep DNA from each final expression vector was linearized by BSPC1 (Stratagene, Pvu I isochizomer) digestion and $1x10^7$ SP2/0 cells were cotransfected by electroporation. Prior to transfection the cells were washed with cold PBS, then resuspended in 0.9 ml of the same cold buffer and placed in a 0.4 cm electrode gap electroporation cuvette (Bio-Rad) with the DNA. For the electrical pulse, the Gene Pulser from Bio-Rad (Bio-Rad, Richmond, California) was set at a capacitance of 960 μF and 200 V. After the pulse the cells were incubated on ice for 10 minutes then washed once in IMDM with 10% calf serum and resuspended in IMDM with 10% calf serum at a concentration of 10^5 cells/ml.

The transfected cells were plated into five 96 well plates at a concentration of 10^4 cells/well. Selection was started after 48 hours. Two plates were selected with 5 mM histidinol (heavy chain selection), 2 plates were selected with 1 μl/ml mycophenolic acid (light chain selection) and 1 plate was selected with histidinol and mycophenolic acid (heavy and light chain selection).

Twelve days post selection supernatants were screened by ELISA to test for the secretion of both chains. Immulon II 96 well plates were coated with 5 μg/ml of goat anti human γ1 in carbonate buffer at pH9.6, and blocked with 3% BSA. Supernatants from the transfectants were added and the plates were

incubated overnight at 4°C. After washing, plates were developed with goat anti-human *k* conjugated with alkaline phosphatase and wells secreting H and L chains identified (Table 7).

Table 7: RESULTS OF TRANSFECTIONS

Results of cotransfection with vectors pAH4602 and pAG4611 in SP2/0 cells. 2 plates were selected with 5mM histidinol (HIS), 2 plates with 1µg/ml mycophenolic acid (HXM) and 1 plate selected with both (HIS+HXM). Wells containing clones were analyzed by ELISA to determine those containing secreted antibody (# positive wells).

	SELECTION		
	HIS	**HXM**	**HIS + HXM**
#WELLS WITH CLONES	78/96 83/96	76/96 64/96	13/96
#POSITIVE WELLS	20/78 25/83	28/76 20/64	10/13

High producers were expanded for further analysis; selected transfectants were subcloned.

ANTIBODY ANALYSIS:

To determine the nature of the protein being produced, transfectants were biosynthetically labelled with ^{35}S methionine, cytoplasmic and secreted antibodies immunoprecipitated with rabbit

anti-human Ig and protein-A and the immunoprecipitates fractioned on SDS polyacrylamide gels.

Clones with the highest production identified by ELISA were expanded to 5 ml petri dishes and removed from selection. $1x10^6$ cells were pelleted at 220xg for 5 minutes at 4°C and washed twice with labelling medium (high glucose DME deficient in methionine: GIBCO). Cells were finally resuspended in 1 ml labeling medium containing 25 μCi ^{35}S-Methionine (Amersham Corp.) and allowed to incorporate label for 3 hours at 37°C under tissue culture atmospheric conditions.

Cells were pelleted and supernatants drawn off for immunoprecipitation of secreted IgG. Cell pellets were lysed in NDET (1% NP-40, 0.4% deoxycholate, 66 mM EDTA, 10 mM Tris, pH 7.4), centrifuged, and the supernatants removed and incubated 1 hour at 4°C with rabbit anti-human IgG Fc polyclonal antiserum (5 μl/ml). To the labelled supernatants, 100 μl/ml of protein A (10% in NDET, IgG Sorb) was added and mixed by rotation at 4°C for 15 minutes. Protein-A bound IgG was washed by centrifuging through 1 ml 30% sucrose in 100 μl NDET + 0.3% SDS. The protein A pellet was then resuspended in 100 μl NDET/3% SDS, transferred to a 1.5 ml polypropylene tube with 100 μl of the same buffer, and the previous tube rinsed with 100 μl. The 300 μl suspension was centrifuged and washed with deionized water. Finally, the protein A pellet was resuspended in 50 μl of loading buffer (25 mMTris pH 6.7, 0.2% SDS, 10% glycerol, 8% μg/100 ml bromophenol

blue) and boiled for two minutes prior to gel loading. Antibodies were analyzed by SDS-PAGE (5% acrylamide gels, 0.1% sodium phosphate buffered) to confirm proper assembly of H and L chains. In addition, a portion of the labelled sample was reduced by treatment with 0.15 M 2-mercaptoethanol, 37°C for 1 hour and analyzed on 12% acrylamide gels to confirm the size of the unassembled H and L chains. The gels were stained, dried and exposed for autoradiograms.

The resultant autoradiograms revealed the expected patterns for fully functional antibodies. The secreted antibodies that were in the cell supernatant exhibited the expected molecular weight pattern of free light chain, light chain dimer and the tetramer formed from two light chains and two heavy chains for fully expressed and assembled functional antibodies. The pattern for antibody parts in the cell cytoplasm was also as expected for fully expressed antibody constitutents.

EXAMPLE 16 - Further Mouse/Human Chimeras of the Anti-Human Transferrin Receptor Antibody 128.1.

As described in Example 15, the initial cloning of the gene encoding the heavy chain of the murine monoclonal antibody 128.1, which binds the human transferrin receptor, involved placing the sequences encoding the variable region of the heavy chain into an expression vector containing the human $\gamma 1$ constant region framework. This created a mouse/human chimera

in which the sequences encoding the variable region of the antibody heavy chain (VH) were derived from a murine source and the sequences encoding CH_1, CH_2 and CH_3 were derived from a human source. Because the different human gamma isotypes (γ-1, -2, -3 and -4) have different biological properties, it was necessary to create chimeric antibodies with constant region sequences from each isotype in order to obtain mouse/human chimeras for each of these isotypes. The production of these chimeras was accomplished by cloning the 400 bp Eco RV-Nhe 1 fragment containing the VH region of antibody 128.1 from plasmid pBSK4600 into expression vectors containing the γ-2, γ-3 and γ-4 constant regions in a fashion similar to that previously described in Example 15 for the cloning of the VH region of antibody 128.1 into the expression vector containing the γ-1 constant region. These clonings with the γ-2, γ-3 and γ-4 constant regions resulted in respective plasmids pAH4625, pAH4807 and pAH4808 whose plasmid maps are shown in Figure 14, Figure 15 and Figure 16, respectively. The antibody coding sequences of the heavy chain expression vectors pAH4625, pAH4807 and pAH4808 are shown in Figure 17, Figure 18 and Figure 19, respectively.

These vectors, in combination with the chimeric light chain vector pAG4611, were transfected into SP2/0 cells and clones selected as described in Example 15. Initial antibody analysis using biosynthetically labeled proteins, immunoprecipitation and SDS-PAGE as previously described gave rise to the appropriate bands for the heavy and light

chains as well as the assembled antibody for the γ-3 and γ-4 chimeras. No detectable protein was made by the γ-2 transfectants.

EXAMPLE 17 - Antibody Production by Transfectants

Antibody production by selected transfectants was assessed by ELISA. Cells were diluted in fresh medium to a density of 10^6 cells/ml and 1 ml was aliquoted into each of 3 wells on a 24-well culture plate. The plates were then incubated for 24 hours at 37°C with 5% CO_2. The media was then collected from the wells and the cells and debris were spun down to give a clarified supernatant. For the ELISA, a 96-well microtiter dish was coated with a goat antisera against human IgG. After blocking with 3% BSA, the plate was washed and a series of dilutions of both the cell supernatants and human IgG standard of known concentration were applied to the plate and incubated for 1 hour at room temperature. The plate was then washed and biotinylated goat antisera against human IgG was added, followed by a mixture of avidin and biotinylated horseradish peroxidase (HRP). The amount of antibody present in the samples was then determined, based on the amount of substrate converted by the HRP.

Three clones resulting from the γ-1 chimera transfection were tested for antibody production. The average values from three experiments were 39, 21 and 24 μg/ml IgG/10^6 cells/24 hours, respectively, for the different clones. One γ-3 clone has been

tested and it was found to produce approximately 1 μg/ml IgG/10^6 cells/24 hours. Two different clones of the γ-4 chimera have been tested and were found to produce 2.8 and 0.2 ng/ml IgG/10^6 cells/24 hours, respectively.

Equivalents

Those skilled in the art will know, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments expressly described herein. These are intended to be within the scope of the invention as described by the claims herein.

CLAIMS

1. An antibody-neuropharmaceutical or diagnostic agent conjugate for the manufacture of a medicament for delivering the neuropharmaceutical or diagnostic agent across the blood-brain barrier to the brain of a host whereby the antibody binds to a transferrin receptor present on brain capillary endothelial cells and the neuropharmaceutical or diagnostic agent is transferred across the blood-brain barrier in a pharmaceutically active form, wherein the antibody is a chimeric antibody that is reactive with said transferrin receptor.

2. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 1 wherein the chimeric antibody is a chimera between the variable region of a murine antibody and the constant region of a separate antibody.

3. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 2 wherein the constant region is of an animal source other than murine.

4. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 3 wherein the animal source is human.

5. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 2 wherein the variable region is from a monoclonal antibody produced by the 128.1 hybridoma.

6. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 4 wherein the variable region is from a monoclonal antibody produced by the 128.1 hybridoma.

7. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 6 wherein the constant region is from plasmids pAH4274 and pAG4270.

8. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 6 wherein the constant region is from plasmids pAH4625 and pAG4270.

9. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 6 wherein the constant region is from plasmids pAH4807 and pAG4270.

10. An antibody-neuropharmaceutical or diagnostic agent conjugate according to Claim 6 wherein the constant region is from plasmids pAH4808 and pAG4270.

11. A delivery system for delivering a neuropharmaceutical or diagnostic agent across the blood brain barrier comprising a chimeric antibody, reactive with a transferrin receptor present on brain capillary endothelial cells, linked to a neuropharmaceutical or diagnostic agent, whereby the delivery system transports the neuropharmaceutical or diagnostic agent across the blood brain barrier when administered in vivo.

12. A delivery system according to Claim 11 wherein the chimeric antibody is a chimera between the variable region of a murine antibody and the constant region of a separate antibody.

13. A delivery system according to Claim 12 wherein the constant region is of an animal source other than murine.

14. A delivery system according to Claim 13 wherein the animal source is human.

15. A delivery system according to Claim 12 wherein the variable region is from a monoclonal antibody produced by the 128.1 hybridoma.

16. A delivery system according to Claim 14 wherein the variable region is from a monoclonal antibody produced by the 128.1 hybridoma.

17. A delivery system according to Claim 16 wherein the constant region is from plasmids pAH4274 and pAG4270.

18. A delivery system according to Claim 16 wherein the constant region is from plasmids pAH4625 and pAG4270.

19. A delivery system according to Claim 16 wherein the constant region is from plasmids pAH4807 and pAG4270.

20. A delivery system according to Claim 16 wherein the constant region is from plasmids pAH4808 and pAG4270.

21. A chimeric antibody comprising a variable region reactive with a transferrin receptor present on brain capillary endothelial cells and a constant region of a separate antibody.

22. A chimeric antibody of Claim 21 wherein the variable region is of murine origin.

23. A chimeric antibody of Claim 22 wherein the constant region is of an animal source other than murine.

24. A chimeric antibody of Claim 23 wherein the animal source is human.

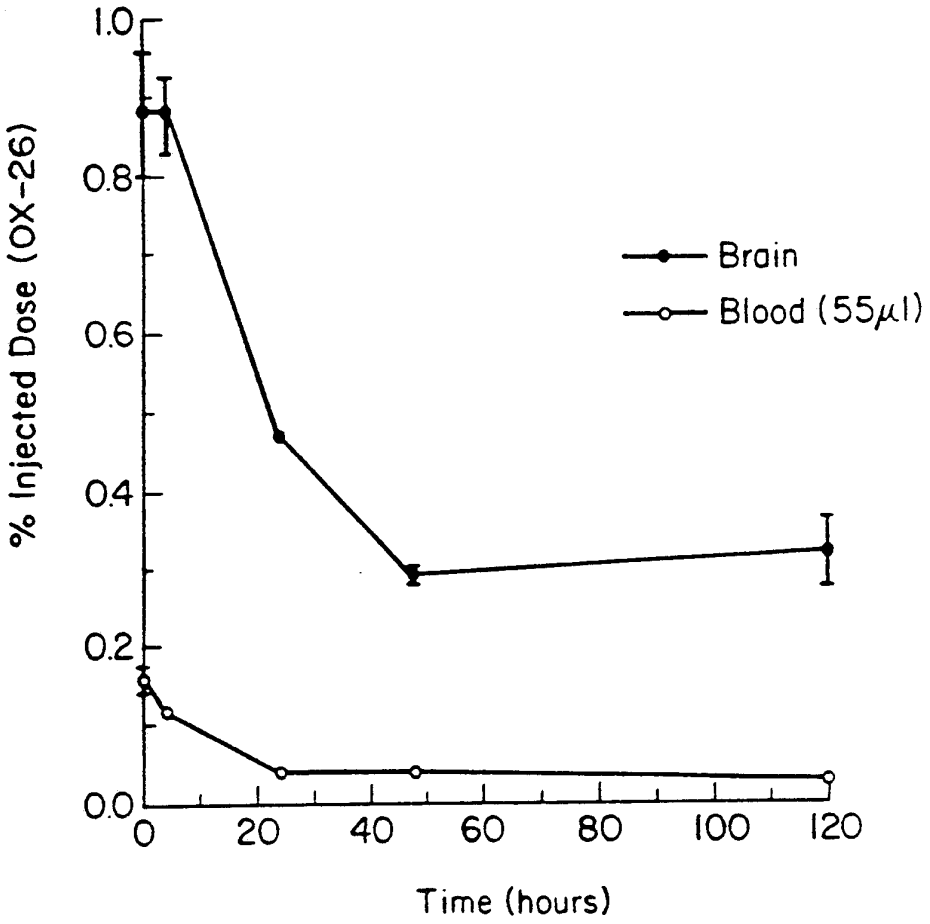

1.0
0.8
0.6
0.4
0.2
0.0
% Injected Dose (OX-26)
0
20
40
60
80
100
120
Time (hours)
Brain
Blood (55μl)

Fig. 1

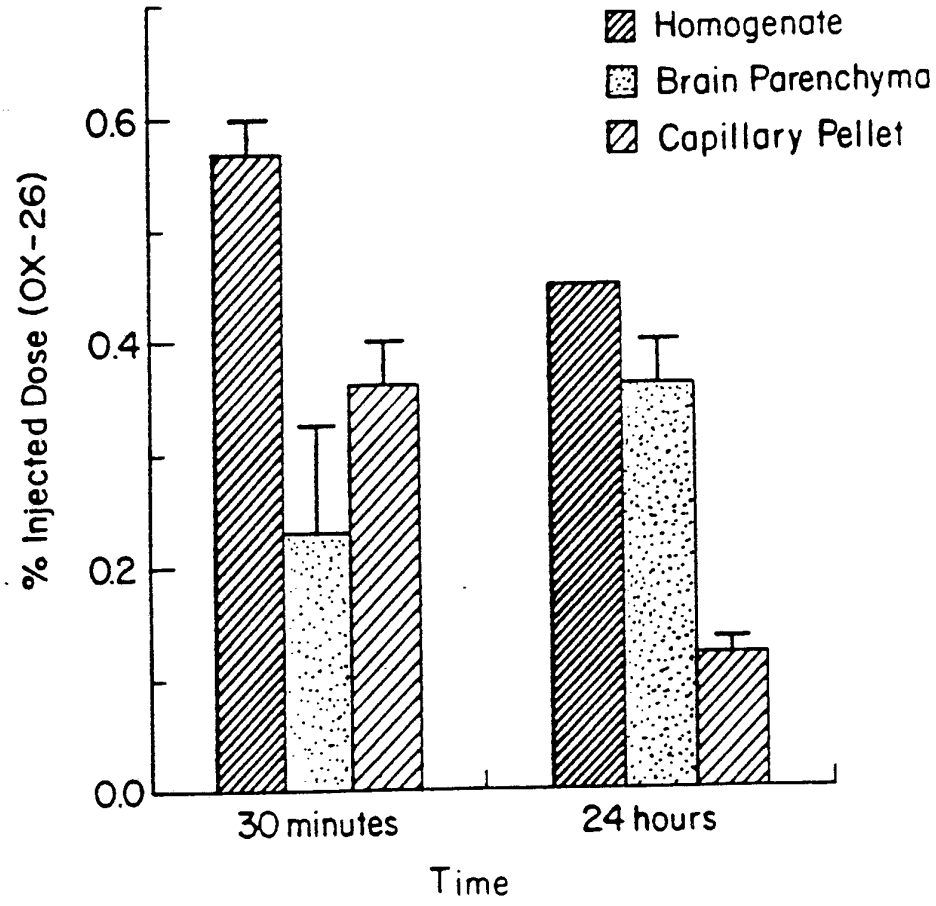

Homogenate
Brain Parenchyma
Capillary Pellet
% Injected Dose (OX-26)
0.6
0.4
0.2
0.0
30 minutes
24 hours
Time

Fig. 2

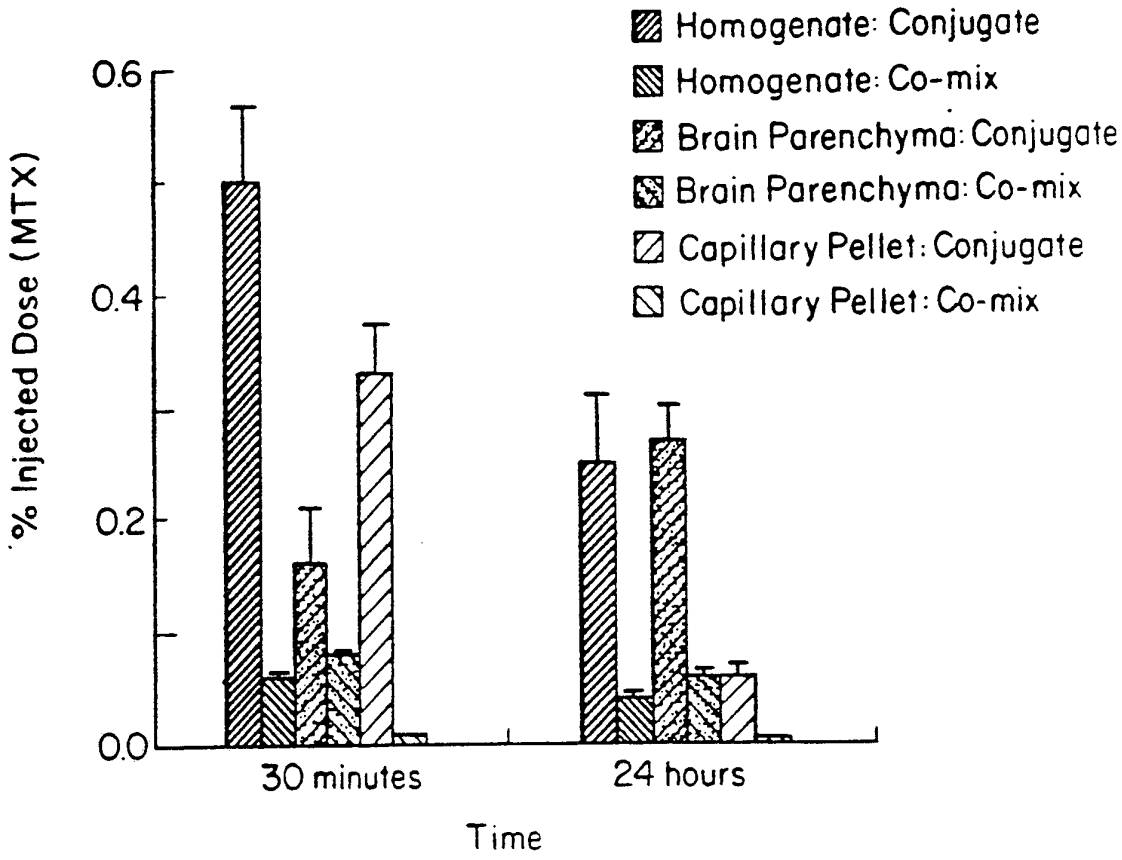

Homogenate: Conjugate
Homogenate: Co-mix
Brain Parenchyma: Conjugate
Brain Parenchyma: Co-mix
Capillary Pellet: Conjugate
Capillary Pellet: Co-mix
% Injected Dose (MTX)
0.6
0.4
0.2
0.0
30 minutes
24 hours
Time

Fig. 3

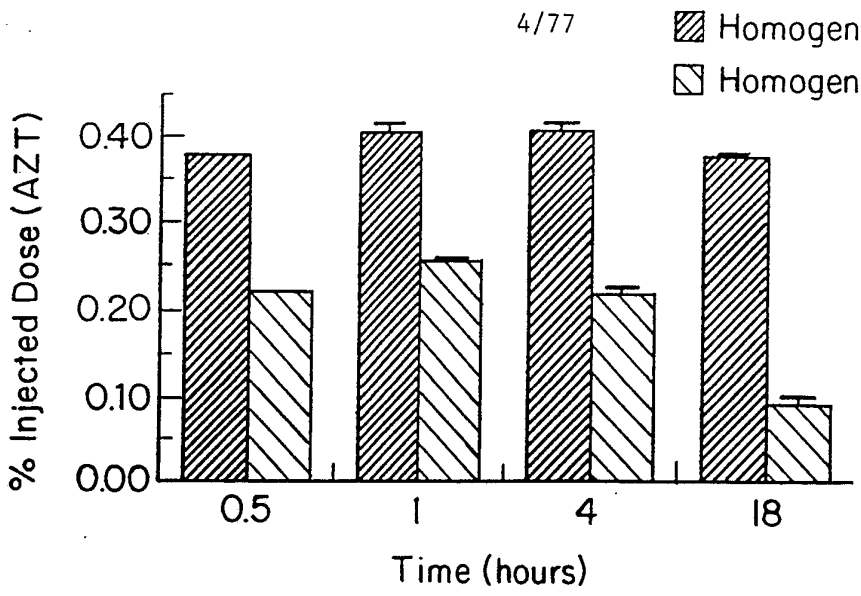


Fig. 4A

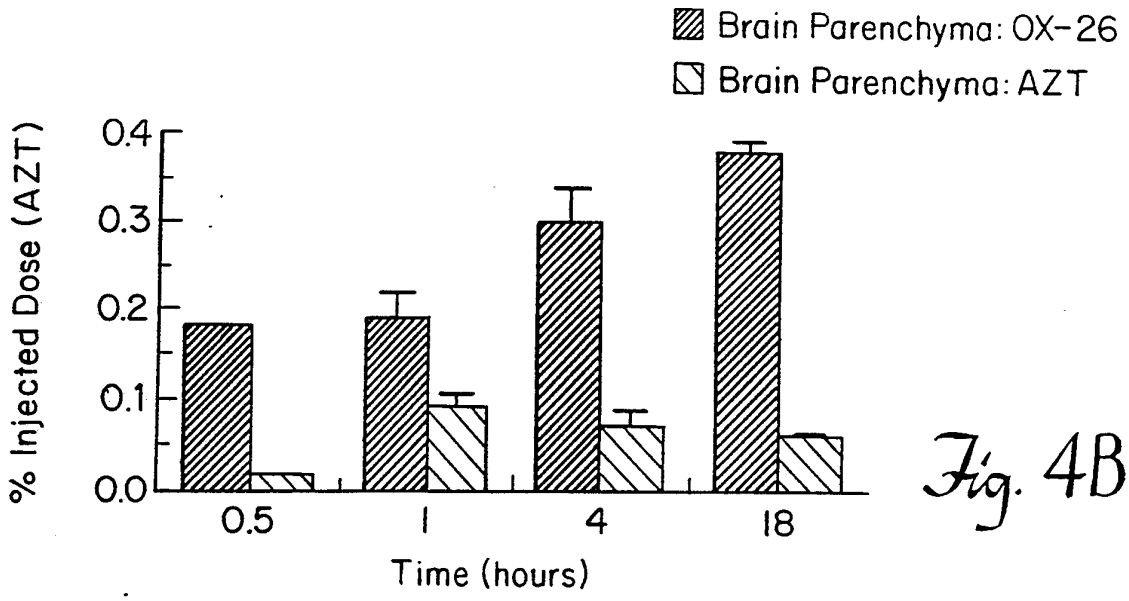


Fig. 4B

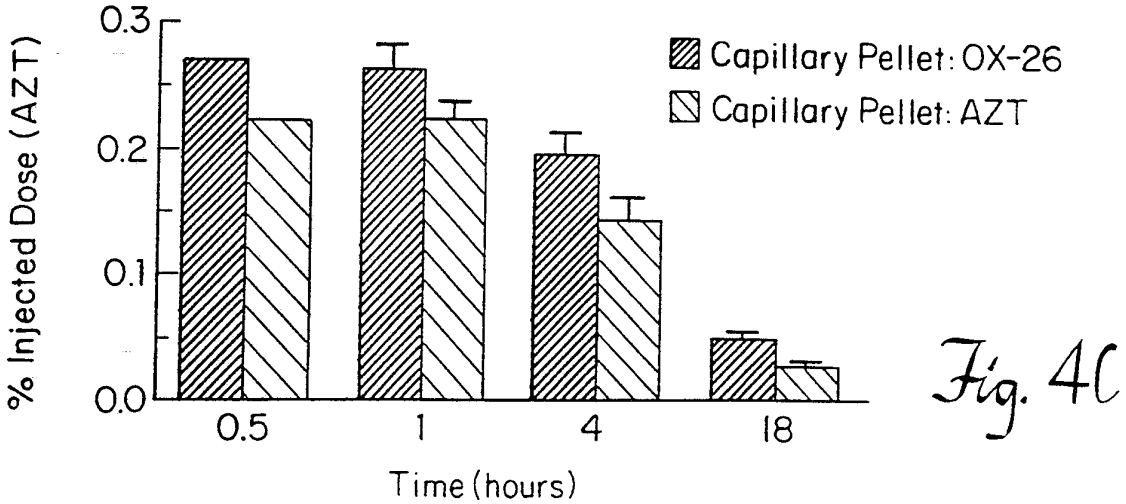


Fig. 4C

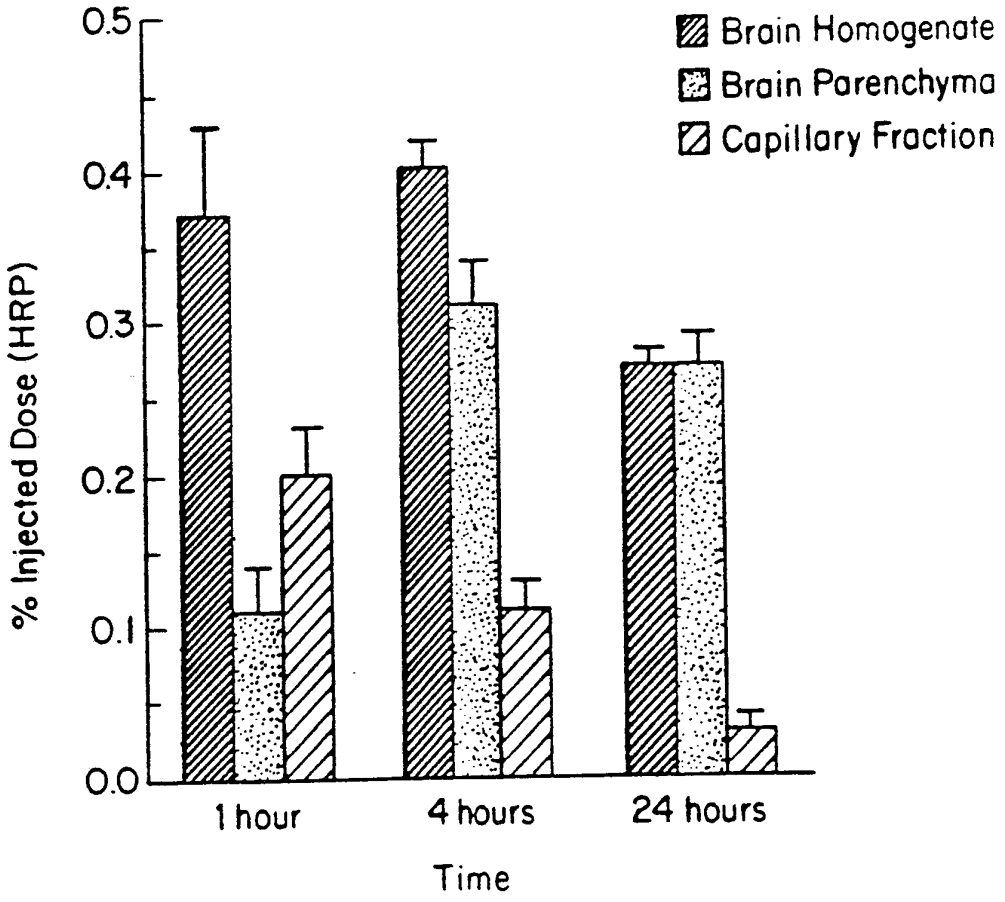

Brain Homogenate
Brain Parenchyma
Capillary Fraction
% Injected Dose (HRP)
0.5
0.4
0.3
0.2
0.1
0.0
1 hour
4 hours
24 hours
Time

Fig. 5

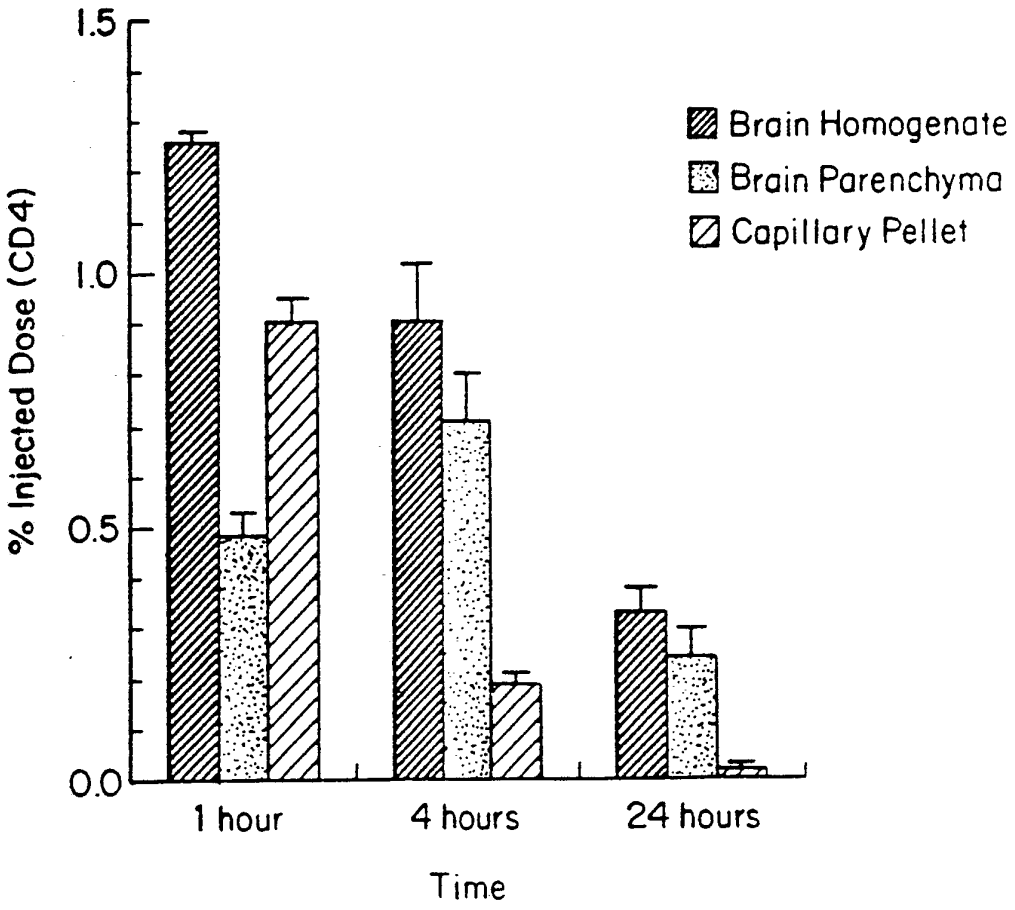

1.5
1.0
0.5
0.0
% Injected Dose (CD4)
Brain Homogenate
Brain Parenchyma
Capillary Pellet
1 hour
4 hours
24 hours
Time

Fig. 6

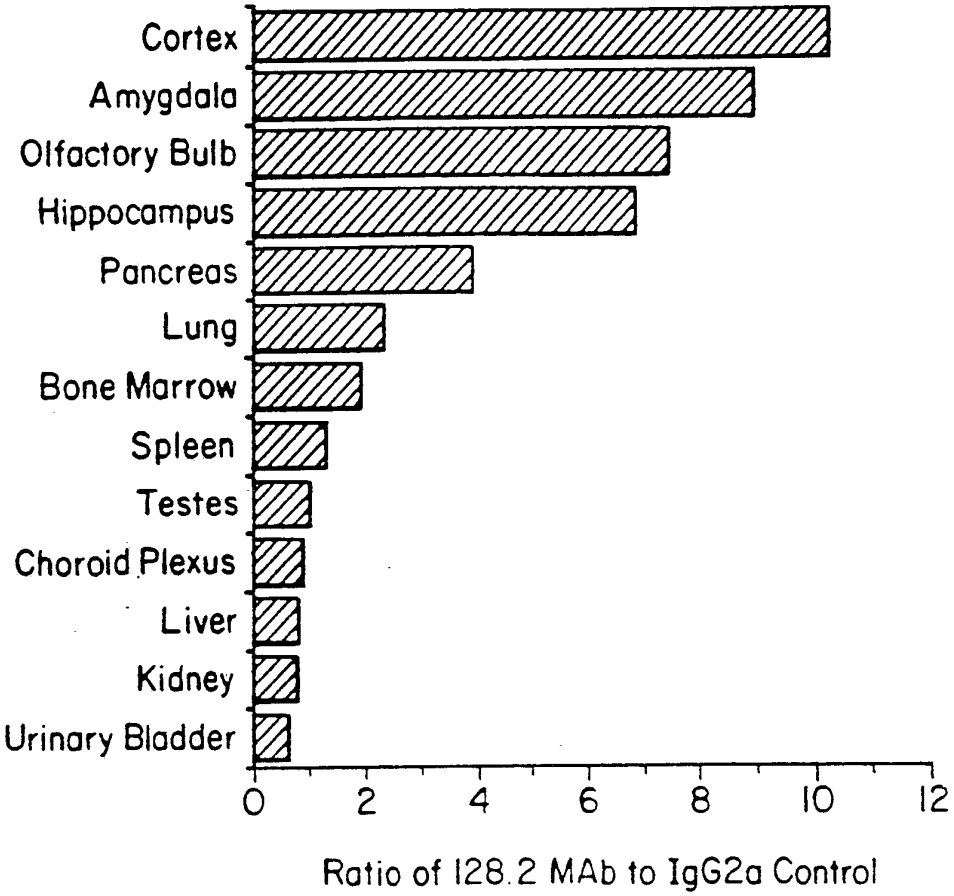
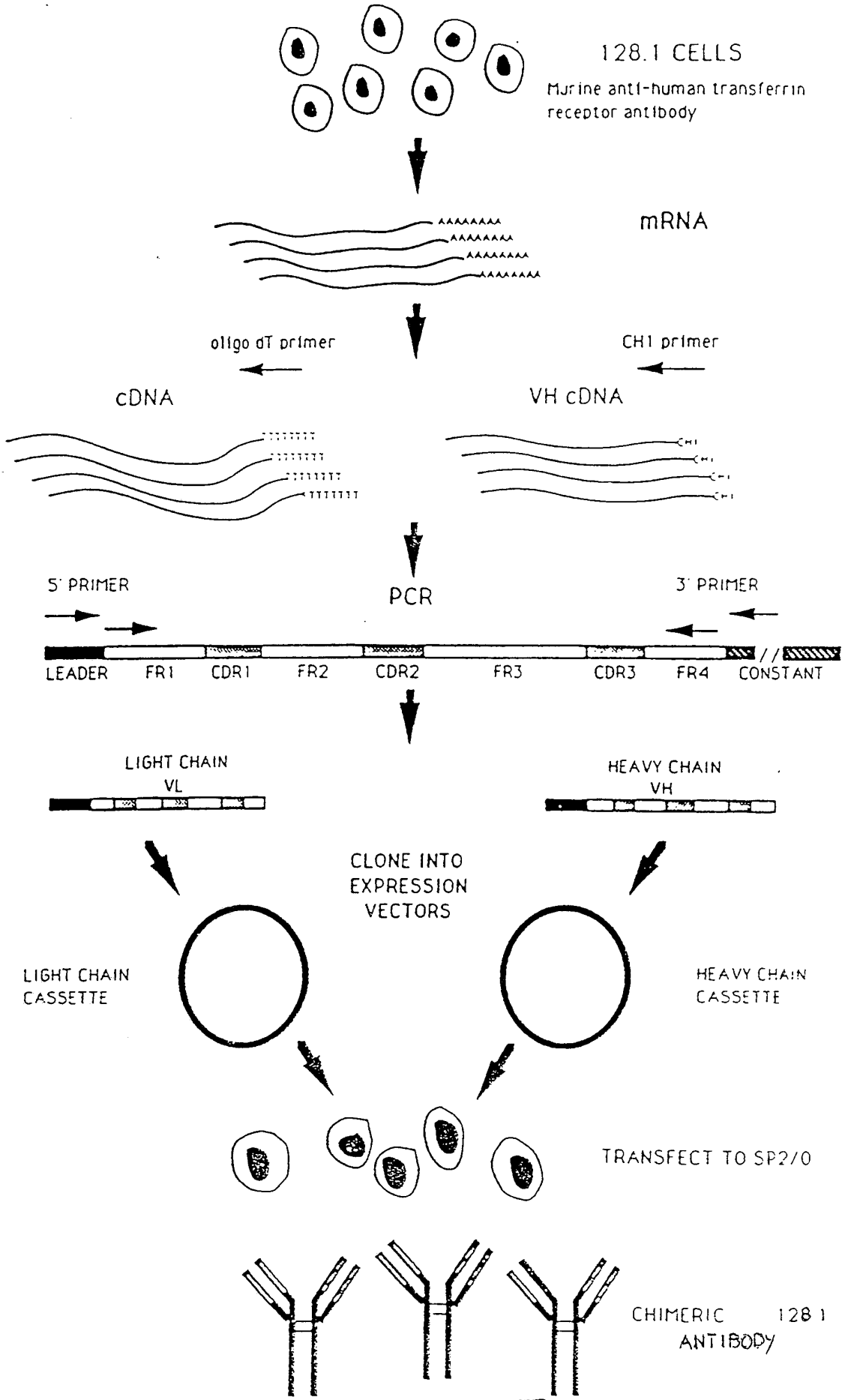

Cortex
Amygdala
Olfactory Bulb
Hippocampus
Pancreas
Lung
Bone Marrow
Spleen
Testes
Choroid Plexus
Liver
Kidney
Urinary Bladder
0
2
4
6
8
10
12
Ratio of 128.2 MAb to IgG2a Control

Fig. 7

FIG. 8


128.1 CELLS
Murine anti-human transferrin receptor antibody
mRNA
oligo dT primer
CH1 primer
cDNA
VH cDNA
5' PRIMER
PCR
3' PRIMER
LEADER
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
CONSTANT
LIGHT CHAIN VL
HEAVY CHAIN VH
CLONE INTO EXPRESSION VECTORS
LIGHT CHAIN CASSETTE
HEAVY CHAIN CASSETTE
TRANSFECT TO SP2/0
CHIMERIC 128.1 ANTIBODY

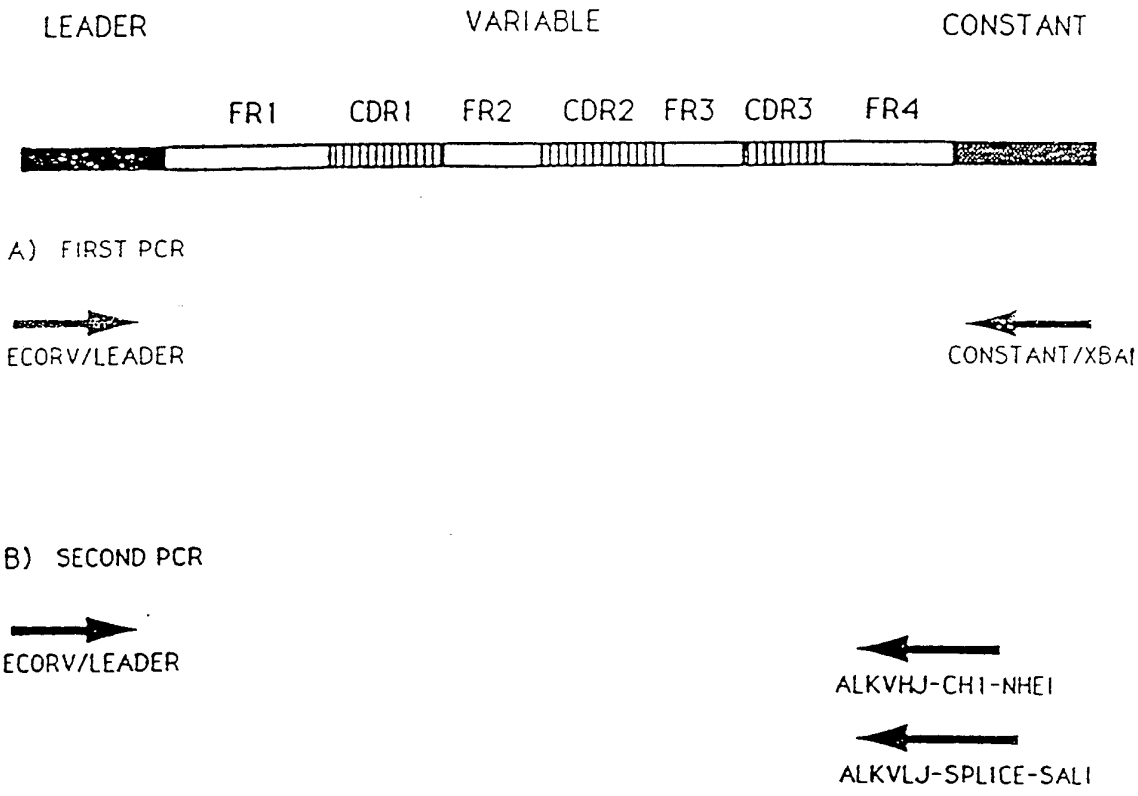

LEADER
VARIABLE
CONSTANT
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
A) FIRST PCR
ECORV/LEADER
CONSTANT/XBAI
B) SECOND PCR
ECORV/LEADER
ALKVHJ-CH1-NHEI
ALKVLJ-SPLICE-SALI

FIG. 9

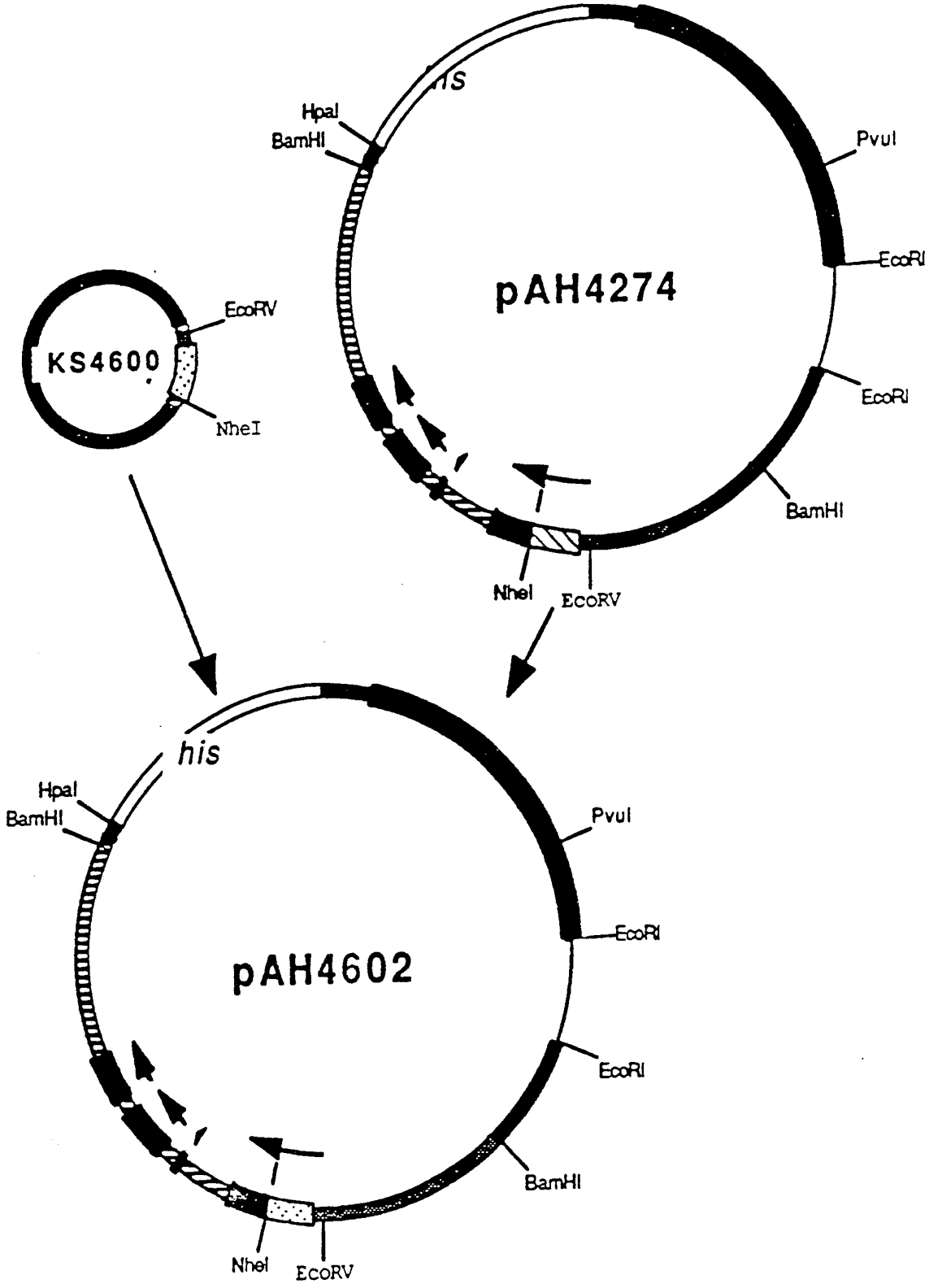

his
HpaI
BamHI
PvuI
EcoRI
pAH4274
EcoRI
BamHI
NheI
EcoRV
KS4600
EcoRV
NheI
his
HpaI
BamHI
PvuI
EcoRI
pAH4602
EcoRI
BamHI
NheI
EcoRV

FIG. 10

CGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT
CCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCT
TGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGG
GCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCAT
CTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGG
AAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACAT
ATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAA
CCATTATTATCATGACATTAACCTAT

EcoRI

AAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCAAGAATTCAGAGAGGTCTGGTGGAGCCTGCAAAAGTCCAGCTTTCA
AAGGAACACAGAAGTATGTGTATGGAATATTAGAAGATGTTGCTTTTACTCTTAAGTTGGTTCCTAGGAAAAATAGTTAA
ATACTGTGACTTTAAAATGTGAGAGGGTTTTCAAGTACTCATTTTTTTAAATGTCCAAAATTTTTGTCAATCAATTTGAG
GTCTTGTTTGTGTAGAACTGACATTACTTAAAGTTTAACCGAGGAATGGGAGTGAGGCTCTCTCATACCCTATTCAGAAC
TGACTTTTAACAATAATAAATTAAGTTTAAAATATTTTTAAATGAATTGAGCAATGTTGAGTTGAGTCAAG

PvuII

ATGGCCGATCAGAACCGGAACACCTGCAGCAGCTGGCAGGAAGCAGGTCATGTGGCAAGGCTATTTGGGGAAGGGAAAAT
AAAACCACTAGGTAAACTTGTAGCTGTGGTTTGAAGAAGTGGTTTTGAAACACTCTGTCCAGCCCCACCAAACCGAAAGT
CCAGGCTGAGCAAAACACCACCTGGGTAATTTGCATTTCTAAAATAAGTTGAGGATTCAGCCGAAACTGGAGAGGTCCTC
TTTTAACTTATTGAGTTCAACCTTTTAATTTTAGCTTGAGTAGTTCTAGTTTCCCCAAACTTAAGTTTATCGACTTCTAA
AATGT

EcoRI

ATTTAGAATTCCTTTGCCTAATATTAATGAGGACTTAACCTGTGGAAATATTTTGATGTGGGAAGCTGTTACTGTTAAAA
CTGAGGTTATTGGGGTAACTGCTATGTTAAACTTGCATTCAGGGACACAAAAAACTCATGAAAATGGTGCTGGAAAACCC
ATTCAAGGGTCAAATTTTCATTTTTTTGCTGTTGGTGGGGAACCTTTGGAGCTGCAGGGTGTGTTAGCAAACTACAGGAC
CAAATATCCTGCTCAAACTGTAACCCCAAAAAATGCTACAGTTGACAGTCAGCAGATGAACACTGACCACAAGGCTGTTT
TGGATAAGGATAATGCTTATCCAGTGGAGTGCTGGGTTCCTGATCCAAGTAAAAATGAAAACACTAGATATTTTGGAACC
TACACAGGTGGGGAAAATGTGCCTCCTGTTTTGCACATTACTAACACAGCAACCACAGTGCTGCTTGATGAGCAGGGTGT
TGGGCCCTTGTGCAAAGCTGACAGCTTGTATGTTTCTGCTGTTGACATTTGTGGGCTGTTTACCAACACTTCTGGAACAC
AGCAGTGGAAGGGACTTCCCAGATATTTTAAAATTACCCTTAGAAAGCGGTCTGTGAAAAACCCCTACCCAATTTCCTTT
TTGTTAAGTGACCTAATTAACAGGAGGACACAGAGGGTGGATGGGCAGCCTATGATTGGAATGTCCTCTCAAGT

FIG. 11A

```
                                               BamHI
AGAGGAGGTTAGGGTTTATGAGGACACAGAGGAGCTTCCTGGGGATCCGATCCNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATATAGC
ACAAAGACATGCAA
```

FIG. 11B

```
                                    HindIII
ATAATATTTCCCTATGCTCATAAAAACAGCCCTGACCATGAAGCTTTGACAGACGCACAACCCTG
                                       EcoRV
GACTCCCAAGTCTTTCTCTTCAGTGACAAACACAGACATAGGATATCCACCATGGAATGGAGCTG
                                          PvuII
GGTAATGCTCTTCCTCCTGTCAGGAACTGCAGGTGTCCGCTCTGAGGTCCAGCTGCAACAGTCTGGACCTGAACTGGTGA
AGCCTGGAGCTTCAATGAAGATTTCCTGCAAGGCTTCTGGTTACTCATTCACTGGCTACACCATGAACTGGGTGAAGCAG
AGCCATGGAGAGAACCTTGAGTGGATTGGACGTATTAATCCTCACAATGGTGGTACTGACTACAACCAGAAGTTCAAGGA
CAAGGCCCCTTTAACTGTAGACAAGTCATCCAACACAGCCTACATGGAGCTCCTCAGTCTGACATCTGAGGACTCTGCAG
TCTATTACTGTGCAAGAGGCTACTATTACTATTCTTTGGACTACTGGGGTCAAGGAACCTCAGTCACCGT
       NheI
CTCCTCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCC
TGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC
ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCAC
CCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGGTGAGAGGCCAGCACAGG
GAGGGAGGGTGTCTGCTGGAAGCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGC
AAGGCAGGCCCCGTCTGCCTCTTCACCCGGAGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGGGTCTTCTGGCTTTTT
CCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCCCTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCT
GCCAAGAGCCATATCCGGGAGGACCCCTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCCTCAGCTCGG
ACACCTTCTCTCCTCCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTGACAAAACTCACACAT
GCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATC
CAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCCATCTCTTCCTCAGCACCTGAACTCCTGGGGGGACCGTCAG
TCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCCTGAGGTCACATGCGTGGTGGTGGACGTG
AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGA
GGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGTGGGACCCGTGGGGTG
CGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGTGACCGCTGTACCAACCTCTGTCCCTACAGG
```

FIG. 11C

GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCC
TGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG
CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAA
CGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAAT
GAGTGCGACGGCCGGCAAGCCCCGCTCCCCGGGCTCTCGCGGTCGCACGAGGATGCTTGGCACGTACCCCCTGTACATAC
TTCCCGGGCGCCCAGCATGGAAATAAAGCACCCAGCGCTGCCCTGGGCCCCTGCGAGACTGTGATGGTTCTTTCCACGGG
TCAGGCCGAGTCTGAGGCCTGAGTGGCATGAGGGAGGCAGAGCGGGTCNAANNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
MboI
BamHI
NNGGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTG
TGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATA

FIG. 11D

```
      HpaI
AACAAGTTAACACAACACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTT
                                  MboI
TTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCTCTAGTCAAGGCACTATACATCAAATATTCC
TTATTAACCCCTTTACAAATTAAAAAGCTAAAGGTACACAATTTTTGAGCATAGTTATTAATAGCAGACACTCTATGCCT
GTGTGGAGTAAGAAAAAACAGTATGTTATGATTATAACTGTTATGCCTACTTATAAAGGTTACAGAATATTTTTCCATAA
TTTTCTTGTATAGCAGTGCAGCTTTTTCCTTTGTGGTGTAAATAGCAAAGCAAGCAAGAGTTCTATTACTAAACACAGCA
TGACTCAAAAAAACTTAGCAATTCTGAAGGAAAGTCCTTGGGGTCTTCTACCTTTCTCTCTTTTTTGGAGGAGTAGAATG
TTGAGAGTCAGCAGTAGCCTCATCACTCACTAGATGGCATTTCTTCTGAGCAAAACAGGTTTTCCTCATTAAAGGCATTCC
ACCACTGCTCCCATTCATCAGTTCCATAGGTTGGAATCTAAAATACACAAACAATTAGAATCAGTAGTTTAACACATTAT
ACACTTAAAAATTTTATATTTACCTTATAGCTTTAAATCTCTGTAGGTAGTTTGTCCAATTATGTCACACCACAGAAGTA
AGGTTCCTTC
      MboI                                  HpaI
ACAAAGATCCGGNNNNNNNNNNNNNNNNNNNNNNNNTCATGCTTGCTCCTTGAGGGCGTTAACGCGCAAGGTAACGGCA
TTTTTATGGGCGGTCAGACGTTCGGCGGCGGCCAGTGTTTCTATGGTTGAAGCCACCGCGGAGAACCCCTCTTTCGACAG
TTCCTGTACGGTCATACGCTTCTGGAAATCTGCCAGCCCGAGGCTGGAACAGGTGGCGGTGTAACCGTAAGTCGGTAGAA
CGTGGTTGGTTCCGGAGGCGTAATCACCTGCCGATTCCGGTGACCAGTCACCAAGAAATACCGAACCGGCGCTGGTGATG
CTATCGACCAGTTCACGGGCGTTGCGGGTCTGAATGATCAGGTGCTCCGGGCCGTACTGATTAGAGATCTCCACGCACTG
CGCTGAATCTTTAGTCACGATCAGGCGGCTGGCGTTCAGTGCCTGGCGGGCGGTTTCGGCACGCCGGCAGTTCCGCCAGTT
GGCGTTCGACGGCCTCGGCAACGCGACGCCGCCATATCAGCAGCGGGCGTCAGTAAAATCACCTGTGAGTCCGGGGCCGTGT
TCAGCCTGAGAGAGCAAATCAGAAG
                                      ClaI
CCACGAAATCCGGCGCGTTGCGCCGCTGTCAGCAATCACCAGCACTTCCGACGGGCCTGCGGGCATATCGATCTCCGCACCG
TCCAGACGCTGGCTCACCTGACGTTTCGCTTCGGTGACAAAGGCGTTACCCGGCCCGAAGATTTTGTCCACTTTTGGCAC
GGATTCCGTACCAAACGCCAGTGCGGCAAT
```

FIG. 11E

PvuII

GGCCTGTGCGCCGCCGACGTTGAACACGTCCTGCACACCGCACAGCTGCGCCGCATAAAGGATCTCATCGGCAATCGGCG
GCGGTGAGCACAGCACCACTTTTTTACAGCCCGCCAATACGCGCCGGAGTCGCCAGCATTAATACCGTTGAGAAGAGCGGG
GCGGAGCCGCCAGGAATATACAACCCAACTGAAGCTACCGGACGCGTGACCTGCTGGCAACGCACGCCTGGCTGCGTTTC
TACATCTACCGGCGGCAGTTTTTGCGCAGTGTGGAAGGTTTCAATATTCTTTACTGCCACCGCCATCGCCTGTTTTAGCT
CGTCGCTCAGGCGTTCGCTGGCGGCGGCGATCTCCTCTGCAGACACCTTCAGCGCGGTAACCGTGGTTTTATCAAACTTC
GCGCTGTATTCCCGCAGGGCCTCATCGCCGCGTGCTTTCACGTTATCGAGAATATCGTTAACAGTGCGGGTAATGCTTT

PvuII

CAGAGGCGGAAATCGCCGGGCGCGTTAACAGCTGGCGTTGTTGCACCGCAGTACAGCTATTCCAGTCAATGATTGTGTTA
AAGCTCATNNNNCCGGATCAGCTTTTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAG
AGGCCGAGGCGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAG
TTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCT
GCTGGGGAGCCTGGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGA
GCCTGGGGGACTTTCCACACCCTAACTGACACACATTCCACAGCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCT
GACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCA
GCGGGTGTTGGCGGGTGTCGGGGCGCAGCCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAACTATGCG
GCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCAT
CAGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA
AGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGG
AACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAG
TCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTC
CGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGG
TATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTT
ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTA
GCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTT
GGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGG
TAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTA

FIG. 11F

```
CGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAG
ATCCTTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTT
AATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTA
CGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCA
GCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTG
TTGCCGGGAAGCTAGAGTAAGTAGTTCGCC
                    PstI
AGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCA
GCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCT
        PvuI
TCGGTCCTCCG
```

FIG. 11G

```
                                                          ATG GAA TGG
                                                          Met Glu Trp
                                                            1

AGC TGG GTA ATG CTC TTC CTC CTG TCA GGA ACT GCA GGT GTC CGC TCT
Ser Trp Val Met Leu Phe Leu Leu Ser Gly Thr Ala Gly Val Arg Ser
      5                  10                  15

GAG GTC CAG CTG CAA CAG TCT GGA CCT GAA CTG GTG AAG CCT GGA GCT
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 20                  25                  30                  35

TCA ATG AAG ATT TCC TGC AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC
Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
                 40                  45                  50

ACC ATG AAC TGG GTG AAG CAG AGC CAT GGA GAG AAC CTT GAG TGG ATT
Thr Met Asn Trp Val Lys Gln Ser His Gly Glu Asn Leu Glu Trp Ile
            55                  60                  65

GGA CGT ATT AAT CCT CAC AAT GGT GGT ACT GAC TAC AAC CAG AAG TTC
Gly Arg Ile Asn Pro His Asn Gly Gly Thr Asp Tyr Asn Gln Lys Phe
        70                  75                  80

AAG GAC AAG GCC CCT TTA ACT GTA GAC AAG TCA TCC AAC ACA GCC TAC
Lys Asp Lys Ala Pro Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
     85                  90                  95

ATG GAG CTC CTC AGT CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT
Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
100                 105                 110                 115

GCA AGA GGC TAC TAT TAC TAT TCT TTG GAC TAC TGG GGT CAA GGA ACC
Ala Arg Gly Tyr Tyr Tyr Tyr Ser Leu Asp Tyr Trp Gly Gln Gly Thr
                120                 125                 130

TCA GTC ACC GTC TCC TCA GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC
Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
            135                 140                 145

CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC
Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
        150                 155                 160

TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
    165                 170                 175

TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180                 185                 190                 195
```

FIG. 11H

```
TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
                200                 205                 210

AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
            215                 220                 225

AAC ACC AAG GTG GAC AAG AAA GTT
Asn Thr Lys Val Asp Lys Lys Val
        230                 235
```

FIG. 11I

```
GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
  1               5                  10                  15
```

FIG. 11J

```
GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
  1               5                  10                  15

CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
             20                  25                  30

GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
         35                  40                  45

GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
     50                  55                  60

CAG TAC AAC AGC ACG TAC CGG GTG GTC AGC GTC CTC ACC GTC CTG CAC
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
 65                  70                  75                  80

CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                 85                  90                  95

GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
         100                 105                 110
```

FIG. 11K

```
                                                GGG CAG CCC CGA GAA CCA
                                                Gly Gln Pro Arg Glu Pro
                                                  1               5

CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
            10                  15                  20

GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        25                  30                  35

GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    40                  45                  50

CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
55                  60                  65                  70

ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                75                  80                  85

GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            90                  95                  100

CTG TCT CCG GGT AAA
Leu Ser Pro Gly Lys
        105
```

FIG. 11L

```
Met Ser Phe Asn Thr Ile Ile Asp Trp Asn Ser Cys Thr Ala Val Gln
 1               5                  10                  15

Gln Arg Gln Leu Leu Thr Arg Pro Ala Ile Ser Ala Ser Glu Ser Ile
            20                  25                  30

Thr Arg Thr Val Asn Asp Ile Leu Asp Asn Val Lys Ala Arg Gly Asp
        35                  40                  45

Glu Ala Leu Arg Glu Tyr Ser Ala Lys Phe Asp Lys Thr Thr Val Thr
    50                  55                  60

Ala Leu Lys Val Ser Ala Glu Glu Ile Ala Ala Ala Ser Glu Arg Leu
65                  70                  75                  80

Ser Asp Glu Leu Lys Gln Ala Met Ala Val Ala Val Lys Asn Ile Glu
                85                  90                  95

Thr Phe His Thr Ala Gln Lys Leu Pro Pro Val Asp Val Glu Thr Gln
            100                 105                 110

Pro Gly Val Arg Cys Gln Gln Val Thr Arg Pro Val Ala Ser Val Gly
        115                 120                 125

Leu Tyr Ile Pro Gly Gly Ser Ala Pro Leu Phe Ser Thr Val Leu Met
    130                 135                 140

Leu Ala Thr Pro Ala Arg Ile Ala Gly Cys Lys Lys Val Val Leu Cys
145                 150                 155                 160

Ser Pro Pro Pro Ile Ala Asp Glu Ile Leu Tyr Ala Ala Gln Leu Cys
                165                 170                 175

Gly Val Gln Asp Val Phe Asn Val Gly Gly Ala Gln Ala Ile Ala Ala
            180                 185                 190

Leu Ala Phe Gly Thr Glu Ser Val Pro Lys Val Asp Lys Ile Phe Gly
        195                 200                 205

Pro Gly Asn Ala Phe Val Thr Glu Ala Lys Arg Gln Val Ser Gln Arg
    210                 215                 220

Leu Asp Gly Ala Glu Ile Asp Met Pro Ala Gly Pro Ser Glu Val Leu
225                 230                 235                 240

Val Ile Ala Asp Ser Gly Ala Thr Pro Asp Phe Val Ala Ser Asp Leu
                245                 250                 255

Leu Ser Gln Ala Glu His Gly Pro Asp Ser Gln Val Ile Leu Leu Thr
            260                 265                 270
```

FIG. 11M

Pro Ala Ala Asp Met Ala Arg Arg Val Ala Glu Ala Val Glu Arg Gln
275 280 285

Leu Ala Glu Leu Pro Arg Ala Glu Thr Ala Arg Gln Ala Leu Asn Ala
290 295 300

Ser Arg Leu Ile Val Thr Lys Asp Ser Ala Gln Cys Val Glu Ile Ser
305 310 315 320

Asn Gln Tyr Gly Pro Glu His Leu Ile Ile Gln Thr Arg Asn Ala Arg
325 330 335

Glu Leu Val Asp Ser Ile Thr Ser Ala Gly Ser Val Phe Leu Gly Asp
340 345 350

Trp Ser Pro Glu Ser Ala Gly Asp Tyr Ala Ser Gly Thr Asn His Val
355 360 365

Leu Pro Thr Tyr Gly Tyr Thr Ala Thr Cys Ser Ser Leu Gly Leu Ala
370 375 380

Asp Phe Gln Lys Arg Met Thr Val Gln Glu Leu Ser Lys Glu Gly Phe
385 390 395 400

Ser Ala Val Ala Ser Thr Ile Glu Thr Leu Ala Ala Ala Glu Arg Leu
405 410 415

Thr Ala His Lys Asn Ala Val Thr Leu Arg Val Asn Ala Leu Lys Glu
420 425 430

Gln Ala

FIG. 11N

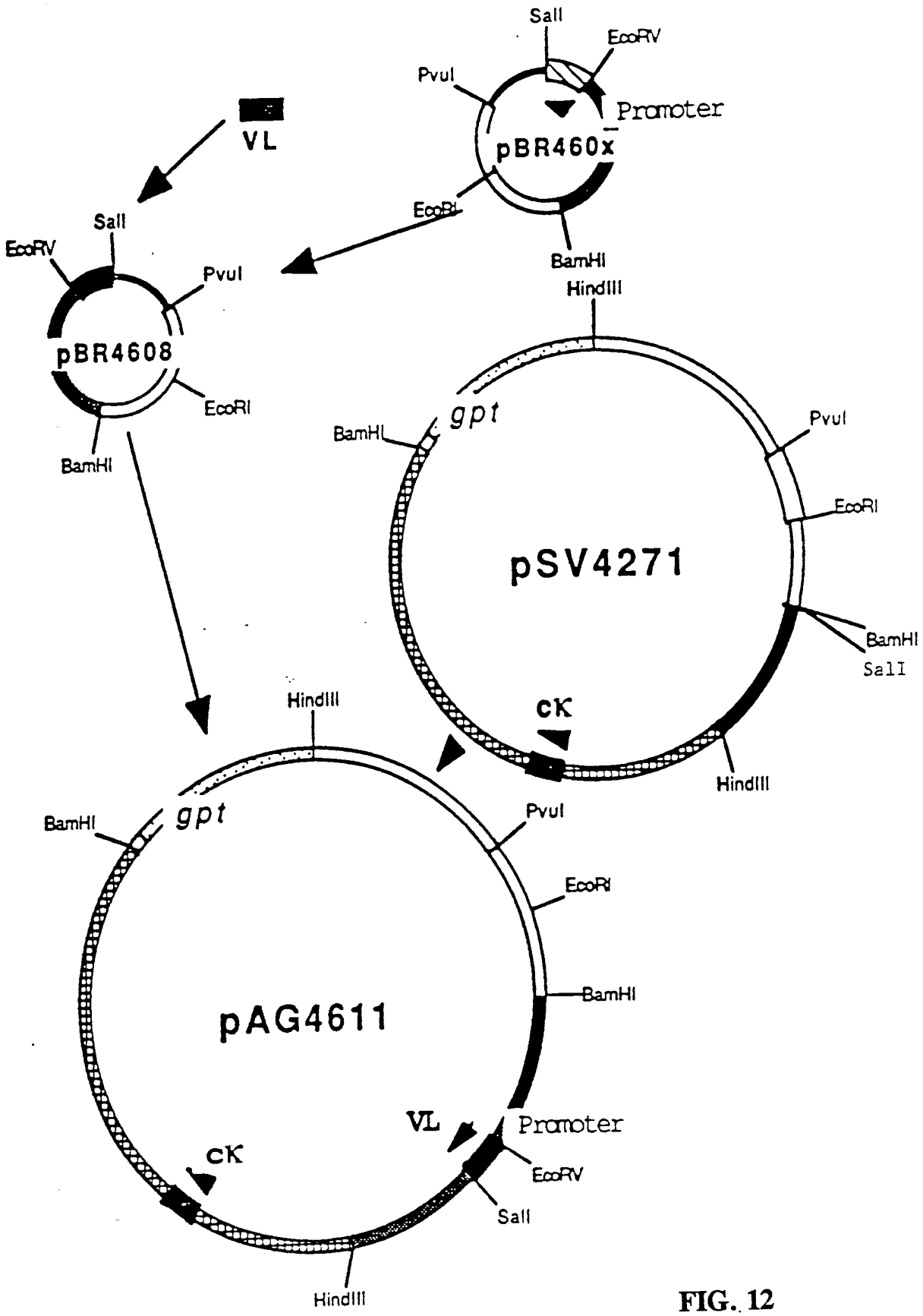

VL
SalI
EcoRV
PvuI
pBR460x
Promoter
EcoRI
BamHI
SalI
EcoRV
PvuI
pBR4608
EcoRI
BamHI
HindIII
gpt
BamHI
PvuI
EcoRI
pSV4271
BamHI
SalI
cK
HindIII
HindIII
gpt
BamHI
PvuI
EcoRI
BamHI
pAG4611
VL
Promoter
EcoRV
SalI
cK
HindIII

FIG. 12

HindIII
TTGCAAGCTTTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGCCT
CGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATG
GGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGG
GGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTC
CACAC
PvuII
CCTAACTGACACACATTCCACAGCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGA
GACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTC
GGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAACTATGCGGCATCAGAGCAGATTGTAC
TGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCC
TCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATC
CACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGT
TGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCG
ACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGG
ATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGG
TCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTT
GAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGG
CGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGA
AGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTT
TGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG
GAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAAT
GAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATC
TCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACC
ATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCG
GAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTA
AGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTCACGCTCGTCGTTTGGTAT
GGCTTCATTCAGCTCCGGTTCCCAA
PvuI
CGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCGATCGTTGTCAGAAGT
AAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTT
TTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAA

FIG. 13A

CACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCA
AGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCAC
CAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATAC
TCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATT
TAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCAT
GACATTAACC

EcoRI

TATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCAAGAATTCCTTTGCCTAATATTAATGAGGACTTAACCTGTGGA
AATATTTTGATGTGGGAAGCTGTTACTGTTAAAACTGAGGTTATTGGGGTAACTGCTATGTTAAACTTGCATTCAGGGAC
ACAAAAAACTCATGAAAATGGTGCTGGAAAACCCATTCAAGGGTCAAATTTTCATTTTTTTGCTGTTGGTGGGGAACCTT
TGGAGCTGCAGGGTGTGTTAGCAAACTACAGGACCAAATATCCTGCTCAAACTGTAACCCCAAAAAATGCTACAGTTGAC
AGTCAGCAGATGAACACTGACCACAAGGCTGTTTTGGATAAGGATAATGCTTATCCAGTGGAGTGCTGGGTTCCTGATCC
AAGTAAAAATGAAAACACTAGATATTTTGGAACCTACACAGGTGGGGAAAATGTGCCTCCTGTTTTGCACATTACTAACA
CAGCAACCACAGTGCTGCTTGATGAGCAGGGTGTTGGGCCCTTGTGCAAAGCTGACAGCTTGTATGTTTCTGCTGTTGAC
ATTTGTGGGCTGTTTACCAACACTTCTGGAACACAGCAGTGGAAGGGACTTCCCAGATATTTTAAAATTACCCTTAGAAA
GCGGTCTGTGAAAAACCCCTACCCAATTTCCTTTTTGTTAAGTGACCTAATTAACAGGAGGACACAGAGGGTGGATGGGC
AGCCTATGATTGGAA

BamHI

TGTCCTCTCAAGTAGAGGAGGTTAGGGTTTATGAGGACACAGAGGAGCTTCCTGGATCCNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

FIG. 13B

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
ATATAGCACAAAGACATGCAAATAATATTTCCCTATGCTCATAAAAACAGCCCTGACCATGAAGCTTTGACAGACGCACA
ACCCTGGACTCCCAAGTCTTTCTCTTCAGTGACAAACACAGACAT

EcoRV
AGGATATCCACCATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCCTCAGTCATACTGTCCAGAGGACA
AATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTCACCATGACCTGCAGTGCCAGCTCAA
GTATAGATTACATTCACTGGTACCAGCAGAAGTCAGGCACCTCCCCCAAAAGATGGATTTATGACACATCCAAACTGGCT
TCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTATTCTCTCACAATCAGCAGCATGGAGCCTGAAGA
TGCTG CCACTTATTACTGCCATCAGCGGAATAGTTACCCATGGACGTTCGGTGGAGGGACCAGGCTGGAA

SalI
ATCAGACGTAAGTCGACTTTCTCATCTTTTTTTATGTGTAAGACACAGGTTTTCATGTTAGGAGTTAAAGTCAGTTCAGA
AAATCTTGAGAAAATGGAGAGGGCTCATTATCAGTTGACGTGGCATACAGTGTCAGATTTTCTGTTTATCAAGCTAGTGA
GATTAGGGGCAAAAAGAGGCTTTAGTTGAGAGGAAAGTAATTAATACTATGGTCACCATCCAAGAGATTGGATCGGAGAA
TAAGCATGAGTAGTTATTGA

XbaI
GATCTGGGTCTGACTGCAGGTAGCGTGGTCTTCTAGACGTTTAAGTGGGAGATTTGGAGGGGATGAGGAATGAAGGAACT
TCAGGATAGAAAAGGGCTGAAGTCAAGTTCAGCTCCTAAAATGGATGTGGGAGCAAACTTTGAAGATAAACTGAATGACC
CAGAGGATGAAACAGCGCAGATCAAAGAGGGGCCTAGAGCTCTGAGAAGAGAAGGAGACTCATCCGTGTTGAGTTTCCAC
AAGTACTGTCTTGAGTTTTGCAATAAAAGTGGGATAGCAGAGTTGAGTGTNAGCCGTANAGTATACTCTCTTTTGTCTCC
TAAGATTTTTATGACTACAAAAATCAGTAGTATGTCCTGAAATAATCATTAAGCTGTTTGAAAGTATGACTGCTTGCCAT
GTAGATACCATGGCTTGCTGAATGATCAGAAGAGGTGTGACTCTTATTCTAAAATTTGTCACAAAATGTCAAAA

PvuII
TGAGAGACTCTGTAGGAACGAGTCCCTTGACAGACAGCTGCAAGGGGTTTTTTCCTTTGTCTCATTTCTACATGAAAGT
AAATTTGAAATGATCNTTTTTTATTATAAGAGTAGAAATACAGTTGGGTTTGAACTATATGTTTTAATNGGCCNCACGGT
TTTGTAAGACATTTGGTCCTTTGTTTTCCCAGTTATTACTCGATTGTAATTTTATATCGCCAGCANTGGTCTGAAACGGT
NNNNNNCGCAACCTCTTCGTTTACTAACTGGGTGACCTTCGGCTGTGCCAGCCATTTGGCGTTCACCCTGCCGCNGGCCN
ATGAGAACCCCCGCGGTAGNNCCCTTGCTCCGCGTGGACCACTTTCCTGAGGACACAGTGATAGGAACAGAGCCACTAAT
CTGAAGAGAACAGAGATGTGACAGACTACACTAATGTGAGAAAAACAAGGAAAGGGTGACTTATTGGAGATTTCAGAAAT
AAAATGCATTTATTATTATATTCCCTTATTTTAATTTTCT

FIG. 13C

HindIII
ATTAGGGAATTAGAAAGGGCATAAACTGCTTTATCCAGTGTTATATTAAAAGCTTNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAATCATTTCAAAATGATTTAGAGAGCCTTTTGAAAACTCTTTTAA
ACACTTTTTAAACTCTATTAAAACTAATAAGATAACTTGAAATAATTTTCATGTCAAATACATTAACTGTTTAATGTTTA
AATGCCAGATGAAAAATGTAAAGCTATCAAGAATTCACCCAGATAGGAGTATCTTCATAGCATGTTTTTCCCTGCTTATT
TTCCAGTGATCACATTATTTTGCTACCATGGTTATTTTATACAATTATCTGAAAAAAATTAGTTATGAAGATTAAAAGAG
AAGAAAATATTAAACATAAGAGATTCAGTCTTTCATGTTGAACTGCTTGGTTAACAGTGAAGTTAGTTTTAAAAA
PvuII
AAAAAAAAACTATTTCTGTTATCAGCTGACTTCTCCCTATCTGTTGACTTCTCCCAGCAAAAGATTCTTACTTATTTTAC
ATTTTAACCTACTGCTCTCCCACCCAACGGGTGGAATCCCCCAGAGGGGGATTTCCAAGAGGCCACCTGGCAGTTGCTGA
GGGTCAGAAGTGAAGCTAGCCACTTCCTCTTAGGCAGGTGGCCAAGATTACAGTTGACCTCTCCTGGTATGGCTGAAAAT
TGCTGCATATGGTTACAGGCCTTGAGGCTTTGGGAGGGCTTAGAGAGAGTTGCTGGAACAGTCAGAAGGTGGAGGGGCTG
ACACCACCCAGGCGCAGAGGCAGGGCTCAGGGCCTGCTCTGCAGGGAGGTTTTAGCCCAGCCCAGCCAAAGTAACCCCCG
GGAGCCTGTTATCCCAGCACAGTCCTGGAAGAGGCACAGGGGAAATAAAAGCGGACGGAGGCTTTCCTTGACTCAGCCGC
TGCCTGGTCTTCTTCAGACCTGTTCTGAATTCTAAACTCTGAGGGGGTCGGATGACGTGGCCATTCTTTGCCTAAAGCAT
TGAGTTTACTGCAAGGTCAGAAAAGCATGCAAAGCCCTCAGAATGGCTGCAAAGAGCTCCAACAAAACAATTTAGAACTT
TATTAAGGAATAGGGGGAAGCTAGGAAGAAACTCAAAACATCAAGATTTTAAATACGCTTCTTGGTCTCCTTGCTATAAT
TATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAACATGCCCTGTGATTATCCGCAAACAACACACCCAAGGGCAGA
ACTTTGTTACTTAAACACCATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA
TCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACA
GTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACA
GCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGC
CTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGGGAGAAGTGCCCCCACCTGCTCCTCAGTTCCAG
CCTGACCCCCTCCCATCCTTTGGCCTCTGACCCTTTTTCCACAGGGGACCTACCCCTATTGCGGTCCTCCAGCTCATCTT
TCACCTCACCCCCCTCCTCCTCCTTGGCTTTAATTATGCTAATGTTGGAGGAGAATGAATAAATAAAGTGAATCTTTGCA
CCTGTGGTTTCTCTCTTTCCTCAATTTAATAATTATTATCTGTTGTTTACCAACTACTCAATTTCTCTTATAAGGGACTA
AATATGTAGTCATCCTAAGGCGCATAACCATTTATAAAAATCATCCTTCATTCTATTTTACCCTATCATCCTCTGCAAGA
CAGTCCTCCCTCAAACCCACAAGCCTTCTGTCCTCACAGTCCCCTGGGCCGTGGTAGGAGAGACTTGCTTCCTTGTTTTC
CCCTCCTCAGCAAGCCCTCATAGTCCTTTTTAAGGGTGACAGGTCTTACGGTCATATATCCTTTGATTCAATTCCCTGGG
AATCAACCAAGGCAAATTTTTCAAAAGAAGAAACCTGCNANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

FIG. 13D

```
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGATTCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
```

FIG. 13E

BamHI

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGATCCAGACATGATAAGATACATT
GATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTT
TGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGT
GGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCTCTAGTCAAGGCACTATACATCA
AATATTCCTTATTAACCCCTTTACAAATTAAAAAGCTAAAGGTACACAATTTTTGAGCATAGTTATTAATAGCAGACACT
CTATGCCTGTGTGGAGTAAGAAAAAACAGTATGTTATGATTATAACTGTTATGCCTACTTATAAAGGTTACAGAATATTTT
TTCCATAATTTTTCTTGTATAGCAGTGCAGCTTTTTCCTTTGTGGTGTAAATAGCAAAGCAAGAGTTTCTATTACTAA
ACACAGCATGACTCAAAAAACTTAGCAATTCTGAAGGAAAGTCCTTGGGGTCTTCTACCTTTCTCTTCTTTTTGGAGGA
GTAGAATGTTGAGAGTCAGCAGTAGCCTCATCACTAGATGGCATTTCTTCTTCTGAGCAAAACAGGTTTTCCTCATTAAA
GGCATTCCACCACTGCTCCCATTCATCAGTTCCATAGGTTGGAATCTAAAATACACAAACAATTAGAATCAGTAGTTTAA
CACATTATACACTTAAAAATTTTATATTTACCTTTATAGCTTTAAATCTCTGTAGGTAGTTTGTCCATTATGTCACACCA
CAGAAGTAAGGTTCCTTCACAAAGATCCGGCTACTGTGCCTGGACGTCGAGGATTTCGCGTTCCGCCACGGTATCCGTA
TCGTATAAATCATCGCGGTACGTTCGGCATCGCTCATCACAATACGTGCCTGGACGTCGAGGATTTCGCGTGGGTCAAT
GCCGCGCCAGATCCACATCAGACGGTTAATCATGCCGATACCAGTGAGGGATGGTTTTACCATCAAGGGCCGACTGCACAG
GCGGTTGTGCGCCGTGATTAAAGCGGCGGACTAGCGTCGAGGTTTCAGGATGTTTAAAGCGGGGTTTGAACAGGGTTTCG
CTCAGGTTTGCCTGTGTCATGGATGCAGCCTCCAGAATACTTACTGGAAACTATTGTAACCCGCCTGAAGTTAAAAAGAA
CAACGCCCGGCAGTGCCAGGCGTTGAAAAGATTAGCGACCGGAGATTGGCGGGACGAATACGACGCCCATATCCCACGGC
TGTTC

EcoRV

AATCCAGGTATCTTGCGGGATATCAACAACATAGTCATCAACCAGCGGACGACCAGCCGGTTTTGCGAAGATGGTGACAA
AGTGCGCTTTTGGATACATTTCACGAATCGCAACCGCAGTACCACCCGGTATCCACCAGGTCATCAATAACGATGAAGCCT
TCGCCATCGCCTTCTGCGCGTTTCAGCACTTTAAGCTCGCGCTGGTTGTCGTGATCGTAGCTGGAAATACAAACGGTATC
GACATGACGAATACCCAGTTCACCGCGCCAGTAACGCACCCGGTACCAGACCGCCACGGCTTACGGCAATAATGCCTTTCC
ATTGTTCAGAAGGCATCAGTCGGCTTGCGAGTTTACGTGCATGGATCTGCAACATGTCCCAGGTGACGATGTATTTTTCG
CTCATGTGAAGTGTCCCAGCCTGTTTATCTACGGCTTAAAAAGTGTTCGAGGGGAAATAGGTTGCGCGAGATTATAGAG
ATCTGGCGCACTAAAAACCAGTATTTCACATGAGTCCGCGTCTTTTTTACGCACTGCCTCTCCCTGACGCGGGATAAAGTG
GTATTCTCAAACATATCTCGCAAGCCTGTCTTGTGTCC

FIG. 13F

```
ATG
Met
 1

GAT TTT CAA GTG CAG ATT TTC AGC TTC CTG CTA ATC AGT GCC TCA GTC
Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser Val
            5                  10                  15

ATA CTG TCC AGA GGA CAA ATT GTT CTC ACC CAG TCT CCA GCA ATC ATG
Ile Leu Ser Arg Gly Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met
        20                  25                  30

TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT GCC AGC TCA
Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser
    35                  40                  45

AGT ATA GAT TAC ATT CAC TGG TAC CAG CAG AAG TCA GGC ACC TCC CCC
Ser Ile Asp Tyr Ile His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro
50                  55                  60                  65

AAA AGA TGG ATT TAT GAC ACA TCC AAA CTG GCT TCT GGA GTC CCT GCT
Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala
                70                  75                  80

CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAT TCT CTC ACA ATC AGC
Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser
            85                  90                  95

AGC ATG GAG CCT GAA GAT GCT GCC ACT TAT TAC TGC CAT CAG CGG AAT
Ser Met Glu Pro Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Arg Asn
        100                 105                 110

AGT TAC CCA TGG ACG TTC GGT GGA GGG ACC AGG CTG GAA ATC AGA
Ser Tyr Pro Trp Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Arg
    115                 120                 125
```

FIG. 13G

```
                                                ACT GTG GCT GCA CCA TCT GTC
                                                Thr Val Ala Ala Pro Ser Val
                                                  1               5

TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT
Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
          10                  15                  20

GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA CAG
Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln
      25                  30                  35

TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT GTC
Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val
 40                  45                  50                  55

ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG
Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu
                60                  65                  70

ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA
Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
            75                  80                  85

GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG
Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
          90                  95                  100

GGA GAG TGT
Gly Glu Cys
    105
```

FIG. 13H

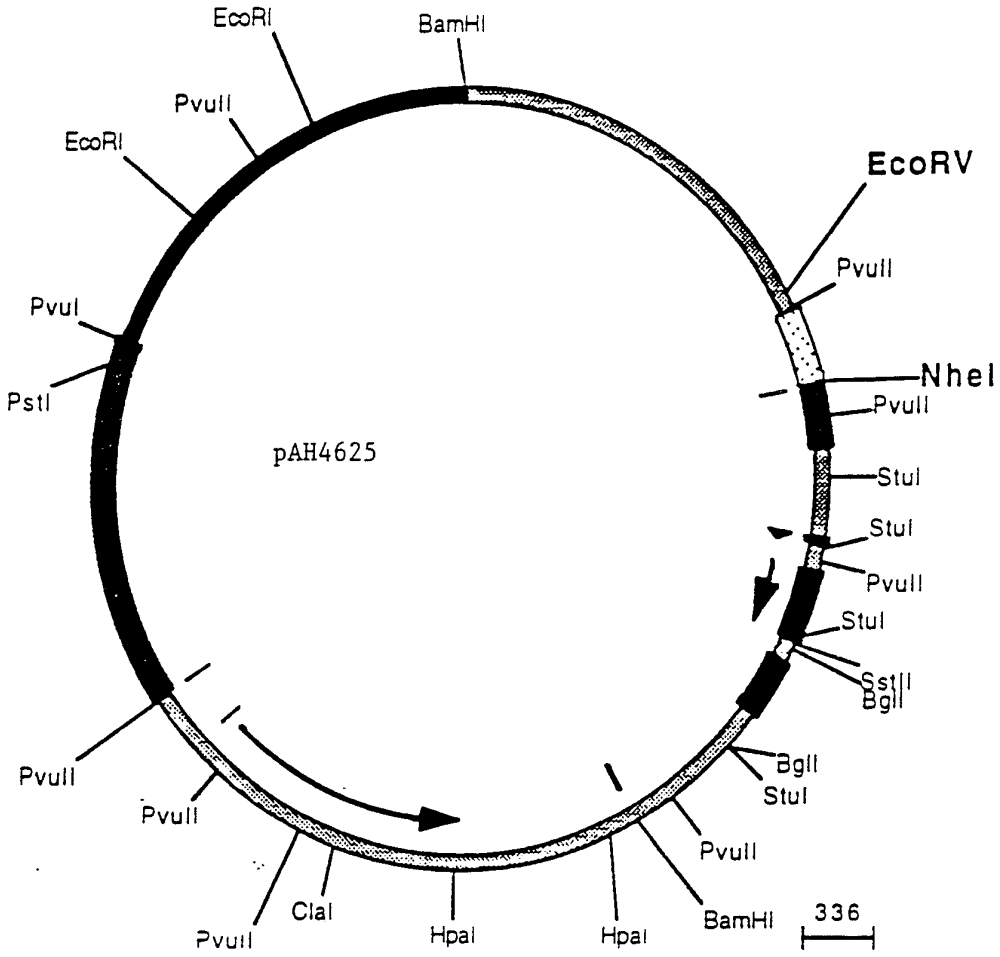

EcoRI
BamHI
PvuII
EcoRI
EcoRV
PvuII
PvuI
NheI
PstI
PvuII
pAH4625
StuI
StuI
PvuII
StuI
SstII
BglI
BglII
StuI
PvuII
PvuII
PvuII
ClaI
HpaI
HpaI
BamHI
336

FIG. 14

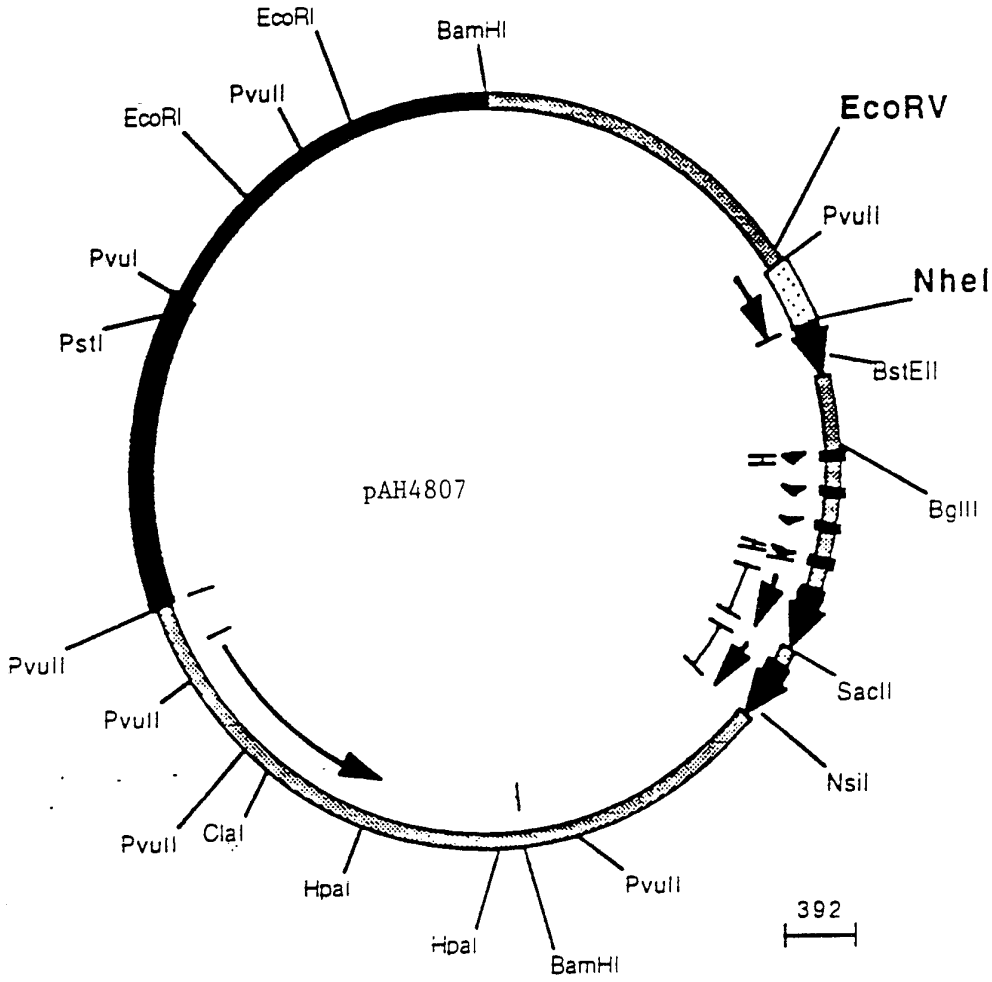

EcoRI
BamHI
PvuII
EcoRI
EcoRV
PvuII
PvuI
NheI
PstI
BstEII
pAH4807
BglII
PvuII
SacII
PvuII
NsiI
PvuII
ClaI
HpaI
PvuII
HpaI
BamHI
392

FIG. 15

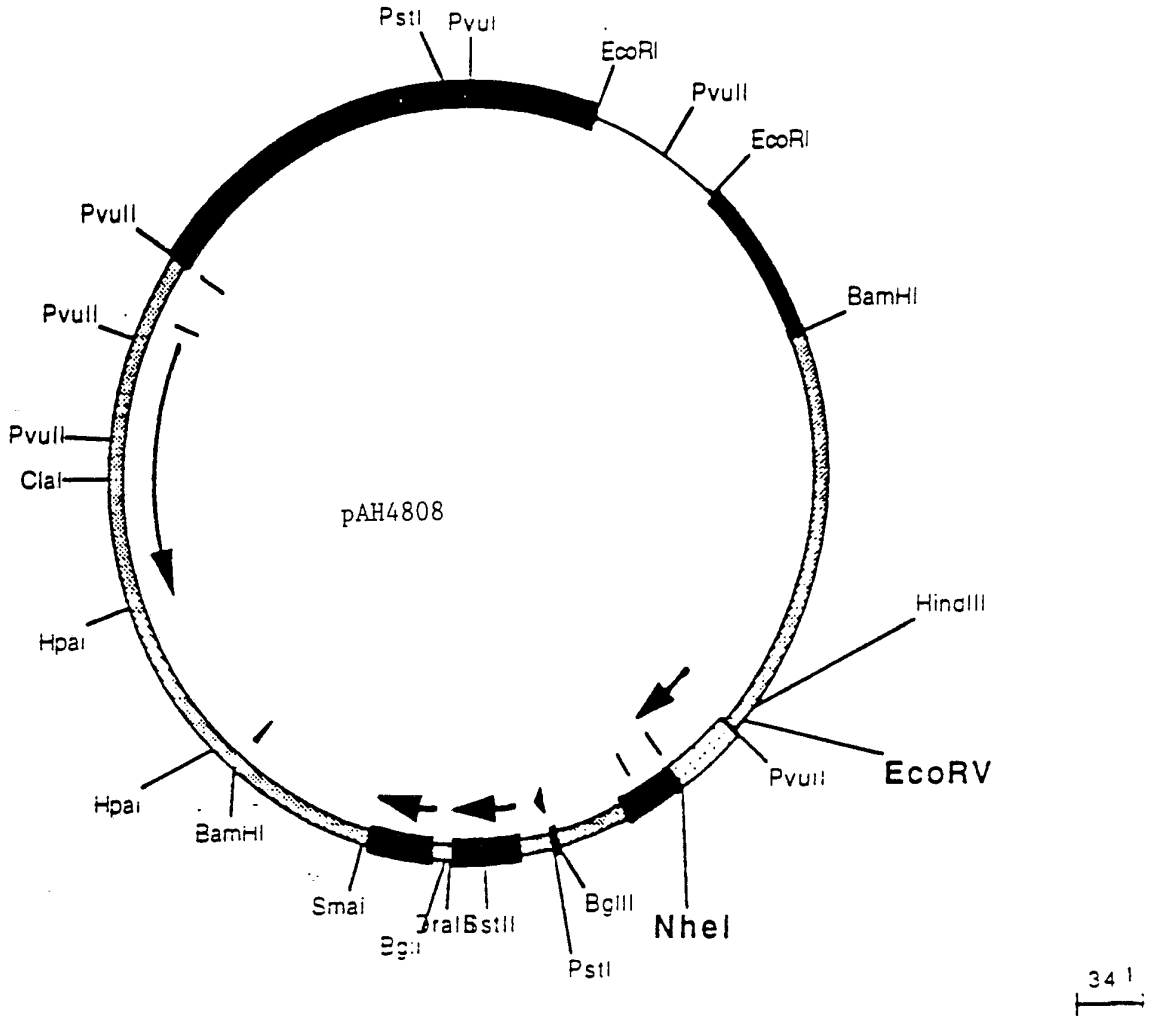

PstI
PvuI
EcoRI
PvuII
EcoRI
BamHI
PvuII
PvuII
PvuII
ClaI
pAH4808
HpaI
HindIII
EcoRV
PvuII
HpaI
BamHI
SmaI
BglII
NheI
PstI
34 1

FIG. 16

GATCCGATCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATATAGCACAAAGACATGCAAATAATATTTCCCTATGCTCATAAAAACAG
CCCTGACCATGAAGCTTTGACAGACGCACAACCCTGGACTCCCAAGTCTTTCTCTTCAGTGA

EcoRV

CAAACACAGACATAGGATATCCACCATGGAATGGAGCTGGGTAATGCTCTTCCTCCTGTCAGGAA

PvuII

CTGCAGGTGTCCGCTCTGAGGTCCAGCTGCAACAGTCTGGACCTGAACTGGTGAAGCCTGGAGCTTCAATGAAGATTTCC
TGCAAGGCTTCTGGTTACTCATTCACTGGCTACACCATGAACTGGGTGAAGCAGAGCCATGGAGAGAACCTTGAGTGGAT
TGGACGTATTAATCCTCACAATGGTGGTACTGACTACAACCAGAAGTTCAAGGACAAGGCCCCTTTAACTGTAGACAAGT
CATCCAACACAGCCTACATGGAGCTCCTCAGTCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAGGCTACTAT
TACTA

FIG. 17A

```
                                                    NheI
TTCTTTGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGG
CGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
GTGTCGTGGAACTCAGGCGCTCTGACCAGCGGCGT
          PvuII
GCACACCTTCCCAGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAACTTCG
GCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGACAGTTGGTGAGAGGCCAGCT
CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCCCCTCCTGCCTGGACGCACCCCGGCTGTGCAGCCCCAGCCCAGG
GCAGCAAGGCAGGCCCCATC
                 StuI
TGTCTCCTCACCCGGAGGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGGGTCTTCTGGCTTTTTCCACCAGGCTCCAG
GCAGGCACAGGCTGGGTGCCCCTACCCCAGGCCCTTCACACACAGGGGCAGGTGCTTGGCTCAGACCTGCCAAAAGCCAT
ATCCGGGAGGACCCTGCCCCTGACCTAAGCCGACCCCAAAGGCCAAACTGTCCACTCCCTCAGCTCGGACACCTTCTCTC
CTCCCAGATCCGAGTAACTC
                                                    StuI
CCAATCTTCTCTCTGCAGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCC
AGC
                                  PvuII
TCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCTGGGTGCTGACACGTCCACCTCCATCT
CTTCCTCAGCACCCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGG
ACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGT
GGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTG
TGCAC
                                        StuI
CAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCCAT
                                                    BglI
CGAGAAAACCATCTCCAAAACCAAAGGTGGGACCCGCGGGGTATGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCC
TCTGCCCCTGGGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGC
CTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAC
ACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGA
ACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
TGAGTGCCACGGCCGGCAAGCCCCCGCTCCCCAGGCTCTCGGGGTCGCGTGAGGATGCTTGGCACGTACCCCGTGTACAT
ACTTCCCAGGCACCCAGCATGGAAATAAAGCACCCAGCGC
```

FIG. 17B

```
                                                                  BglI
                                                                 StuI
TGCCCTGGGCCCCTGCGAGACTGTGATGGTTCTTTCCGTGGGTCAGGCCGAGTCTGAGGCCTGAGTGGCATGAGGGAGGC
AGAGTGGGTCANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
 PvuII
NCAGCTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
            BamHI
NNNNNNNNNNNNGGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAAT
GCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATA
                 HpaI
AGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTA
AAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCTCTAGTCAAGGCACTATACATCAAATATTCCTTATT
AACCCCTTTACAAATTAAAAAGCTAAAGGTACACAATTTTTGAGCATAGTTATTAATAGCAGACACTCTATGCCTGTGTG
GAGTAAGAAAAAACAGTATGTTATGATTATAACTGTTATGCCTACTTATAAAGGTTACAGAATATTTTTCCATAATTTTC
TTGTATAGCAGTGCAGCTTTTTCCTTTGTGGTGTAAATAGCAAAGCAAGCAAGAGTTCTATTACTAAACACAGCATGACT
CAAAAAACTTAGCAATTCTGAAGGAAAGTCCTTGGGGTCTTCTACCTTTCTCTTCTTTTTTGGAGGAGTAGAATGTTGAG
AGTCAGCAGTAGCCTCATCATCACTAGATGGCATTTCTTCTGAGCAAAACAGGTTTTCCTCATTAAAGGCATTCCACCAC
TGCTCCCATTCATCAGTTCCATAGGTTGGAATCTAAAATACACAAACAATTAGAATCAGTAGTTTAACACATTATACACT
TAAAAATTTTATATTTACCTTATAGCTTTAAATCTCTGTAGGTAGTTTGTCCAATTATGTCACACCACAGAAGTAAGGTT
CCTTCACAAAGATCCGGNNNNNNNNNNNNNNNNNNNNNNNNTCATGCTTGCTCCTTGAG
   HpaI
GGCGTTAACGCGCAAGGTAACGGCATTTTTATGGGCGGTCAGACGTTCGGCGGCGGCCAGTGTTTCTATGGTTGAAGCCA
CCGCGGAGAACCCCTCTTTCGACAGTTCCTGTACGGTCATACGCTTCTGGAAATCTGCCAGCCCGAGGCTGGAACAGGTG
GCGGTGTAACCGTAAGTCGGTAGAACGTGGTTGGTTCCGGAGGCGTAATCACCTGCCGATTCCGGTGACCAGTCACCAAG
AAATACCGAACCGGCGCTGGTGATGCTATCGACCAGTTCACGGGCGTTGCGGGTCTGAATGATCAGGTGCTCCGGGCCGT
ACTGATTAGAGATCTCCACGCACTGCGCTGAATCTTTAGTCACGATCAGGCGGCTGGCGTTCAGTGCCTGGCGGGCGGTT
TCGGCACGCGGCAGTTCCGCCAGTTGGCGTTCGACGGCCTCGGCAACGCGACGCGCCATATCAGCAGCGGGCGTCAGTAA
AATCACCTGTGAGTCCGGGCCGTGTTCAGCCTGAGAGAGCAAATCAGAAGCCACGAAAT
```

FIG. 17C

ClaI
CCGGCGTTGCGCCGCTGTCAGCAATCACCAGCACTTCCGACGGGCCTGCGGGCATATCGATCTCCGCACCGTCCAGACGC
TGGCTCACCTGACGTTTCGCTTCGGTGACAAAGGCGTTACCCGGCCCGAAGATTTTGTCCACTTTTGGCACGGATTCCGT
ACCAAACGCCAGTGCGGCAATGGCCTGTGCGCCGC
PvuII
CGACGTTGAACACGTCCTGCACACCGCACAGCTGCGCCGCATAAAGGATCTCATCGGCAATCGGCGGCGGTGAGCACAGC
ACCACTTTTTTACAGCCCGCAATACGCGCCGGAGTCGCCAGCATTAATACCGTTGAGAAGAGCGGGGCGGAGCCGCCAGG
AATATACAACCCAACTGAAGCTACCGGACGCGTGACCTGCTGGCAACGCACGCCTGGCTGCGTTTCTACATCTACCGGCG
GCAGTTTTTGCGCAGTGTGGAAGGTTTCAATATTCTTTACTGCCACCGCCATCGCCTGTTTTAGCTCGTCGCTCAGGCGT
TCGCTGGCGGCGGCGATCTCCTCTGCAGACACCTTCAGCGCGGTAACCGTGGTTTTATCAAACTTCGCGCTGTATTCCCG
CAGGGCCTCATCGCCGCGTGCTTTCACGTTATCGAGAATATCGTTAACAGTGCGGGTAATGCTTTCAGAGGCGGAAATCG
CCGGGCGCGTTAA
PvuII
CAGCTGGCGTTGTTGCACCGCAGTACAGCTATTCCAGTCAATGATTGTGTTAAAGCTCATNNNNCCGGATCAGCTTTTTG
CAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGCCTCGGCCTCTGCATAA
ATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGG
CGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACC
TGGTTGCTGACTAATTGAGATGCATGCTTTGC
PvuII
ATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACAGCTGCCTCGCGCGTTTCGG
TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGAC
AAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTG
TATACTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGC
GTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCG
AGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAA
AAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCAT
CACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTC
CCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTT
CTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTT
CAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGC
AGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCT
ACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCC
GGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGA
AGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTAT

FIG. 17D

TCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACT
CCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCT
CACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCC
TCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAA

PstI

GTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATG
GCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCC

PvuI

CATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCA
TGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACC
AAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACACGGGATAATACCGCGCCACATAG
CAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCA
GTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACA
GGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTA
TTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTC
CGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACC

EcoRI

ATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCAAGAATTCAGAGAGGTCTGGTG
GAGCCTGCAAAAGTCCAGCTTTCAAAGGAACACAGAAGTATGTGTATGGAATATTAGAAGATGTTGCTTTTACTCTTAAG
TTGGTTCCTAGGAAAAATAGTTAAATACTGTGACTTTAAAATGTGAGAGGGTTTTCAAGTACTCATTTTTTTAAATGTCC
AAAATTTTTGTCAATCAATTTGAGGTCTTGTTTGTGTAGAACTGACATTACTTAAAGTTTAACCGAGGAATGGGAGTGAG
GCTCTCTCATACCCTATTCAGAACTGACTTTTAACAATAATAAATTAAGTTTAAAATATTTTTAAATGAA

PvuII

TTGAGCAATGTTGAGTTGAGTCAAGATGGCCGATCAGAACCGGAACACCTGCAGCAGCTGGCAGGAAGCAGGTCATGTGG
CAAGGCTATTTGGGGAAGGGAAAATAAAACCACTAGGTAAACTTGTAGCTGTGGTTTGAAGAAGTGGTTTTGAAACACTC
TGTCCAGCCCCACCAAACCGAAAGTCCAGGCTGAGCAAAACACCACCTGGGTAATTTGCATTTCTAAAATAAGTTGAGGA
TTCAGCCGAAACTGGAGAGGTCCTCTTTTAACTTATTGAGTTCAACCTTTTAATTTTAGCTTGAGTAGTTCTAGTTTCCC
CAAAC

EcoRI

TTAAGTTTATCGACTTCTAAAATGTATTTAGAATTCCTTTGCCTAATATTAATGAGGACTTAACCTGTGGAAATATTTTG
ATGTGGGAAGCTGTTACTGTTAAAACTGAGGTTATTGGGGTAACTGCTATGTTAAACTTGCATTCAGGGACACAAAAAAC
TCATGAAAATGGTGCTGGAAAACCCATTCAAGGGTCAAATTTTCATTTTTTTGCTGTTGGTGGGGAACCTTTGGAGCTGC
AGGGTGTGTTAGCAAACTACAGGACCAAATATCCTGCTCAAACTGTAACCCCAAAAAATGCTACAGTTGACAGTCAGCAG
ATGAACACTGACCACAAGGCTGTTTTGGATAAGGATAATGCTTATCCAGTGGAGTGCTGGGTTCCTGATCCAAGTAAAAA

FIG. 17E

```
TGAAAACACTAGATATTTTGGAACCTACACAGGTGGGGAAAATGTGCCTCCTGTTTTGCACATTACTAACACAGCAACCA
CAGTGCTGCTTGATGAGCAGGGTGTTGGGCCCTTGTGCAAAGCTGACAGCTTGTATGTTTCTGCTGTTGACATTTGTGGG
CTGTTTACCAACACACTTCTGGAACACAGCAGTGGAAGGGACTTCCCAGATATTTTAAATTACCCTTAGAAAGCGGTCTGT
GAAAAACCCCTACCCAATTTCCTTTTTGTTAAGTGACCTAATTAACAGGAGGACACAGAGGGTGGATGGGCAGCC
                                                    BamHI
TATGATTGGAATGTCCTCTCAAGTAGAGGAGGTTAGGGTTTATGAGGACACAGAGGAGCTTCCTGGG
```

FIG. 17F

```
                                        ATG GAA TGG AGC TGG GTA ATG CTC
                                        Met Glu Trp Ser Trp Val Met Leu
                                          1               5

TTC CTC CTG TCA GGA ACT GCA GGT GTC CGC TCT GAG GTC CAG CTG CAA
Phe Leu Leu Ser Gly Thr Ala Gly Val Arg Ser Glu Val Gln Leu Gln
     10                  15                  20

CAG TCT GGA CCT GAA CTG GTG AAG CCT GGA GCT TCA ATG AAG ATT TCC
Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser
 25                  30                  35                  40

TGC AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC ACC ATG AAC TGG GTG
Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Thr Met Asn Trp Val
                 45                  50                  55

AAG CAG AGC CAT GGA GAG AAC CTT GAG TGG ATT GGA CGT ATT AAT CCT
Lys Gln Ser His Gly Glu Asn Leu Glu Trp Ile Gly Arg Ile Asn Pro
             60                  65                  70

CAC AAT GGT GGT ACT GAC TAC AAC CAG AAG TTC AAG GAC AAG GCC CCT
His Asn Gly Gly Thr Asp Tyr Asn Gln Lys Phe Lys Asp Lys Ala Pro
         75                  80                  85

TTA ACT GTA GAC AAG TCA TCC AAC ACA GCC TAC ATG GAG CTC CTC AGT
Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr Met Glu Leu Leu Ser
     90                  95                  100

CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA GGC TAC TAT
Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Tyr
105                 110                 115                 120

TAC TAT TCT TTG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC
Tyr Tyr Ser Leu Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
                125                 130                 135

TCA GCT AGC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCG CCC TGC TCC
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser
            140                 145                 150

AGG AGC ACC TCC GAG AGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC
Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
        155                 160                 165

TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCT CTG ACC
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
    170                 175                 180

AGC GGC GTG CAC ACC TTC CCA GCT GTC CTA CAG TCC TCA GGA CTC TAC
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
185                 190                 195                 200
```

FIG. 17G

```
TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AAC TTC GGC ACC CAG
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe Gly Thr Gln
                205                 210                 215

ACC TAC ACC TGC AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC
Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp
            220                 225                 230

AAG ACA GTT
Lys Thr Val
        235
```

FIG. 17H

```
GAG CGC AAA TGT TGT GTC GAG TGC CCA CCG TGC CCA
Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro
  1               5                  10
```

FIG. 17I

```
                                                    GCA CCA CCT GTG GCA
                                                    Ala Pro Pro Val Ala
                                                      1               5

GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
                 10                  15                  20

ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG GAC GTG AGC CAC
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
             25                  30                  35

GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG
Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val
         40                  45                  50

CAT AAT GCC AAG ACA AAG CCA CGG GAG GAG CAG TTC AAC AGC ACG TTC
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
     55                  60                  65

CGT GTG GTC AGC GTC CTC ACC GTT GTG CAC CAG GAC TGG CTG AAC GGC
Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
 70                  75                  80                  85

AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GGC CTC CCA GCC CCC ATC
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
                 90                  95                 100

GAG AAA ACC ATC TCC AAA ACC AAA
Glu Lys Thr Ile Ser Lys Thr Lys
             105
```

FIG. 17J

```
Met Ser Phe Asn Thr Ile Ile Asp Trp Asn Ser Cys Thr Ala Val Gln
 1               5                  10                  15

Gln Arg Gln Leu Leu Thr Arg Pro Ala Ile Ser Ala Ser Glu Ser Ile
            20                  25                  30

Thr Arg Thr Val Asn Asp Ile Leu Asp Asn Val Lys Ala Arg Gly Asp
        35                  40                  45

Glu Ala Leu Arg Glu Tyr Ser Ala Lys Phe Asp Lys Thr Thr Val Thr
    50                  55                  60

Ala Leu Lys Val Ser Ala Glu Glu Ile Ala Ala Ala Ser Glu Arg Leu
65                  70                  75                  80

Ser Asp Glu Leu Lys Gln Ala Met Ala Val Ala Val Lys Asn Ile Glu
                85                  90                  95

Thr Phe His Thr Ala Gln Lys Leu Pro Pro Val Asp Val Glu Thr Gln
            100                 105                 110

Pro Gly Val Arg Cys Gln Gln Val Thr Arg Pro Val Ala Ser Val Gly
        115                 120                 125

Leu Tyr Ile Pro Gly Gly Ser Ala Pro Leu Phe Ser Thr Val Leu Met
    130                 135                 140

Leu Ala Thr Pro Ala Arg Ile Ala Gly Cys Lys Lys Val Val Leu Cys
145                 150                 155                 160

Ser Pro Pro Pro Ile Ala Asp Glu Ile Leu Tyr Ala Ala Gln Leu Cys
                165                 170                 175

Gly Val Gln Asp Val Phe Asn Val Gly Gly Ala Gln Ala Ile Ala Ala
            180                 185                 190

Leu Ala Phe Gly Thr Glu Ser Val Pro Lys Val Asp Lys Ile Phe Gly
        195                 200                 205

Pro Gly Asn Ala Phe Val Thr Glu Ala Lys Arg Gln Val Ser Gln Arg
    210                 215                 220

Leu Asp Gly Ala Glu Ile Asp Met Pro Ala Gly Pro Ser Glu Val Leu
225                 230                 235                 240

Val Ile Ala Asp Ser Gly Ala Thr Pro Asp Phe Val Ala Ser Asp Leu
                245                 250                 255

Leu Ser Gln Ala Glu His Gly Pro Asp Ser Gln Val Ile Leu Leu Thr
            260                 265                 270
```

FIG. 17K

```
Pro Ala Ala Asp Met Ala Arg Arg Val Ala Glu Ala Val Glu Arg Gln
        275                 280                 285

Leu Ala Glu Leu Pro Arg Ala Glu Thr Ala Arg Gln Ala Leu Asn Ala
    290                 295                 300

Ser Arg Leu Ile Val Thr Lys Asp Ser Ala Gln Cys Val Glu Ile Ser
305                 310                 315                 320

Asn Gln Tyr Gly Pro Glu His Leu Ile Ile Gln Thr Arg Asn Ala Arg
                325                 330                 335

Glu Leu Val Asp Ser Ile Thr Ser Ala Gly Ser Val Phe Leu Gly Asp
            340                 345                 350

Trp Ser Pro Glu Ser Ala Gly Asp Tyr Ala Ser Gly Thr Asn His Val
        355                 360                 365

Leu Pro Thr Tyr Gly Tyr Thr Ala Thr Cys Ser Ser Leu Gly Leu Ala
    370                 375                 380

Asp Phe Gln Lys Arg Met Thr Val Gln Glu Leu Ser Lys Glu Gly Phe
385                 390                 395                 400

Ser Ala Val Ala Ser Thr Ile Glu Thr Leu Ala Ala Ala Glu Arg Leu
                405                 410                 415

Thr Ala His Lys Asn Ala Val Thr Leu Arg Val Asn Ala Leu Lys Glu
            420                 425                 430

Gln Ala
```

FIG. 17L

```
GATCCGATCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATATAGCACAAAGACATGCAAATAATATTTCCCTATGCTCATAAAAACAG
CCCTGACCATGAAGCTTTGACAGACGCACAACCCTGGACTCCCAAGTCTTTCTCTTCAGTGA
                 EcoRV
CAAACACAGACATAGGATATCCACCATGGAATGGAGCTGGGTAATGCTCTTCCTCCTGTCAGGAACTGCAGGTGTCCGCT
CTGAGGTCCAGCTGCAACAGTCTGGACCTGAACTGGTGAAGCCTGGAGCTTCAATGAAGATTTCCTGCAAGGCTTCTGGT
TACTCATTCACTGGCTACACCATGAACTGGGTGAAGCAGAGCCATGGAGAGAACCTTGAGTGGATTGGACGTATTAATCC
TCACAATGGTGGTACTGACTACAACCAGAAGTTCAAGGACAAGGCCCCTTTAACTGTAGACAAGTCATCCAACACAGCCT
ACATGGAGCTCCTCAGTCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAGGCTACTATTACTATTCTTTGGAC
TACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAG
GCGCCCTGACCAGCGGCGTGCACAC
```

FIG. 18A

SUBSTITUTE SHEET

BstEII

CTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCC
AGACCTACACCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAGCGCAGGGA
GGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCCCTCCTGCCTGGACGCATCCCGGCTGTGCAGTCCCAGCCCAGGGCACCA
AGGCAGGCCCCGTCTGACTCCTCACCCGGAGGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGGGTCTTCTGGCTTTTT
CCACCAGGCTCCGGGCAGGCACAGGCTGGATGCCCCTACCCCAGGCCCTTCACACACAGGGGCAGGTGCTGCGCTCAGAG
CTGCCAAGAGCCATATCCAGGAGGACCCTGCCCCTGACCTAAGCCCACCCCAAAG

BglII

GCCAAACTCTCTACTCACTCAGCTCAGACACCTTCTCTCTTCCCAGATCTGAGTAACTCCCAATCTTCTCTCTGCAGAGC
TCAAAACCCCACTTGGTGACACAACTCACACATGCCCACGGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCA
AGGCGGGACAAGAGCCCTAGAGTGGCCTGAGTCCAGGGACAGGCCCCAGCAGGGTGCTGACGCATCCACCTCCATCCCAG
ATCCCCGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTGACACACCTCCCCCGTGCCCAAGGTGCCCAGGTAA
GCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCAGGACAGGTGCCCTAGAGTGGCCTGAGTCCAGGGACAGGCCCCAGCAG
GGTGCTGACGCATCCACCTCCATCCCAGATCCCCGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTGACACAC
CTCCCCCGTGCCCAAGGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCAGGACAGGTGCCCTAGAGTG
GCCTGAGTCCAGGGACAGGCCCCAGCAGGGTGCTGACGCATCCACCTCCATCCCAGATCCCCGTAACTCCCAATCTTCTC
TCTGCAGAGCCCAAATCTTGTGACACACCTCCCCCGTGCCCAAGGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAG
CTCAAGGCAGGACAGGTGCCCTAGAGTGGCCTGCATCCAGGGACAGGTCCCAGTCGGGTGCTGACACATCTGCCTCCATC
TCTTCCTCAGCACCTGAACTCCTGGGAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGATACCCTTATGATTTC
CCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAAGTGGTACGTGGACG
GCGTGGAGGTGCATAATGCCAAGACAAAGCTGCGGGAGGAGCAGTACAACAGCACGTTCCGTGTGGTCAGCGTCCTCACC
GTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAA
AAC

SacII

CATCTCCAAAGCCAAAGGTGGGACCCGCGGGGTATGAGGGCCACGTGGACAGAGGCCAGCTTGACCCACCCTCTGCCCTG
GGAGTGACCGCTGTGCCAACCTCTGTCCCTACAGGACAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGA
GGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGA
GCAATGGGCAGCCGGAGAACAACTACAACACCACGCCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG
CTCAC

NsiI

CGTGGACAAGAGCAGGTGGCAGCAGGGGAACATCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCGCTACACCC
AGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACAGCCGGCAAGCCCCCGCTCCCCGGGCTCTCGGGGTCGCGCG
AGGATGCTTGGCACGTACCCCGTGTACATACTTCCCGGGCACCCAGCATGGAAATAAAGCACCCAGCGCTGCCCTGGGCC
CCTGTGAGACTGTGATGGTTCTTTCCACGGGTCAGGCCGAGTCTGAGGCCTGAGTGACATGAGGGAGGCAGAGCGGGTCN

FIG. 18B

```
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAGCTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
                                                   BamHI
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGATCCAGACATGATAAGATACATTG
ATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTA
                                   HpaI
TTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAG
GGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCTCTAGTCAAGGCA
CTATACATCAAATATTCCTTATTAACCCCTTTACAAATTAAAAAGCTAAAGGTACACAATTTTTGAGCATAGTTATTAAT
AGCAGACACTCTATGCCTGTGTGGAGTAAGAAAAAACAGTATGTTATGATTATAACTGTTATGCCTACTTATAAAGGTTA
CAGAATATTTTTCCATAATTTTCTTGTATAGCAGTGCAGCTTTTTCCTTTGTGGTGTAAATAGCAAAGCAAGCAAGAGTT
CTATTACTAAACACAGCATGACTCAAAAAACTTAGCAATTCTGAAGGAAAGTCCTTGGGGTCTTCTACCTTTCTCTTCTT
TTTTGGAGGAGTAGAATGTTGAGAGTCAGCAGTAGCCTCATCATCACTAGATGGCATTTCTTCTGAGCAAAACAGGTTTT
CCTCATTAAAGGCATTCCACCACTGCTCCCATTCATCAGTTCCATAGGTTGGAATCTAAAATACACAAACAATTAGAATC
AGTAGTTTAACACATTATACACTTAAAAATTTTATATTTACCTTATAGCTTTAAATCTCTGTAGGTAGTTTGTCCAATTA
TGTCACACCACAGAAGTAAGGTTCCTTCACAAAGATCCGGNNNNNNNNNNNNNNNNNN
                   HpaI
NNNNNTCATGCTTGCTCCTTGAGGGCGTTAACGCGCAAGGTAACGGCATTTTTATGGGCGGTCAGACGTTCGGCGGCGGC
CAGTGTTTCTATGGTTGAAGCCACCGCGGAGAACCCCTCTTTCGACAGTTCCTGTACGGTCATACGCTTCTGGAAATCTG
CCAGCCCGAGGCTGGAACAGGTGGCGGTGTAACCGTAAGTCGGTAGAACGTGGTTGGTTCCGGAGGCGTAATCACCTGCC
GATTCCGGTGACCAGTCACCAAGAAATACCGAACCGGCGCTGGTGATGCTATCGACCAGTTCACGGGCGTTGCGGGTCTG
```

FIG. 18C

```
AATGATCAGGTGCTCCGGGCCGTACTGATTAGAGATCTCCACGCACTGCGCTGAATCTTTAGTCACGATCAGGCGGCTGG
CGTTCAGTGCCTGGCGGGCGGTTTCGGCACGCGGCAGTTCCGCCAGTTGGCGTTCGACGGCCTCGGCAACGCGACGCGCC
ATATCAGCAGCGGGCGTCAGTAAAATCACCTGTGAGTCCGGGCCGTGTTCAGCCTGAGAGAGCAAATCAGAAGCCACGAA
ATCCGGCGTTGCGCCGCTGTCAGCA
                                    ClaI
ATCACCAGCACTTCCGACGGGCCTGCGGGCATATCGATCTCCGCACCGTCCAGACGCTGGCTCACCTGACGTTTCGCTTC
GGTGACAAAGGCGTTACCCGGCCCGAAGATTTTGTCCACTTTTGGCACGGATTCCGTACCAAACGCCAGTGCGGCAATGG
CCTGTGCGCCGCCGACGTTGAACACGTCCTGCACA
     PvuII
CCGCACAGCTGCGCCGCATAAAGGATCTCATCGGCAATCGGCGGCGGTGAGCACAGCACCACTTTTTTACAGCCCGCAAT
ACGCGCCGGAGTCGCCAGCATTAATACCGTTGAGAAGAGCGGGGCGGAGCCGCCAGGAATATACAACCCAACTGAAGCTA
CCGGACGCGTGACCTGCTGGCAACGCACGCCTGGCTGCGTTTCTACATCTACCGGCGGCAGTTTTTGCGCAGTGTGGAAG
GTTTCAATATTCTTTACTGCCACCGCCATCGCCTGTTTTAGCTCGTCGCTCAGGCGTTCGCTGGCGGCGGCGATCTCCTC
TGCAGACACCTTCAGCGCGGTAACCGTGGTTTTATCAAACTTCGCGCTGTATTCCCGCAGGGCCTCATCGCCGCGTGCTT
TCACGTTATCGAGAATATCGTTAACAGTGCGGGTAATGCTTTCAGAGGCGGAAAT
                PvuII
CGCCGGGCGCGTTAACAGCTGGCGTTGTTGCACCGCAGTACAGCTATTCCAGTCAATGATTGTGTTAAAGCTCATNNNNC
CGGATCAGCTTTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGCC
TCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGAT
GGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTG
GGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCC
                                    PvuII
TGGGGACTTTCCACACCCTAACTGACACACATTCCACAGCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGAC
ACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCG
GGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAACTATGCGGCA
TCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAG
GCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGG
CGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAAC
CGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA
GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGA
CCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTAT
CTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATC
CGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCA
GAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGT
```

FIG. 18D

```
ATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAG
CGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGG
GGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATC
CTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAAT
CAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGA
TACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCA
ATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTG
CCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTG
PstI
CTGCAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGA
                                                              PvuI
TCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAA
GTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTT
CTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAACA
CGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAG
GATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCA
GCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTC
ATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTA
GAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGA
CATTAACCTA
                                        EcoRI
TAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCAAGAATTCAGAGAGGTCTGGTGGAGCCTGCAAAAGTCCAGCTTTC
AAAGGAACACAGAAGTATGTGTATGGAATATTAGAAGATGTTGCTTTTACTCTTAAGTTGGTTCCTAGGAAAAATAGTTA
AATACTGTGACTTTAAAATGTGAGAGGGTTTTCAAGTACTCATTTTTTTAAATGTCCAAAATTTTTGTCAATCAATTTGA
GGTCTTGTTTGTGTAGAACTGACATTACTTAAAGTTTAACCGAGGAATGGGAGTGAGGCTCTCTCATACCCTATTCAGAA
CTGACTTTTAACAATAATAAATTAAGTTTAAAATATTTTTAAATGAATTGAGCAATGTTGAGTTGAGTCA
                                      PvuII
AGATGGCCGATCAGAACCGGAACACCTGCAGCAGCTGGCAGGAAGCAGGTCATGTGGCAAGGCTATTTGGGGAAGGGAAA
ATAAAACCACTAGGTAAACTTGTAGCTGTGGTTTGAAGAAGTGGTTTTGAAACACTCTGTCCAGCCCCACCAAACCGAAA
GTCCAGGCTGAGCAAAACACCACCTGGGTAATTTGCATTTCTAAAATAAGTTGAGGATTCAGCCGAAACTGGAGAGGTCC
TCTTTTAACTTATTGAGTTCAACCTTTTAATTTTAGCTTGAGTAGTTCTAGTTTCCCCAAACTTAAGTTTATCGACTTCT
AAAAT
```

FIG. 18E

EcoRI

GTATTTAGAATTCCTTTGCCTAATATTAATGAGGACTTAACCTGTGGAAATATTTTGATGTGGGAAGCTGTTACTGTTAA
AACTGAGGTTATTGGGGTAACTGCTATGTTAAACTTGCATTCAGGGACACAAAAAACTCATGAAAATGGTGCTGGAAAAC
CCATTCAAGGGTCAAATTTTCATTTTTTGCTGTTGGTGGGGAACCTTTGGAGCTGCAGGGTGTGTTAGCAAACTACAGG
ACCAAATATCCTGCTCAAACTGTAACCCCAAAAAATGCTACAGTTGACAGTCAGGCAGATGAACACTGACCACACAAGGCTGT
TTTGGATAAGGATAATGCTTATCCAGTGGAGTGCTGGGTTCCTGATCCAAGTAAAAATGAAAACACTAGATATTTTGGAA
CCTACACAGGTGGGGAAAATGTGCCTCCTGTTTTGCACATTACTAACACAGCAACCACAGTGCTGCTTGATGAGCAGGGT
GTTGGGCCCTTGTGCAAAGCTGACAGCTTGTATGTTTCTGCTGTTGACATTTGTGGGCTGTTTACCAACACTTCTGGAAC
ACAGCAGTGGAAGGGACTTCCCAGATATTTTAAAATTACCCTTAGAAAGCGGTCTGTGAAAAACCCCTACCCAATTTCCT
TTTTGTTAAGTGACCTAATTAACAGGAGGACACAGAGGGTGGATGGGCAGCCTATGATTGGAATGTCCTCTCAAGTAGAG

BamHI

GAGGTTAGGGTTTATGAGGACACAGAGGAGCTTCCTGGG

FIG. 18F

```
                                        ATG GAA TGG AGC TGG GTA ATG CTC
                                        Met Glu Trp Ser Trp Val Met Leu
                                          1               5

TTC CTC CTG TCA GGA ACT GCA GGT GTC CGC TCT GAG GTC CAG CTG CAA
Phe Leu Leu Ser Gly Thr Ala Gly Val Arg Ser Glu Val Gln Leu Gln
     10                  15                  20

CAG TCT GGA CCT GAA CTG GTG AAG CCT GGA GCT TCA ATG AAG ATT TCC
Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Met Lys Ile Ser
 25                  30                  35                  40

TGC AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC ACC ATG AAC TGG GTG
Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr Thr Met Asn Trp Val
                45                  50                  55

AAG CAG AGC CAT GGA GAG AAC CTT GAG TGG ATT GGA CGT ATT AAT CCT
Lys Gln Ser His Gly Glu Asn Leu Glu Trp Ile Gly Arg Ile Asn Pro
            60                  65                  70

CAC AAT GGT GGT ACT GAC TAC AAC CAG AAG TTC AAG GAC AAG GCC CCT
His Asn Gly Gly Thr Asp Tyr Asn Gln Lys Phe Lys Asp Lys Ala Pro
        75                  80                  85

TTA ACT GTA GAC AAG TCA TCC AAC ACA GCC TAC ATG GAG CTC CTC AGT
Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr Met Glu Leu Leu Ser
     90                  95                  100

CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT GCA AGA GGC TAC TAT
Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg Gly Tyr Tyr
105                 110                 115                 120

TAC TAT TCT TTG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC
Tyr Tyr Ser Leu Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
                125                 130                 135

TCA ACC AAG GGC CCA TCG GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC
Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser
            140                 145                 150

ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC
Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe
        155                 160                 165

CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC
Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly
    170                 175                 180
```

FIG. 18G

```
GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC
Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
185                 190                 195                 200

AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC
Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr
                205                 210                 215

ACC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA
Thr Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg
            220                 225                 230

GTT
Val
```

FIG. 18H

```
                                                    GAG CTC AAA ACC
                                                    Glu Leu Lys Thr
                                                      1

CCA CTT GGT GAC ACA ACT CAC ACA TGC CCA CGG TGC CCA
Pro Leu Gly Asp Thr Thr His Thr Cys Pro Arg Cys Pro
  5                  10                  15
```

FIG. 18I

```
                    GAG CCC AAA TCT TGT GAC ACA CCT CCC CCG TGC CCA
                    Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro
                      1               5                  10

AGG TGC CCA
Arg Cys Pro
        15
```

FIG. 18J

```
                                                    GAG CCC AAA TCT
                                                    Glu Pro Lys Ser
                                                      1

TGT GAC ACA CCT CCC CCG TGC CCA AGG TGC CCA
Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
  5                  10                  15
```

FIG. 18K

```
GAG CCC AAA TCT TGT GAC ACA CCT CCC CCG TGC CCA AGG TGC CCA
Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro Cys Pro Arg Cys Pro
  1               5                  10                  15
```

FIG. 18L

```
GCA CCT GAA CTC CTG GGA GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
  1               5                  10                  15

CCC AAG GAT ACC CTT ATG ATT TCC CGG ACC CCT GAG GTC ACG TGC GTG
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            20                  25                  30

GTG GTG GAC GTG AGC CAC GAA GAC CCC GAG GTC CAG TTC AAG TGG TAC
Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Lys Trp Tyr
        35                  40                  45

GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CTG CGG GAG GAG
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Leu Arg Glu Glu
    50                  55                  60

CAG TAC AAC AGC ACG TTC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC
Gln Tyr Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His
65                  70                  75                  80

CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                85                  90                  95

GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
            100                 105                 110
```

FIG. 18M

```
                                                GGA CAG CCC CGA GAA CCA
                                                Gly Gln Pro Arg Glu Pro
                                                  1               5

CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln
            10                  15                  20

GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        25                  30                  35

GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAC ACC ACG
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Asn Thr Thr
    40                  45                  50

CCT CCC ATG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC
Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
55                  60                  65                  70

ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC ATC TTC TCA TGC TCC
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Ile Phe Ser Cys Ser
                75                  80                  85

GTG ATG CAT GAG GCT CTG CAC AAC CGC TAC ACC CAG AAG AGC CTC TCC
Val Met His Glu Ala Leu His Asn Arg Tyr Thr Gln Lys Ser Leu Ser
            90                  95                  100

CTG TCT CCG GGT AAA
Leu Ser Pro Gly Lys
        105
```

FIG. 18N

```
Met Ser Phe Asn Thr Ile Ile Asp Trp Asn Ser Cys Thr Ala Val Gln
 1               5                  10                  15

Gln Arg Gln Leu Leu Thr Arg Pro Ala Ile Ser Ala Ser Glu Ser Ile
            20                  25                  30

Thr Arg Thr Val Asn Asp Ile Leu Asp Asn Val Lys Ala Arg Gly Asp
        35                  40                  45

Glu Ala Leu Arg Glu Tyr Ser Ala Lys Phe Asp Lys Thr Thr Val Thr
    50                  55                  60

Ala Leu Lys Val Ser Ala Glu Glu Ile Ala Ala Ala Ser Glu Arg Leu
65                  70                  75                  80

Ser Asp Glu Leu Lys Gln Ala Met Ala Val Ala Val Lys Asn Ile Glu
                85                  90                  95

Thr Phe His Thr Ala Gln Lys Leu Pro Pro Val Asp Val Glu Thr Gln
            100                 105                 110

Pro Gly Val Arg Cys Gln Gln Val Thr Arg Pro Val Ala Ser Val Gly
        115                 120                 125

Leu Tyr Ile Pro Gly Gly Ser Ala Pro Leu Phe Ser Thr Val Leu Met
    130                 135                 140

Leu Ala Thr Pro Ala Arg Ile Ala Gly Cys Lys Lys Val Val Leu Cys
145                 150                 155                 160

Ser Pro Pro Pro Ile Ala Asp Glu Ile Leu Tyr Ala Ala Gln Leu Cys
                165                 170                 175

Gly Val Gln Asp Val Phe Asn Val Gly Gly Ala Gln Ala Ile Ala Ala
            180                 185                 190

Leu Ala Phe Gly Thr Glu Ser Val Pro Lys Val Asp Lys Ile Phe Gly
        195                 200                 205

Pro Gly Asn Ala Phe Val Thr Glu Ala Lys Arg Gln Val Ser Gln Arg
    210                 215                 220

Leu Asp Gly Ala Glu Ile Asp Met Pro Ala Gly Pro Ser Glu Val Leu
225                 230                 235                 240

Val Ile Ala Asp Ser Gly Ala Thr Pro Asp Phe Val Ala Ser Asp Leu
                245                 250                 255

Leu Ser Gln Ala Glu His Gly Pro Asp Ser Gln Val Ile Leu Leu Thr
            260                 265                 270
```

FIG. 18O

```
Pro Ala Ala Asp Met Ala Arg Arg Val Ala Glu Ala Val Glu Arg Gln
        275                 280                 285

Leu Ala Glu Leu Pro Arg Ala Glu Thr Ala Arg Gln Ala Leu Asn Ala
    290                 295                 300

Ser Arg Leu Ile Val Thr Lys Asp Ser Ala Gln Cys Val Glu Ile Ser
305                 310                 315                 320

Asn Gln Tyr Gly Pro Glu His Leu Ile Ile Gln Thr Arg Asn Ala Arg
                325                 330                 335

Glu Leu Val Asp Ser Ile Thr Ser Ala Gly Ser Val Phe Leu Gly Asp
            340                 345                 350

Trp Ser Pro Glu Ser Ala Gly Asp Tyr Ala Ser Gly Thr Asn His Val
        355                 360                 365

Leu Pro Thr Tyr Gly Tyr Thr Ala Thr Cys Ser Ser Leu Gly Leu Ala
    370                 375                 380

Asp Phe Gln Lys Arg Met Thr Val Gln Glu Leu Ser Lys Glu Gly Phe
385                 390                 395                 400

Ser Ala Val Ala Ser Thr Ile Glu Thr Leu Ala Ala Ala Glu Arg Leu
                405                 410                 415

Thr Ala His Lys Asn Ala Val Thr Leu Arg Val Asn Ala Leu Lys Glu
            420                 425                 430

Gln Ala
```

FIG. 18P

CGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCAT
CCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCT
TGCCCGGCGTCAACACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGG
GCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCAT
CTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGG
AAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACAT
ATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAA
CCATTATTATCATGACATTAACCTA

EcoRI

TAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCAAGAATTCAGAGAGGTCTGGTGGAGCCTGCAAAAGTCCAGCTTTC
AAAGGAACACAGAAGTATGTGTATGGAATATTAGAAGATGTTGCTTTTACTCTTAAGTTGGTTCCTAGGAAAAATAGTTA
AATACTGTGACTTTAAAATGTGAGAGGGTTTTCAAGTACTCATTTTTTTAAATGTCCAAAATTTTTGTCAATCAATTTGA
GGTCTTGTTTGTGTAGAACTGACATTACTTAAAGTTTAACCGAGGAATGGGAGTGAGGCTCTCTCATACCCTATTCAGAA
CTGACTTTTAACAATAATAAATTAAGTTTAAAATATTTTTAAATGAATTGAGCAATGTTGAGTTGAGTCAAGATGGCCGA

PvuII

TCAGAACCGGAACACCTGCAGCAGCTGGCAGGAAGCAGGTCATGTGGCAAGGCTATTTGGGGAAGGGAAAATAAAACCAC
TAGGTAAACTTGTAGCTGTGGTTTGAAGAAGTGGTTTTGAAACACTCTGTCCAGCCCCACCAAACCGAAAGTCCAGGCTG
AGCAAAACACCACCTGGGTAATTTGCATTTCTAAAATAAGTTGAGGATTCAGCCGAAACTGGAGAGGTCCTCTTTTAACT
TATTGAGTTCAACCTTTTAATTTTAGCTTGAGTAGTTCTAGTTTCCCCAAACTTAAGTTTATCGACTTCTAAAATGTATT

EcoRI

TAGAATTCCTTTGCCTAATATTAATGAGGACTTAACCTGTGGAAATATTTTGATGTGGGAAGCTGTTACTGTTAAAACTG
AGGTTATTGGGGTAACTGCTATGTTAAACTTGCATTCAGGGACACAAAAAACTCATGAAAATGGTGCTGGAAAACCCATT
CAAGGGTCAAATTTTCATTTTTTTGCTGTTGGTGGGGAACCTTTGGAGCTGCAGGGTGTGTTAGCAAACTACAGGACCAA
ATATCCTGCTCAAACTGTAACCCCAAAAAATGCTACAGTTGACAGTCAGCAGATGAACACTGACCACAAGGCTGTTTTGG
ATAAGGATAATGCTTATCCAGTGGAGTGCTGGGTTCCTGATCCAAGTAAAAATGAAAACACTAGATATTTTGGAACCTAC
ACAGGTGGGGAAAATGTGCCTCCTGTTTTGCACATTACTAACACAGCAACCACAGTGCTGCTTGATGAGCAGGGTGTTGG
GCCCTTGTGCAAAGCTGACAGCTTGTATGTTTCTGCTGTTGACATTTGTGGGCTGTTTACCAACACTTCTGGAACACAGC
AGTGGAAGGGACTTCCCAGATATTTTAAAATTACCCTTAGAAAGCGGTCTGTGAAAAACCCCTACCCAATTTCCTTTTTG
TTAAGTGACCTAATTAACAGGAGGACACAGAGGGTGGATGGGCAGCCTATGATTGGAATGTCCTCTCAAG

FIG. 19A

```
                                                 BamHI
TAGAGGAGGTTAGGGTTTATGAGGACACAGAGGAGCTTCCTGGGATCCGATCCNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATATAG
CACAAAGACATGCAA
                                              HindIII
ATAATATTTCCCTATGCTCATAAAAACAGCCCTGACCATGAAGCTTTGACAGACGCACAACCCTG
                                               EcoRV
GACTCCCAAGTCTTTCTCTTCAGTGACAAACACAGACATAGGATATCCACCATGGAATGGAGCTGGGTAATGCTCTTCCT
CCTGTCAGGAACTGCAGGTGTCCGCTCTGAGGTCCAGCTGCAACAGTCTGGACCTGAACTGGTGAAGCCTGGAGCTTCAA
TGAAGATTTCCTGCAAGGCTTCTGGTTACTCATTCACTGGCTACACCATGAACTGGGTGAAGCAGAGCCATGGAGAGAAC
CTTGAGTGGATTGGACGTATTAATCCTCACAATGGTGGTACTGACTACAACCAGAAGTTCAAGGACAAGGCCCCTTTAAC
TGTAGACAAGTCATCCAACACAGCCTACATGGAGCTCCTCAGTCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAA
GAGGCTACTATTACTATTCTTTGGACTACTGGGGTCAAGGAACCTCAGTCACCGT
```

FIG. 19B

SUBSTITUTE SHEET

```
        NheI
CTCCTCAGCTAGCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGGACCTCCGAGAGCACAGCCGCCC
TGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC
ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCAC
GAAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAGCACAGG
GAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCCCTCCTGCCTGGACGCACCCCGGCTGTGCAGCCCCAGCCCAGGGCAG
CAAGGGCCCCATCTGTCTCCTCACCCGGAGGCCTCTGACCACCCCACTCATGCTCAGGGAGAGGGTCTTCTGGATTTTTC
CACCAGGCTCCCGGCACCACAGGCTGGATGCCCCTACCCCAGGCCCTGCGCATACAGGGCAGGTGCTGCGCTCAGACCTG
CCAAGAGCCATATCCGGGAGGACCCTGCCCCTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCAGA
CACCTTCTCT
      BglII                          PstI
CCTCCCAGATCTGAGTAACTCCCAATCTTCTCTCTGCAGAGTCCAAATATGGTCCCCCATGCCCATCATGCCCAGGTAAG
CCAACCCAGGCCTCGCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGG
GTGCTGACGCATCCACCTCCATCTCTTCCTCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAA
CCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGT
CCAGT
                                                    SstII
TCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGT
GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCT
CCCGTCCTCCATCGAGAAAACCATCTCCAAAGCCA
          DraIII                              BglI
AAGGTGGGACCCACGGGGTGCGAGGGCCACACGGACAGAGGCCAGCTCGGCCCACCCTCTGCCCTGGGAGTGACCGCTGT
GCCAACCTCTGTCCCTACAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGA
ACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCG
GAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAA
GAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG
                                                        SmaI
CAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCCAGGGCCGGCAAGCCCCCGCTCCCCGGGCTCTCGGGGTCGCGC
GAGGATGCTTGGCACGTACCCCGTCTACATACTTCCCAGGCACCCAGCATGGAAATAAAGCACCCACCACTGCCCTGGGC
CCCTGTGAGACTGTGATGGTTCTTTCCACGGGTCAGGCCGAGTCTGAGGCCTGAGTGACATGAGGGAGGCAGAGCGGGTC
CCACTGTCCCCACACTGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCAGCTGNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
```

FIG. 19C

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNN

BamHI

NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACT
AGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGC

HpaI

TTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGG
AGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCTCTAGTCAAGGCACTAT
ACATCAAATATTCCTTATTAACCCCTTTACAAATTAAAAAGCTAAAGGTACACAATTTTTGAGCATAGTTATTAATAGCA
GACACTCTATGCCTGTGTGGAGTAAGAAAAAACAGTATGTTATGATTATAACTGTTATGCCTACTTATAAAGGTTACAGA
ATATTTTTCCATAATTTTCTTGTATAGCAGTGCAGCTTTTTCCTTTGTGGTGTAAATAGCAAAGCAAGCAAGAGTTCTAT
TACTAAACACAGCATGACTCAAAAAACTTAGCAATTCTGAAGGAAAGTCCTTGGGGTCTTCTACCTTTCTCTTCTTTTTT
GGAGGAGTAGAATGTTGAGAGTCAGCAGTAGCCTCATCATCACTAGATGGCATTTCTTCTGAGCAAAACAGGTTTTCCTC
ATTAAAGGCATTCCACCACTGCTCCCATTCATCAGTTCCATAGGTTGGAATCTAAAATACACAAACAATTAGAATCAGTA
GTTTAACACATTATACACTTAAAAATTTATATTTACCTTATAGCTTTAAATCTCTGTAGGTAGTTTGTCCAATTATGTC
ACACCACAGAAGTAAGGTTCCTTCACAAAGATCCGGNNNNNNNNNNNNNNNNNNNNNNNN

HpaI

NTCATGCTTGCTCCTTGAGGGCGTTAACGCGCAAGGTAACGGCATTTTTATGGGCGGTCAGACGTTCGGCGGCGGCCAGT
GTTTCTATGGTTGAAGCCACCGCGGAGAACCCCTCTTTCGACAGTTCCTGTACGGTCATACGCTTCTGGAAATCTGCCAG
CCCGAGGCTGGAACAGGTGGCGGTGTAACCGTAAGTCGGTAGAACGTGGTTGGTTCCGGAGGCGTAATCACCTGCCGATT
CCGGTGACCAGTCACCAAGAAATACCGAACCGGCGCTGGTGATGCTATCGACCAGTTCACGGGCGTTGCGGGTCTGAATG
ATCAGGTGCTCCGGGCCGTACTGATTAGAGATCTCCACGCACTGCGCTGAATCTTTAGTCACGATCAGGCGGCTGGCGTT
CAGTGCCTGGCGGGCGGTTTCGGCACGCGGCAGTTCCGCCAGTTGGCGTTCGACGGCCTCGGCAACGCGACGCGCCATAT
CAGCAGCGGGCGTCAGTAAAATCACCTGTGAGTCCGGGCCGTGTTCAGCCTGAGAGAGCAAATCAGAAGCCACGAAATCC
GGCGTTGCGCCGCTGTCAGCAATCA

ClaI

CCAGCACTTCCGACGGGCCTGCGGGCATATCGATCTCCGCACCGTCCAGACGCTGGCTCACCTGACGTTTCGCTTCGGTG
ACAAAGGCGTTACCCGGCCCGAAGATTTGTCCACTTTTGGCACGGATTCCGTACCAAACGCCAGTGCGGCAATGGCCTG
TGCGCCGCCGACGTTGAACACGTCCTGCACACCGC

PvuII

ACAGCTGCGCCGCATAAAGGATCTCATCGGCAATCGGCGGCGGTGAGCACAGCACCACTTTTTTACAGCCCGCAATACGC
GCCGGAGTCGCCAGCATTAATACCGTTGAGAAGAGCGGGGCGGAGCCGCCAGGAATATACAACCCAACTGAAGCTACCGG
ACGCGTGACCTGCTGGCAACGCACGCCTGGCTGCGTTTCTACATCTACCGGCGGCAGTTTTTGCGCAGTGTGGAAGGTTT

FIG. 19D

```
CAATATTCTTTACTGCCACCGCCATCGCCTGTTTTAGCTCGTCGCTCAGGCGTTCGCTGGCGGCGGCGATCTCCTCTGCA
GACACCTTCAGCGCGGTAACCGTGGTTTTATCAAACTTCGCGCTGTATTCCCGCAGGGCCTCATCGCCGCGTGCTTTCAC
GTTATCGAGAATATCGTTAACAGTGCGGGTAATGCTTTCAGAGGCGGAAATCGCC
          PvuII
GGGCGCGTTAACAGCTGGCGTTGTTGCACCGCAGTACAGCTATTCCAGTCAATGATTGTGTTAAAGCTCATNNNNCCGGA
TCAGCTTTTTGCAAAAGCCTAGGCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGCCTCGG
CCTCTGCATAAATAAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGGC
GGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGA
CTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCAC
ACCCTAACT
          PvuII
GACACACATTCCACAGCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTC
ACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCGC
AGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATACTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGT
GCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCTCTTCCGCTTCCTCGCTCA
CTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAA
TCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGC
GTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGAC
TATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTG
TCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCG
CTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA
ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT
ACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGT
TACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGC
AGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTCGACGCTCAGTGGAACGAA
AACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTT
TAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGA
TCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGC
CCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGC
CGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGT
```

FIG. 19E

PstI

TGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTGCAGGCATCGTGGTGTC

ACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAA

PvuI

GGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGAT

FIG. 19F

```
                                                          ATG GAA TGG
                                                          Met Glu Trp
                                                            1

AGC TGG GTA ATG CTC TTC CTC CTG TCA GGA ACT GCA GGT GTC CGC TCT
Ser Trp Val Met Leu Phe Leu Leu Ser Gly Thr Ala Gly Val Arg Ser
      5                  10                  15

GAG GTC CAG CTG CAA CAG TCT GGA CCT GAA CTG GTG AAG CCT GGA GCT
Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 20                  25                  30                  35

TCA ATG AAG ATT TCC TGC AAG GCT TCT GGT TAC TCA TTC ACT GGC TAC
Ser Met Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Gly Tyr
                 40                  45                  50

ACC ATG AAC TGG GTG AAG CAG AGC CAT GGA GAG AAC CTT GAG TGG ATT
Thr Met Asn Trp Val Lys Gln Ser His Gly Glu Asn Leu Glu Trp Ile
            55                  60                  65

GGA CGT ATT AAT CCT CAC AAT GGT GGT ACT GAC TAC AAC CAG AAG TTC
Gly Arg Ile Asn Pro His Asn Gly Gly Thr Asp Tyr Asn Gln Lys Phe
        70                  75                  80

AAG GAC AAG GCC CCT TTA ACT GTA GAC AAG TCA TCC AAC ACA GCC TAC
Lys Asp Lys Ala Pro Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
     85                  90                  95

ATG GAG CTC CTC AGT CTG ACA TCT GAG GAC TCT GCA GTC TAT TAC TGT
Met Glu Leu Leu Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
100                 105                 110                 115

GCA AGA GGC TAC TAT TAC TAT TCT TTG GAC TAC TGG GGT CAA GGA ACC
Ala Arg Gly Tyr Tyr Tyr Tyr Ser Leu Asp Tyr Trp Gly Gln Gly Thr
                120                 125                 130

TCA GTC ACC GTC TCC TCA GCT AGC ACC AAG GGC CCA TCC GTC TTC CCC
Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
            135                 140                 145

CTG GCG CCC TGC TCC AGG AGG ACC TCC GAG AGC ACA GCC GCC CTG GGC
Leu Ala Pro Cys Ser Arg Arg Thr Ser Glu Ser Thr Ala Ala Leu Gly
        150                 155                 160

TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC
Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
    165                 170                 175
```

FIG. 19G

```
TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG
Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
180                 185                 190                 195

TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC
Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
                200                 205                 210

AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC AAG CCC AGC
Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser
            215                 220                 225

AAC ACC AAG GTG GAC AAG AGA GTT
Asn Thr Lys Val Asp Lys Arg Val
        230                 235
```

FIG. 19H

```
GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA
Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
  1               5                  10
```

FIG. 19I

```
                                                                GCA CCT GAG
                                                                Ala Pro Glu
                                                                  1

TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA CCC AAG GAC
Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
      5                  10                  15

ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 20                  25                  30                  35

GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAT GGC
Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
                 40                  45                  50

GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
            55                  60                  65

AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
        70                  75                  80

CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GGC CTC CCG
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
     85                  90                  95

TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA
Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
100                 105                 110
```

FIG. 19J

```
                    GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG
                    Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
                      1               5                  10

CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC
Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
            15                  20                  25

CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
        30                  35                  40

AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
    45                  50                  55

TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser
60                  65                  70                  75

AGG TGG CAG GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
                80                  85                  90

CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
            95                  100                 105
```

FIG. 19K

```
Met Ser Phe Asn Thr Ile Ile Asp Trp Asn Ser Cys Thr Ala Val Gln
 1               5                  10                  15

Gln Arg Gln Leu Leu Thr Arg Pro Ala Ile Ser Ala Ser Glu Ser Ile
            20                  25                  30

Thr Arg Thr Val Asn Asp Ile Leu Asp Asn Val Lys Ala Arg Gly Asp
        35                  40                  45

Glu Ala Leu Arg Glu Tyr Ser Ala Lys Phe Asp Lys Thr Thr Val Thr
    50                  55                  60

Ala Leu Lys Val Ser Ala Glu Glu Ile Ala Ala Ala Ser Glu Arg Leu
65                  70                  75                  80

Ser Asp Glu Leu Lys Gln Ala Met Ala Val Ala Val Lys Asn Ile Glu
                85                  90                  95

Thr Phe His Thr Ala Gln Lys Leu Pro Pro Val Asp Val Glu Thr Gln
            100                 105                 110

Pro Gly Val Arg Cys Gln Gln Val Thr Arg Pro Val Ala Ser Val Gly
        115                 120                 125

Leu Tyr Ile Pro Gly Gly Ser Ala Pro Leu Phe Ser Thr Val Leu Met
    130                 135                 140

Leu Ala Thr Pro Ala Arg Ile Ala Gly Cys Lys Lys Val Val Leu Cys
145                 150                 155                 160

Ser Pro Pro Pro Ile Ala Asp Glu Ile Leu Tyr Ala Ala Gln Leu Cys
                165                 170                 175

Gly Val Gln Asp Val Phe Asn Val Gly Gly Ala Gln Ala Ile Ala Ala
            180                 185                 190

Leu Ala Phe Gly Thr Glu Ser Val Pro Lys Val Asp Lys Ile Phe Gly
        195                 200                 205

Pro Gly Asn Ala Phe Val Thr Glu Ala Lys Arg Gln Val Ser Gln Arg
    210                 215                 220

Leu Asp Gly Ala Glu Ile Asp Met Pro Ala Gly Pro Ser Glu Val Leu
225                 230                 235                 240

Val Ile Ala Asp Ser Gly Ala Thr Pro Asp Phe Val Ala Ser Asp Leu
                245                 250                 255

Leu Ser Gln Ala Glu His Gly Pro Asp Ser Gln Val Ile Leu Leu Thr
            260                 265                 270
```

FIG. 19L

```
Pro Ala Ala Asp Met Ala Arg Arg Val Ala Glu Ala Val Glu Arg Gln
        275                 280                 285

Leu Ala Glu Leu Pro Arg Ala Glu Thr Ala Arg Gln Ala Leu Asn Ala
    290                 295                 300

Ser Arg Leu Ile Val Thr Lys Asp Ser Ala Gln Cys Val Glu Ile Ser
305                 310                 315                 320

Asn Gln Tyr Gly Pro Glu His Leu Ile Ile Gln Thr Arg Asn Ala Arg
                325                 330                 335

Glu Leu Val Asp Ser Ile Thr Ser Ala Gly Ser Val Phe Leu Gly Asp
            340                 345                 350

Trp Ser Pro Glu Ser Ala Gly Asp Tyr Ala Ser Gly Thr Asn His Val
        355                 360                 365

Leu Pro Thr Tyr Gly Tyr Thr Ala Thr Cys Ser Ser Leu Gly Leu Ala
    370                 375                 380

Asp Phe Gln Lys Arg Met Thr Val Gln Glu Leu Ser Lys Glu Gly Phe
385                 390                 395                 400

Ser Ala Val Ala Ser Thr Ile Glu Thr Leu Ala Ala Ala Glu Arg Leu
            405                 410                 415

Thr Ala His Lys Asn Ala Val Thr Leu Arg Val Asn Ala Leu Lys Glu
            420                 425                 430

Gln Ala
```

FIG. 19M

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/10206

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K47/48

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	PROC. NATL. ACAD. SCI. U. S. A., VOL. 88, NO. 11, PAGE(S) 4771-5, 1 June 1991, FRIDEN P M ET AL. 'Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier.' see abstract ---	1-24
Y	WO,A,9 103 259 (ALKERMES INC) 21 March 1991 see the whole document ---	1-24
Y	WO,A,9 104 753 (CETUS CORP.) 18 April 1991 see page 12, line 17 - line 31; claims 8-9,12-14 ---	1-24

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 APRIL 1993	03. 05. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	DULLAART A.W.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	J. IMMUNOL., VOL. 144 NO. 8, PAGES 3211-17, 15 April 1990, US HOOGENBOOM, HENNIE R. ET AL. 'Cloning and expression of a chimeric antibody directed against the human transferrin receptor' see the whole document	1-24
Y	WO,A,9 109 965 (UNITED STATES OF AMERICA; DEPARTMENT OF COMMERCE) 11 July 1991 see tables	1-24
Y	US,A,4 545 985 (PASTAN ET AL.) 8 October 1985 see column 6, line 41 - column 7, line 30; examples 1,5	1-24
Y	EP,A,0 449 769 (SANDOZ LTD.; ROYAL FREE HOSPITAL SCHOOL OF MEDECINE) 2 October 1991 see page 13 - page 17; claims	1-24
P,Y	WO,A,9 213 570 (BOEHRINGER INGELHEIM INTERNATIONAL GMBH) 20 August 1992 see examples	1-24

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9210206
SA 67926

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 13/04/93
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9103259	21-03-91	US-A-	5154924	13-10-92
		AU-A-	6445990	08-04-91
		CA-A-	2066244	08-03-91
		EP-A-	0490998	24-06-92
		US-A-	5182107	26-01-93
WO-A-9104753	18-04-91	None		
WO-A-9109965	11-07-91	AU-A-	7172391	24-07-91
		EP-A-	0506854	07-10-92
US-A-4545985	08-10-85	None		
EP-A-0449769	02-10-91	AU-A-	7290991	19-09-91
		JP-A-	4316600	06-11-92
WO-A-9213570	20-08-92	DE-A-	4104186	13-08-92