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(54) Titre : PLASMIDES DE VECTEURS MULTIGENIQUES ET METHODES D'ACCROISSEMENT DE L'EXPRESSION DE POLYPEPTIDES RECOMBINANTS
(54) Title: METHODS VECTORS PLASMIDS AND METHODS FOR INCREASING EXPRESSION OF RECOMBINANT POLYPEPTIDES

(57) **Abrégé/Abstract:**

The present invention provides novel methods and materials for increasing the expression of recombinant polypeptides. Methods and materials of the invention allow increased expression of transcription units that include recombinant DNA sequences which encode polypeptides of interest. The present invention provides expression vectors which contain multiple copies of a transcription unit encoding a polypeptide of interest separated by at least one selective marker gene and methods for sequentially transforming or transfecting host cells with expression vectors to increase transcription unit dosage and expression.

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(54) Title: METHODS VECTORS PLASMIDS AND METHODS FOR INCREASING EXPRESSION OF RECOMBINANT POLYPEPTIDES

(57) Abstract: The present invention provides novel methods and materials for increasing the expression of recombinant polypep-
tides. Methods and materials of the invention allow increased expression of transcription units that include recombinant DNA se-
quences which encode polypeptides of interest. The present invention provides expression vectors which contain multiple copies of
a transcription unit encoding a polypeptide of interest separated by at least one selective marker gene and methods for sequentially
transforming or transfecting host cells with expression vectors to increase transcription unit dosage and expression.

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MULTIGENIC VECTORS PLASMIDS AND METHODS FOR INCREASING EXPRESSION OF
RECOMBINANT POLYPEPTIDES

RELATED APPLICATIONS

[001] This application claims the priority of U.S. Provisional Application No. 60/368,530 filed March 29, 2002, the disclosure of which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

[002] Recombinant polypeptide compositions are increasingly being used in a wide variety of treatments or therapies across the health-related fields. Recombinant polypeptides are being used in diagnostic procedures, as tools in preventative medicine, and to directly save lives through administrative therapies. In addition, recombinant polypeptides are found in a wide array of both health and cosmetic products, used to increase the quality of life. Complex polypeptide products are also routinely used in research laboratories both as end-products of analyses themselves and as agents in assays for the study or preparation of other molecules. Such uses often lead to the discovery of the causes of disease and an understanding of the underlying disease mechanism(s), furthering development toward diagnoses and/or viable treatments. Recombinant polypeptide products also play a vital role in a variety of industrial settings, in areas ranging from farming to the processing of food materials and the raising of livestock to the catalytic degradation of both natural and synthetic by-products and waste materials.

[003] The production of polypeptides for preclinical and clinical evaluation often requires multigram quantities [Kelley, *Bio/Technology* 14: 28-31 (1996)]. Industrial applications using such polypeptides generally require even greater quantities and the costs of production are often prohibitive. While there are a variety of ways to chemically synthesize simple polypeptides when the amino acid sequence is known, this method of production has problems with respect to larger polypeptides, e.g., uncertain deviations from native conformational folding, an absence of intracellular

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post-translational modification, and reduced or limited bioactivity. For these and other reasons, recombinant DNA technology is the most common production method of choice and offers the greatest potential for large-scale production at high efficiency and reasonable cost. Accordingly, the production of useful quantities of these important polypeptides is typically generated through standard recombinant DNA technology, especially where the polypeptide of interest is ultimately modified or where it only occurs naturally in very small amounts.

[004] Current recombinant DNA techniques used for expressing polypeptides can exhibit numerous limitations, including, for example, significant production costs for materials and reagents and low product yield. In addition, production can be time consuming and can require substantial monitoring with limited control. These, as well as other problems and limitations involved in production of recombinant polypeptides, along with inefficiencies in the current methods for producing recombinant polypeptides, ultimately can result in a significant toll in costs, resources, health, and life itself. Thus, given the fundamental role and countless uses for recombinant polypeptides and the limitations current methods for recombinant polypeptide production, it is of primary importance to employ a method of recombinant gene expression that maximizes protein production and, ultimately, saves time, money and other resources. Accordingly, there exists a need for methods that can increase, maximize and/or optimize the production of recombinant polypeptides.

[005] Several factors can influence recombinant expression in mammalian cells, including promoter strength, the context of the translation initiation region, the efficiency of the 3' untranslated region to polyadenylate and terminate transcription, the insertion site of the randomly integrated recombinant gene in the host chromosome, and the number of integrated copies of the gene that is being expressed. Of these factors, the choice of promoter and 3' untranslated regions can significantly impact expression levels. Viral promoters are often used because they are thought to promote high expression levels. The optimal translation initiation sequence, ACCATGG, also known as the Kozak box, can promote more efficient polypeptide synthesis and, therefore, higher expression levels.

[006] One strategy employed for increasing expression of polypeptides uses expression vectors containing multiple integrated copies of a desired gene.

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Improvements in recombinant polypeptide expression in mammalian cells can be achieved in this manner by effectively increasing the gene dosage in a transfected host cell. Increases in gene copy number are most commonly achieved by gene amplification using cell lines deficient in an enzyme such as dihydrofolate reductase (DHFR) or glutamine synthetase (GS) in conjunction with expression vectors containing genes encoding these enzymes and agents such as methotrexate (MTX), which inhibits DHFR, and methionine sulfoxamine (MSX), which inhibits GS. Using expression vectors containing the recombinant gene under control of a strong promoter and genes encoding DHFR or GS, DHFR⁺ or GS⁺ transfectants, respectively, are first obtained and gene amplification is then achieved by growing the transfectants in progressively increasing concentrations of MTX or MSX.

[007] While gene amplification can result in higher levels of expression, it has several drawbacks. First, cell lines that have mutations in the genes encoding the selective enzymes are generally used for gene amplification. In the case of DHFR, both chromosomal copies need to be mutated and, consequently, these cell lines can be less robust than wildtype cells. This can ultimately lead to cells which secrete lower net amounts of the protein of interest as compared to more robust cells that thrive and are stable. In the case of GS, the lymphoid cell line NSO is naturally GS⁻, but CHO-K1, another commonly-used cell line, is GS⁺ and requires selection directly for MSX-resistant transfectants. A second problem with current methods of gene amplification is that the amplification can result in cell lines that are unstable in the absence of selective pressure, thus requiring the maintenance of selective pressure. Finally, current methods for gene amplification can be exceedingly time-consuming and can require up to several months to complete with a proportional allocation of both resources and costs. Another strategy for increasing expression of polypeptides uses sequential transfections with expression vectors, each containing single copies of a desired gene but with different selective marker genes such as *gpt*, *neo* and *his*. Gene copy number for the first transfection is one, from the second transfection, two and so on. While this approach can achieve higher levels of expression, it has drawbacks in that the number of gene copies increases only modestly even after three sequential transfections.

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[008] Therefore, in view of all these problems, novel ways to increase gene expression through increased gene copy number by methods that do not depend on the use of mutant cell lines and current methods for gene amplification would be highly desirable.

[009] For all the foregoing reasons, there is a need in the art for improved methods of expressing polypeptides of interest at increased net concentrations from stable cell lines, while maximizing efficiency and minimizing expenditure of time, resources, and costs in production. Furthermore, there is a need for expression vectors and stable cell lines harboring and/or integrating into their chromosomes such vectors that are capable of efficiently producing secreting high concentrations of valuable and useful polypeptide products.

SUMMARY OF THE INVENTION

[010] The present invention provides novel methods and materials for increasing the expression of recombinant polypeptides. In one aspect, the present invention is directed to novel methods for efficiently increasing the expression of recombinant polypeptides from host cells, thus satisfying the need for producing increased concentrations of valuable proteins and polypeptides of interest. The benefits of such methods can include minimizing time, resources and/or costs of production. In another aspect, the present invention is directed to novel vectors comprising multiple copies of a recombinant DNA sequence encoding, for example, a recombinant polypeptide of interest, separated by at least one selective marker gene and cell lines harboring such vector constructs, thus satisfying the need for vectors and host cells, including stable cell lines, which are capable of efficiently producing increased concentrations of valuable and/or useful polypeptide products.

[011] According to the present invention, the number of transcription units, *i.e.*, recombinant DNA sequences which encode a recombinant polypeptide of interest, introduced into a host cell is increased so that there are more copies of the transcription unit to express. This is achieved by incorporating at least two copies of the transcription unit on the same vector. In certain embodiments, each transcription unit copy is placed under control of its own promoter and 3' untranslated region. Increasing transcription unit dosage and expression is achieved by transfection with

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such a vector. Performing additional transformations or transfections with additional vectors, for example, a second and then third sequential transformation or transfection with such vectors, each containing at least two transcription unit copies can yield further increases in transcription unit dosage and expression relative to a vector with only a single transcription unit copy. Preferably, each additional vector for each sequential transformation or transfection contains a different selective marker gene.

[012] While integration into a host cell chromosome of a linear DNA sequence containing two transcription unit copies on the same vector can increase gene dosage and therefore expression, it could also lead to instability due to homologous recombination. According to the present invention, such potential instability, especially during the early stages of cell line development, is overcome by the design of the vector so that a gene encoding a selective marker is positioned between at least two identical or similar transcription units encoding a recombinant polypeptide of interest. Preferably, the vector is linearized (*e.g.*, via a unique restriction enzyme site prior to transfection or transformation such that the selective marker gene is positioned between at least two and no more than 8 transcription unit copies, each transcription unit encoding recombinant polypeptide(s) of interest. For example, the vector comprises as [(transcription unit)_x - selective marker gene - (transcription unit)_x], wherein $x = 1-4$. Using such a vector, any homologous recombination between, for example, two identical or similar transcription unit DNA sequences would then potentially delete or inactivate the gene encoding resistance to the selective agent. Thus, cells transfected or transformed with such a vector and that underwent homologous recombination would not be able to grow in the presence of the selective agent. Application of at least some selective pressure should promote maintenance of transcription unit copies from such novel vectors.

[013] One aspect of the invention involves the design, construction and use of vectors, preferably mammalian expression vectors containing at least two copies of a transcription unit encoding a recombinant polypeptide of interest to be expressed, each under control of a promoter and a 3' untranslated region and positioned so that, when the vector is linearized by digestion at a unique restriction enzyme site, at least two transcription unit copies are separated by a selective marker gene in the linear sequence. According to this aspect of the invention, the design or configuration

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includes positioning the selective marker gene between at least two transcription units for example, [(transcription unit) – selective marker gene – (transcription unit)], which will in turn stabilize the linear vector DNA with that position of the selective marker gene between transcription units integrated into a host cell chromosome. In contrast, homologous recombination between at least two copies of the transcription unit can lead to the loss of at least one of the transcription units and the selective marker gene. As an additional benefit, this aspect of the present invention can yield a vector that has multiple enhancers. As an example, the multiple enhancers can include several from promoters controlling transcription unit expression and one from a promoter controlling the selective marker gene(s). Because enhancers are known to act bi-directionally and also at a distance, this vector design or configuration can lead to increased transcription and consequently increased net concentrations of the polypeptide of interest.

[014] Another aspect of the invention also provides for the use of vectors containing two or more transcription units (preferably between two and no more than eight) with various selective marker genes, including, for example, *gpt*, *neo* or *his*, for selection of mammalian cell transfectants. By use of these additional selective marker genes, transfectants can be obtained with multiple copies of a transcription unit and further increased by performing a number of sequential transfections with vectors according to the invention.

[015] In addition to selective marker genes such as *gpt*, *neo* and *his* for selection of transfectants, the vector configuration as described herein can be used in conjunction with genes encoding amplifiable selective marker genes such as DHFR or GS. Amplification of such vectors may yield transfectants with vector DNA integrated into a host cell chromosome so that transcription units are separated by the DHFR or GS marker gene.

[016] The present invention provides multiple transcription unit vectors, including, those containing for example, two, three, four, five, six, seven and eight and so on, copies of a transcription unit with DNA sequences encoding a recombinant polypeptide of interest. Preferably, there are at least two and no more than eight transcription units. Such vectors are constructed with at least one selective marker gene positioned between or separating the transcription units in a manner that allows

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the stable integration into a host cell chromosome and reduction, avoidance or nullification of homologous recombination. Vectors comprising multiple copies of a transcription unit each separated by a selective marker gene, wherein the transcription unit encodes a polypeptides are provided by the invention. All such permutations of multiple transcription unit copies are contemplated by this invention as one skilled in the art would recognize from the disclosure herein.

[017] An aspect of the present invention includes vectors containing transcription units which encode subunits of dimeric or higher order multimeric proteins. According to the invention, transcription units encoding different subunits of a multimeric protein, each under control of a promoter and a 3' untranslated region, are separated by a selective marker gene. For multimeric proteins encoded by at least two distinct genes (for instance, immunoglobulin light and heavy chains or at least the variable regions of immunoglobulin light and heavy chains), transcription units encoding the desired subunits are first linked with or without an internal ribosome entry site (IRES) and this bi (or poly) transcription unit can be placed under the control of a promoter and a 3' untranslated region. These transcription units then can be combined to construct vectors with at least two copies of a bi-cistronic unit separated by a selective marker gene. For example, vectors comprise [(transcription unit – IRES – transcription unit) – selective marker gene – (transcription unit – IRES – transcription unit)]. Also, for example, vectors can comprise [(transcription unit – transcription unit) – selective marker – (transcription unit – transcription unit)]. In certain embodiments, each transcription unit copy is placed under control of its own promoter (P) and 3' untranslated region (3' UT). For example, vectors can comprise [(P – transcription unit – 3' UT) – (CP – transcription unit – 3' UT) – selective marker gene – (P – transcription unit – 3' UT) – (CP – transcription unit – 3' UT) –].

[018] The present invention provides methods of producing a recombinant polypeptide and/or of increasing the expression of a transcription unit comprising the following steps:

- (a) culturing under selective conditions cells which have been transformed or transfected with a vector containing multiple copies of a transcription unit separated by at least one selective marker gene wherein the transcription unit encodes a polypeptide; and

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(b) expressing the polypeptide from the multiple copies of the transcription unit.

According to the present invention, the number of copies of the transcription unit on the vector is at least two, but can be three, four, five, six, seven, eight, etc. Preferably, the number of copies of the transcription unit is at least two and not more than eight on any vector. Each vector containing at least two transcription units separated by a selective marker gene can be introduced into host cells. Additional vectors, each preferably with a different selective marker gene, can be sequentially introduced into the host cells to further increase gene dosage and expression. Preferred host cells include Chinese hamster ovary (CHO) cells, such as CHO-K1 cells. The methods of the present invention also allow for transcription units encoding any polypeptide product of interest, including, for example, multimeric protein products, such as immunoglobulins.

[019] The present invention provides methods for constructing vectors or segments thereof comprising building an expression vector or cloning vector with multiple copies of a transcription unit separated by at least one selective marker gene. According to the invention, the number of multiple copies of the transcription unit is at least two. Preferably, the number of multiple copies of the transcription unit on a single vector is less than or equal to eight.

[020] The present invention provides vectors comprising multiple copies of a transcription unit encoding recombinant mammalian polypeptides of interest separated by at least one selective marker gene. According to the invention, the number of multiple copies of the transcription unit on the vector or transformed/transfected into host cells is at least two. Preferably, the number of multiple copies of the transcription unit is equal to or less than eight. Multiple vectors can be used and multiple transformations or transfections can be carried out to produce clones and cell lines expressing the polypeptide of interest. Exemplary vectors are described herein containing a transcriptional unit encoding a BPI protein product, for example, a BPI fragment, BPI analog, BPI variant or BPI-derived peptide. An exemplary expression vector described herein contains a transcriptional unit encoding rBPI₂₁. The present invention further provides vectors containing transcription units encoding different subunits of a multimeric protein, including

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vectors containing transcription units encoding antibody light and heavy chains, or at least the variable regions of light and heavy chains.

[021] According to the present invention, vectors contain selective marker genes which can vary, but may include a *gpt*, *neo* or *his* gene. A vector according to the present invention can also include multiple copies of a transcription unit in a direct repeat orientation and/or in an inverted repeat orientation. Additionally, the present invention provides a vector containing multiple copies of transcription units that are identical or similar. Such copies can be homologous copies, for example, at least 25% homologous. Accordingly, the copies can be identical or similar by any percentage between 25% and 100% homologous, preferably identical or similar by at least 80%, including 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%.

[022] The present invention further embodies all variations of vectors or segments thereof within the scope and spirit of this disclosure or as may be understood or contemplated by one skilled in the art through this disclosure and/or the art.

[023] The present invention provides host cells comprising vectors or segments thereof, [(transcription unit)_x - selective marker gene - (transcription unit)_x], wherein x = 1-4 as disclosed herein. According to the invention, host cells include any or all eukaryotic host cell types. By way of example, eukaryotic host cell types may include any mammalian cell, for example, the Chinese hamster ovary (CHO) cell line, the DHFR⁺ CHO-K1 cell line, the DHFR⁻ DUKX-B11 (DXB11) cell line or DG-44 cell line. Eukaryotic host cell types may also include plant, insect, and yeast cells. In addition, the present invention provides any stable cell line comprising vectors or segments thereof within the scope and spirit of this disclosure or as may be understood or contemplated by one skilled in the art through this disclosure and/or the art. Such cells may be propagated by any means, and may include cells in an attached or suspension state with any growth or support medium or sustaining solution.

[024] In view of all the current problems and limitations in the art, including those discussed hereinabove, there are many advantages of the present invention over the art, including increased recombinant polypeptide production, increased production efficiency, greater control and/or regulation over the quantities of polypeptide expressed, increased stability of cell lines, and/or decreased costs for materials,

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reagents, and/or other resources. For example, the present invention provides methods of vector construction that, when such vectors are linearized, minimize or avoid homologous recombination of multiple copies of transcription units by separating transcription units containing DNA sequences encoding polypeptides of interest with at least one selective marker gene. As another example, the present invention provides methods to further increase expression through sequential transfections with multiple-transcription unit constructs. This leads to clones and/or cell lines which can produce substantially increased levels of polypeptides of interest, while still maintaining viability and stability in growth medium.

[025] While various modifications may be suggested by those versed in the art, it should be understood that this invention contemplates all embodiments within the scope of the patent warranted hereon and all such modifications as reasonably and properly come within the scope of this contribution to the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[026] These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

[027] Figure 1 depicts a construction map for vector pING1733.

[028] Figure 2 depicts a construction map for vector pING1737.

[029] Figure 3A shows the structure of vector pING1737 linearized with *Xba*I.

[030] Figure 3B shows the structure of vector pING1737 linearized with *Not*I.

[031] Figure 4 depicts a construction map for vector pING1753.

[032] Figure 5 depicts a construction map for vector pING1744.

[033] Figure 6 shows the structure of vector pING1744 linearized with *Xba*I.

[034] Figure 7 shows a linear map of vector pING4155 showing the location of binding of an rBPI₂₁ probe.

[035] Figure 8 shows a Northern blot of rBPI₂₁ RNA isolated from untransfected CHO-K1 cells, Clone 228 and Clone 689.

[036] Figure 9 depicts a construction map for vector pING1928.

[037] Figure 10 depicts a construction map for vector pING1931.

[038] Figure 11 depicts construction maps for vector pING1932 and pING1932R.

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[039] Figure 12 depicts a construction map for vector pING1933.

[040] Figure 13 shows the amino acid changes (underlined) made during human engineering of the ING-1 light chain variable region, including mouse and human *light* chain variable region sequences, risk line for human engineered method, and low as well as moderate risk changes.

[041] Figure 14 depicts a construction map for vector pING1936.

[042] Figure 15 shows the amino acid changes (underlined) made during human engineering of the ING-1 heavy chain variable region, including mouse and human *heavy* chain variable region sequences, risk line for human engineered method, and low as well as moderate risk changes..

[043] Figure 16 depicts a construction map for vector pING1937.

[044] Figure 17 depicts a construction map for vector pING1959.

[045] Figure 18 depicts a construction map for vector pING1957.

[046] Figure 19 depicts a construction map for vector pING1963.

[047] Figure 20 depicts a construction map for vector pING1964.

[048] Figure 21 depicts a construction map for vector pING1965.

[049] Figure 22 shows the structure of vector pING1964 linearized with *Not*1.

[050] Figure 23 shows competition binding results for human engineered low risk ING-1.

[051] Figure 24 shows competition binding results for human engineered anti-Ep-CAM antibodies, including low risk as well as low plus moderate risk ING-1 as compared with mouse-human chimeric ING-1.

[052] Figure 25 shows competition binding results for ING-1 with combinations of light and heavy chains modified at either low or low plus moderate risk positions. ING-1 (heMab) has both heavy and light chains modified at low risk positions.

[053] Figure 26 shows competition binding results for human engineered low risk ht chain with additional P2 or P3) or pair combinations (P1P2 or P1P3) of moderate risk proline changes.

[054] Figure 27 depicts a construction map for vector pING1954.

[055] Figure 28 shows a direct binding ELISA for human engineered (low risk) ING-1 with soluble Ep-CAM.

[056] Figure 29 depicts a construction map for vector pING1732-R1ClaRV.

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- [057] Figure 30 depicts a construction map for vector pING1736-R1C1aKpnl.
- [058] Figure 31 depicts a construction map for vector pING2050.
- [059] Figure 32 depicts a construction map for vector pING2051.
- [060] Figure 33 depicts the DNA and amino acid sequence for an anti-CD18 light chain (designated LDP-01 or LDP-1 LC).
- [061] Figure 34 depicts the DNA and amino acid sequence for an anti-CD18 heavy chain (designated LDP-01 or LDP-1 HC).
- [062] Figure 35 depicts a construction map for vector pING2052.
- [063] Figure 36 depicts a construction map for vector pING2057.
- [064] Figure 37 shows the structure of vector pING2052 linearized with *Xba*I.
- [065] Figure 38 shows the growth and productivity of various anti-CD18 producing clones, including 1-15, 1-37, 66B9, 84C4, 128D12, 90B6.
- [066] Figure 39 shows the level of expression from clones 264EZ and 254G12 in additional commercially available media, including ProCH04 and ProCH05.
- [067] Figure 40 depicts a construction map for vector pING2053.
- [068] Figure 41 depicts a construction map for vector pING2054.
- [069] Figure 42 depicts a construction map for vector pING2055.
- [070] Figure 43 depicts a construction map for vector pING2056.
- [071] Figure 44 shows the structure of vectors pING2054 and pING2055 linearized with *Xba*I.
- [072] Figure 45 shows the growth and productivity of Clone 3G8 *neo* Subclone G5F1.

DETAILED DESCRIPTION

[073] The present invention provides novel materials and methods for increasing the expression of recombinant polypeptides, including expression from stable cell lines. Novel expression vectors are provided comprising multiple copies of a transcription unit containing a recombinant DNA sequence which encodes a polypeptide of interest, separated by a selective marker gene. Cell lines harboring these vector constructs are also provided that are capable of expressing the polypeptides encoded by the transcription units. Methods of increasing expression of any polypeptide of interest, including multimeric polypeptides such as immunoglobulins, are provided.

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An exemplary polypeptide described herein is a BPI protein product, such as rBPI₂₁. Another exemplary polypeptide is an immunoglobulin, or a polypeptide comprising at least the variable regions of the light and heavy chains of an immunoglobulin. As described herein, two or more copies of a recombinant gene are inserted into an expression vector wherein the genes are separated by various selective marker genes such as *gpt*, *neo* or *his*, in order to prepare and/or select cell transformants or transfectants, preferably mammalian cell transformants or transfectants and to reduce, minimize or avoid homologous recombination. An increase in net yield of recombinant polypeptide product from host cells harboring these vector constructs is achieved.

[074] Examples of polypeptides expressed through use of the methods of the present invention can include any polypeptide of interest. Expressed recombinant polypeptides of the invention can include any sequences known or contemplated in the art. Polypeptides of interest can be produced by any means through use of the methods disclosed herein, including transformation or transfection of host cells such as mammalian cells with the disclosed vector constructs. Polypeptide production can be provided by any means in a host cell, including accumulation in an intracellular compartment or secretion from the cell into a culture supernatant. Host cells of the present invention may be propagated or cultured by any method known or contemplated in the art, including but not limited to growth in culture tubes, flasks, roller bottles, shake flasks or fermentors. Isolation and/or purification of polypeptide products may be conducted by any means known or contemplated in the art.

[075] In accordance with the invention, vectors were designed that contain multiple copies of a transcription unit encoding a polypeptides of interest. Two-transcription unit vectors were constructed and used to prepare cell lines expressing the encoded polypeptides. Two of these two-transcription unit vectors were used in sequential transfections with cells to produce cell lines, which produced approximately twice the level of polypeptide as a cell line which contained a single two-transcription unit vector or two one-transcription unit vectors as a result of sequential transfection. This increased productivity appeared to result from a two-fold increase in specific productivity and not merely from an increase in cell density. The examples herein demonstrate that increased expression can be achieved through increased transcription

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unit dosage in a non-amplified expression system through the methods of the present invention in at least two different employed strategies with surprising effectiveness. First, by positioning the selective marker gene between multiple copies of transcription units encoding the gene of interest, the problem of homologous recombination can be avoided and high producing, stable cell lines can be developed which express multiple copies of this gene. Second, by sequentially transfecting cells in the methods of the present invention, the number of multiple integrated copies of transcription units can be increased stepwise to increase expression levels. The present invention contemplates the use of any transcription unit encoding any polypeptide of interest.

[076] The present invention thus provides vectors comprising multiple copies of transcription units that are identical or similar. An aspect of the present invention includes vectors containing transcription units which encode subunits of dimeric or higher order multimeric proteins. Such copies can be homologous copies, for example, at least 25% homologous. Accordingly, the copies can be identical or similar by any percentage between 25% and 100% homologous. Percent sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST or FASTA, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences, or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff *et al.*, in: *Atlas of Protein Sequence and Structure*, vol 5, supp.3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the longer sequences in order to align the two sequences.

[077] Transcription Unit refers to a DNA sequence encoding a polypeptide and can include a promoter and/or a 3' untranslated region.

[078] DNA sequence or recombinant DNA sequence refers to any natural or synthetic DNA sequence derived using molecular biology and/or cloning techniques

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including, but not limited to, cDNA sequences, genomic sequences, polymerase chain reaction (PCR)-amplified DNA sequences, as well as any chemically-synthesized DNA sequences obtained using techniques known by those of ordinary skill in the art. Recombinant DNA sequences for use in the present invention may be derived from any native or natural source, including prokaryotic DNA sequences or eukaryotic DNA sequences, from sources such as mammalian, plant, yeast, bacterial or viral sources, or from any synthetic or non-natural source, such as chemically-synthesized oligonucleotides.

[079] Polypeptide refers to a molecule comprising a polymer of amino acids linked together by a peptide bond(s). Polypeptides include polypeptides of any length, including proteins (*e.g.*, ≥ 50 amino acids) and peptides (*e.g.*, 2-49 amino acids) [Alberts *et al.*, *Molecular Biology of the Cell*, 3rd Ed., (1994)]. Polypeptides include proteins or peptides of any activity or bioactivity, including, for example, bioactive polypeptides, such as enzymatic proteins or peptides (*e.g.*, proteases, kinases, phosphatases), receptor proteins or peptides, transporter proteins or peptides, bactericidal and/or endotoxin-binding proteins or peptides, structural proteins or peptides, immune proteins or peptides (*e.g.*, antibodies or antigen-binding portions thereof), cytokine proteins or peptides, toxins, chemotherapeutic agents, antibiotics, hormones, growth factors, vaccines, or the like. For example, polypeptides include, but are not limited to, full length proteins, fragments, analogs, variants or derivatives of proteins, fusion proteins, chimeric proteins, multimeric proteins or their subunits. Peptides include, but are not limited to, antimicrobial peptides, herbicidal peptides, peptide hormones, neuropeptides, toxins or the like.

[080] Bioactive polypeptide refers to a polypeptide product exhibiting bioactivity akin to that of a protein or peptide innately produced within a living organism for a physiological purpose, as well as to intermediates which can be processed into such proteins and peptides, as by cleavage away of DNA sequences encoding superfluous protein, folding, combination (as in the case of the A and B chains of insulin or the heavy and light chains of immunoglobulins), post-translational modification, etc.

[081] Vector refers to an agent or vehicle for carrying polynucleotide sequences (*e.g.*, DNA or RNA). Thus, vectors are said to contain (*i.e.*, comprise) such polynucleotide sequences.

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[082] Cloning vector refers to an agent or vehicle that may be capable of autonomously replicating, including but not limited to plasmids, phagemids, or phage, comprising a polynucleotide molecules, including a DNA molecule, to which one or more additional polynucleotide segments, including DNA segments, can be or have been added.

[083] Expression vector refers to a vector into which one or more transcriptional and translational regulation sequence(s) have been incorporated.

[084] Promoter refers to a site on a DNA molecule to which an RNA polymerase and/or any associated factors attaches and at which transcription is initiated. Promoters may include those well-known in the art, including from SV40, HSV, bovine growth hormone, thymidine kinase, MPSV, mouse beta globin, human EF1, MSV-LTR, RSV, MMTV-LTR, CMV, MLV, Chinese hamster elongation factor, mouse Abelson LTR, human C-fos promoter or the like. Promoters for yeast, insect, plant and/or bacterial expression are also well-known in the art.

[085] Enhancer refers to a control element that can increase expression of a gene or genetic sequence.

[086] Selective marker or selectable marker refers to a gene or any genetic material that encodes for a phenotype that may encourage or inhibit the growth of a cell or organism in the absence or presence of a chosen compound or condition. Selective marker genes may include *gpt*, *res*, *his* genes, or also genes for adenosine deaminase (ADA), thymidine kinase (TK), adenine phosphoribosyl transferase (APRT), zeocin resistance gene, hygromycin resistance gene, or puromycin resistance gene.

[087] Transformation or transfection refers to the introduction of genetic material, such as a vector, including a cloning or expression vector, into a recipient host cell that changes the genotype and results in a phenotypic change in the recipient cell. A host cell or those colonies resulting from a host cell that has undergone successful transformation or transfection is said to be transformed or transfected or may be referred to as a transformant or transfectant.

[088] The present invention specifically provides for the development of exemplary vectors, including two and four transcription unit expression vectors which are functional and selectable in a host cell.

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[089] A series of exemplary vectors have been constructed containing two copies of a gene encoding a BPI protein product, rBPI₂₁, each under the control of a hCMV promoter and mouse light chain 3' untranslated region with 0, 1 or 2 copies of a human heavy chain enhancer and either a *gpt* or *neo* gene for selection of transformants or transfectants. The vectors also differed in the orientation of rBPI₂₁ genes on the vector. Initial testing of these vectors indicated that a preferred configuration was represented by the *gpt* vector, pING1737 (Figure 2). This vector and its *neo* version, pING1733 (Figure 1) were selected for use in full-scale cell line development.

[090] According to the present invention, the presence of two copies of a rBPI₂₁ gene and flanking promoter and 3' untranslated region sequences in pING1737 and pING1733 should promote higher expression through increased gene dosage. However, one potentially adverse consequence of this configuration could be instability of the integrated DNA as a result of recombination between two homologous rBPI₂₁ and flanking sequences leading to elimination of one of the rBPI₂₁ copies and lower expression. To reduce the chance of recombination, these vectors have been designed according to the invention so that when linearized at a unique *Xba*I site, the rBPI₂₁ genes would be separated by the selective marker gene as integrated into a host cell chromosome. This configuration would have the greatest potential stability in the chromosome, at least as long as the transfectants were maintained in the presence of selective agent(s), because homologous recombination between the gene copies of rBPI₂₁ would also eliminate selective marker gene(s). To evaluate the effect of gene placement on both gene expression and clone stability, both configurations were tested in cell line development for rBPI₂₁ expression described in the examples that follow.

[091] Bactericidal/permeability-increasing protein (BPI) is a protein isolated from the granules of mammalian polymorphonuclear leukocytes (PMNs or neutrophils), which are blood cells essential in the defense against invading microorganisms. Human BPI protein has been isolated from PMNs by acid extraction combined with either ion exchange chromatography [Elsbach, *J. Biol. Chem.*, 254: 11000 (1979)] or *E. coli* affinity chromatography [Weiss, *et al.*, *Blood* 69: 652 (1987)]. BPI obtained in such a manner is referred to herein as natural BPI and was initially shown by Elsbach

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and Weiss to have bactericidal activity against gram-negative bacteria. The molecular weight of human BPI is approximately 55,000 daltons (55 kD). The amino acid sequence of the entire human BPI protein and the nucleic acid sequence of DNA encoding the protein have been reported in Figure 1 of [Gray, *et al.*, *J. Biol. Chem.* 264: 9505 (1989)].

[092] BPI protein products have a wide variety of beneficial activities. BPI protein product refers to and includes naturally and recombinantly produced BPI protein; natural, synthetic, and recombinant biologically active polypeptide fragments of BPI protein; biologically active polypeptide variants of BPI protein or fragments thereof, including hybrid fusion proteins and multimer of monomers; biologically active polypeptide analogs of BPI protein or fragments or variants thereof, including cysteine-substituted analogs; and BPI-derived peptides. U.S. Patent Nos. 5,198,541 and 5,641,874, the disclosures of which are incorporated herein by reference, discloses recombinant genes encoding and methods for expression of BPI proteins including recombinant BPI holoprotein, referred to as rBPI and recombinant fragments of BPI. U.S. Patent No. 5,439,807 and corresponding International Publication No. WO 93/23540 (PCT/US93/04752), which are all incorporated herein by reference, disclose novel methods for the purification of recombinant BPI protein products expressed in and secreted from genetically transformed or transfected mammalian host cells in culture and disclose how one may produce large quantities of recombinant BPI products suitable for incorporation into stable, homogeneous pharmaceutical preparations.

[093] Biologically active fragments of BPI (BPI fragments) include biologically active molecules that have the same or similar amino acid sequence as a natural human BPI holoprotein, except that the fragment molecule lacks amino-terminal amino acids, internal amino acids, and/or carboxy-terminal amino acids of the holoprotein, including those described in U.S. Patent Nos. 5,198,541 and 5,641,874. Nonlimiting examples of such fragments include an N-terminal fragment of natural human BPI of approximately 25 kD, described in [Ooi *et al.*, *J. Exp. Med.*, 174: 649 (1991)], or the recombinant expression product of DNA encoding N-terminal amino acids from 1 to about 193 to 199 of natural human BPI, described in [Gazzano-Santoro *et al.*, *Infect. Immun.* 60: 4754-4761 (1992)], and referred to as rBPI₂₃. In that publication, an expression vector was used as a source of DNA encoding a

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recombinant expression product (rBPI₂₃) having the 31-residue signal sequence and the first 199 amino acids of the N-terminus of the mature human BPI, as set out in Figure 1 of [Gray et al., *supra*], except that valine at position 151 is specified by GTG rather than GTC and residue 185 is glutamic acid (specified by GAG) rather than lysine (specified by AAG). Recombinant holoprotein (rBPI) has also been produced having the sequence (SEQ ID NOS: 1 and 2) set out in Figure 1 of [Gray et al., *supra*], with the exceptions noted for rBPI₂₃ and with the exception that residue 417 is alanine (specified by GCT) rather than valine (specified by GTT). Another fragment consisting of residues 10-193 of BPI has been described in U.S. Patent No. 6,013,631, continuation-in-part U.S. Application Serial No. 09/336,402, filed June 18, 1999, and corresponding International Publication No. WO 99/66044 (PCT/US99/13860), all of which are incorporated herein by reference. Other examples include dimeric forms of BPI fragments, as described in U.S. Patent Nos. 5,447,913, 5,703,038, and 5,856,302 and corresponding International Publication No. WO 95/24209 (PCT/US95/03125), all of which are incorporated herein by reference. Preferred dimeric products include dimeric BPI protein products wherein the monomers are amino-terminal BPI fragments having the N-terminal residues from about 1 to 175 to about 1 to 199 of BPI holoprotein. A particularly preferred dimeric product is the dimeric form of the BPI fragment having N-terminal residues 1 through 193, designated rBPI₄₂ dimer.

[094] Biologically active variants of BPI (BPI variants) include but are not limited to recombinant hybrid fusion proteins, comprising BPI holoprotein or biologically active fragment thereof and at least a portion of at least one other polypeptide, or dimeric forms of BPI variants. Examples of such hybrid fusion proteins and dimeric forms are described in U.S. Patent No. 5,643,570 and corresponding International Publication No. WO 93/23434 (PCT/US93/04754), which are all incorporated herein by reference and include hybrid fusion proteins comprising, at the amino-terminal end, a BPI protein or a biologically active fragment thereof and, at the carboxy-terminal end, at least one constant domain of an immunoglobulin heavy chain or allelic variant thereof.

[095] Biologically active analogs of BPI (BPI analogs) include but are not limited to BPI protein products wherein one or more amino acid residues have been replaced by a different amino acid. For example, U.S. Patent Nos. 5,420,019, 5,674,834 and

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5,827,816 and corresponding International Publication No. WO 94/18323 (PCT/US94/01235), all of which are incorporated herein by reference, disclose polypeptide analogs of BPI and BPI fragments wherein a cysteine residue is replaced by a different amino acid. A stable BPI protein product has been described that is the expression product of DNA encoding from amino acid 1 to approximately 193 or 199 of the N-terminal amino acids of BPI holoprotein, but wherein the cysteine at residue number 132 is substituted with alanine. This product is designated rBPI₂₁Δcys or rBPI₂₁. Production of this N-terminal analog of BPI, rBPI₂₁, has been described in [Horwitz et al., *Protein Expression Purification*, 8: 28-40 (1996)]. Similarly, an analog consisting of residues 10-193 of BPI in which the cysteine at position 132 is replaced with an alanine (designated rBPI(10-193)C132A or rBPI(10-193)ala¹³²) has been described in U.S. Patent No. 6,013,631, continuation-in-part U.S. Application Serial No. 09/336,402, filed June 18, 1999, and corresponding International Publication No. WO 99/66044 (PCT/US99/13860), all of which are incorporated herein by reference. Other examples include dimeric forms of BPI analogs; e.g. U.S. Patent Nos. 5,447,913, 5,703,038, and 5,856,302 and corresponding International Publication No. WO 95/24209 (PCT/US95/03125), all of which are incorporated herein by reference.

[096] Other BPI protein products useful according to the methods of the invention are peptides derived from or based on BPI produced by synthetic or recombinant means (BPI-derived peptides), such as those described in International Publication No. WO 97/04008 (PCT/US96/03845), which corresponds to U.S. Application Serial No. 08/621,259 filed March 21, 1996, and International Publication No. WO 96/08509 (PCT/US95/09262), which corresponds to U.S. Patent No. 5,858,974, and International Publication No. WO 95/19372 (PCT/US94/10427), which corresponds to U.S. Patent Nos. 5,652,332 and 5,856,438, and International Publication No. WO94/20532 (PCT/US94/02465), which corresponds to U.S. Patent No. 5,763,567 which is a continuation of U.S. Patent No. 5,733,872, which is a continuation-in-part of U.S. Application Serial No. 08/183,222, filed January 14, 1994, which is a continuation-in-part of U.S. Application Serial No. 08/093,202 filed July 15, 1993 (corresponding to International Publication No. WO 94/20128 (PCT/US94/02401)), which is a continuation-in-part of U.S. Patent No. 5,348,942, as well as International

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Application No. PCT/US97/05287, which corresponds to U.S. Patent No. 5,851,802, the disclosures of all of which are incorporated herein by reference. Methods of recombinant peptide production are described in U.S. Patent No. 5,851,802 and International Publication No. WO 97/35009 (PCT/US97/05287), the disclosures of which are incorporated herein by reference. Three separate functional domains within the BPI sequence have been identified and designate regions of the amino acid sequence of BPI that contribute to the total biological activity of the protein (Domain I - from about amino acid 17 to about amino acid 45; Domain II - from about amino acid 65 to about amino acid 99; and Domain III - from about amino acid 142 to about amino acid 169). The biological activities of peptides derived from or based on these functional domains (*i.e.*, BPI-derived peptides) may include LPS binding, LPS neutralization, heparin binding, heparin neutralization or antimicrobial including antifungal and antibacterial (including *e.g.*, anti-gram-positive and anti-gram-negative) activity.

[097] Many utilities of BPI protein products, including rBPI₂₃ and rBPI₂₁, have been described due to the wide variety of biological activities of these products.

[098] Exemplary vectors have been developed according to the invention, with multiple transcription units encoding an exemplary polypeptide of interest, rBPI₂₁. Using such vectors, exemplary clones and cell lines have been developed. For example, a CHO-K1 cell line, Clone 689, has been developed, as detailed in the following examples, which secretes up to ~125 µg/ml of rBPI₂₁ in shake flasks. This cell line was developed using a novel expression vectors containing two copies of the rBPI₂₁ gene, each under the control of the human immediate early cytomegalovirus (hCMV) promoter and the mouse light chain 3' untranslated region, and either the *gpt* (pING1737) or *neo* (pING1733) genes for selection of mycophenolic acid or G418-resistant transfectants, respectively. These vectors were designed so that when linearized at a unique *Xba*I restriction site prior to transfection, the two copies of the rBPI₂₁ transcription unit are separated by the selective marker gene. This configuration provides a selective pressure against recombination because recombination between the two rBPI₂₁ genes would result in deletion of the selective marker gene, and thereby results in enhanced stability of clones developed with these vectors.

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[0099] As detailed in the following examples, a total of ~500 mycophenolic acid-resistant transfectants were screened from a pING1737 transfection. The highest-secreting transfectants, based on the 24 well plate cultures, were adapted to suspension growth in Ex-Cell 301 medium supplemented with 2% Fetal Bovine Serum (FBS) and tested in shake flask tests with S-Sepharose beads. The highest producer, Clone 51, consistently secreted ~50 µg/ml in shake flask tests. This level is similar to that produced by Clone 228 which contains two rBPI₂₁ gene copies as a result of a sequential transfection with vectors each containing one rBPI₂₁ gene. Clone 51 maintained this productivity level in the absence of selection for up to 14 weeks and was subsequently re-transfected with the *neo* vector, pING1733, for selection of G418-resistant transfectants. A total of 1253 clones were screened and Clone 689 was the highest producer at ~100 µg/ml based on results of initial shake flask tests. Clone 689 was adapted to growth in Ex-Cell 301 medium without FBS and re-designated Clone 689b. This clone maintained its productivity during passage in the absence of selection for at least 12 weeks.

[0100] As further detailed in the following examples, the results of initial shake flask tests indicated that after adaptation to Ex-Cell 301 medium without FBS, Clone 689b secreted ~75-80 µg/ml compared to ~50 µg/ml for Clone 228. Typically, these tests were performed with 250 ml flasks that are closed for the entire 12 days of incubation until the beads are harvested. It was discovered that by periodically opening the flasks (thus allowing gas exchange to occur), the expression levels for Clone 689 increased to ~125 µg/ml compared to ~60 µg/ml for Clone 228 under the same conditions. Clones 228 and 689 produced ~4 and 9 pg/cell/day, respectively, during the period when the cells were at their highest levels of viability, indicating that increased gene dosage resulted in increased specific productivities. Consistent with these results, Northern blot analysis revealed that Clone 689 expressed almost 2-fold higher levels of rBPI₂₁ mRNA than Clone 228. Several research cell banks based on these clones have been prepared.

[0101] An additional series of exemplary vectors comprising multiple transcription units encoding anti-Ep-CAM immunoglobulin polypeptides were developed, including vectors containing two genes encoding mouse-human chimeric or human engineered anti-Ep-CAM antibody light plus heavy chains, each under the control of a

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hCMV promoter and mouse light chain 3' untranslated region with 0, 1 or 2 copies of a human heavy chain enhancer and either a *gpt* or *neo* gene for selection of transformants or transfectants. The vectors also differed in the orientation of light and heavy chains genes on the vector.

[0102] ING-1 is a mouse-human chimeric antibody comprising variable regions from the mouse antibody Br-1 and human constant regions (*see, e.g.*, U.S. Patent No. 5,576,184). It is now known that the mouse anti-carcinoma antibody Br-1 binds the Epithelial Cell Adhesion Molecule (Ep-CAM). This murine antibody was first made and characterized by Colcher et al. from the B38.1 hybridoma cell line (described in U.S. Patent No. 4,612,282). The ING-1 antibody is a mouse-human chimeric version of Br-1 and was previously developed and expressed in Sp2/0 cells using vectors pING2207 encoding the mouse-human chimeric ING-1 light chain mammalian and pING2225 encoding the mouse-human chimeric ING-1 heavy chain (*see, e.g.*, U.S. Patent No. 5,576,184).

[0103] Antibody products that target Ep-CAM derived from the ING-1 antibody produced by cell line HB9812 as deposited with the ATCC (*see, e.g.*, U.S. Patent No. 5,576,184) have a variety of beneficial activities for diagnostic, prognostic and/or therapeutic uses involving diseases, disorders or conditions related to the expression of Ep-CAM, including for use with Ep-CAM-positive tumor cells, particularly the metastasis of Ep-CAM positive tumor cells. ING-1 antibody product refers to and includes an antibody heavy and/or light chain protein comprising at least an antibody variable region wherein the heavy and/or light chain variable region binds to Ep-CAM and wherein the heavy and/or light chain variable region shares at least 80% (including, for example, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the murine heavy and/or light chain variable regions of the ING-1 antibody produced by cell line HB9812 as deposited with the ATCC.

[0104] Exemplary vectors have been developed according to the invention, with multiple transcription units encoding an exemplary immunoglobulin polypeptide of interest, including mouse-human chimeric or human engineered anti-Ep-CAM antibodies derived from the ING-1 antibody produced by cell line HB9812 as deposited with the ATCC. Using such vectors, exemplary clones and cell lines have been

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developed. For example, a CHO-K1 cell line, Clone 146, has been developed, as detailed in the following examples, which secretes up to ~60 µg/ml of immunoglobulin polypeptides in shake flasks and about 200 mg/L in a fermentor. This cell line was developed using a novel expression vector pING1937 containing two genes, one copy each of the low risk human engineered ING-1 light and heavy chain genes and a *neo* (G418-resistant) gene, each under the control of a human cytomegalovirus (hCMV) promoter and mouse light chain 3' untranslated region, and a *neo* gene for selection of G418-resistant transfectants.

[0105] The present invention provides for increasing expression and production of human engineered anti-Ep-CAM immunoglobulin polypeptides through a second transfection of an exemplary cell line with a second multiple transcription unit vector. For example, Clone 373 was developed, as detailed in the following examples, by transfecting a subclone of Clone 146, Clone 146.3, with the expression vector pING1959 which is similar to pING1937 except that it contains a *gpt* selective marker gene. Clone 373 expressed ~225 and ~257 µg/ml of the human engineered anti-Ep-CAM antibody as determined by shake flask results in Ex-Cell 301 medium.

[0106] A series of exemplary vectors have also been constructed containing four genes, two copies of each of the low risk human engineered ING-1 light and heavy chain genes (pING1965, four gene vector), each under the control of a hCMV promoter and mouse light chain 3' untranslated region with 0, 1 or 2 copies of a human heavy chain enhancer and either a *gpt* or *neo* gene for selection of transformants or transfectants. Using such vectors, exemplary clones and cell lines have been developed. For example, a CHO-K1 cell line, Clone 17, has been developed, as detailed in the following examples, which secretes up to ~216 µg/ml in ExCell 301 medium supplemented with 1% FBS and ~214 µg/ml in ExCell 301 medium without FBS supplementation. This cell line was developed using a novel expression vector pING1964 containing four genes, two copies each of the low risk human engineered ING-1 light and heavy chain genes and the *neo* (G418-resistant) gene, each under the control of a human cytomegalovirus (hCMV) promoter and mouse light chain 3' untranslated region.

[0107] An additional series of exemplary vectors comprising multiple transcription units encoding anti-CD18 immunoglobulin polypeptides, including vectors containing

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two genes encoding anti-CD18 antibody light and heavy chains, each under the control of a hCMV promoter and mouse light chain 3' untranslated region with 0, 1 or 2 copies of a human heavy chain enhancer and either a *gpt* or *neo* gene for selection of transformants or transfectants. The vectors also differed in the orientation of light and heavy chains genes on the vector. The anti-CD18 antibody was originally developed as a rat antibody YFC51.1.1 to the human lymphocyte surface antigen CD18 and later humanized by CDR grafting as described in U.S. Patent Nos. 5,985,279 and 5,997,867.

[0108] Antibody products that target CD18 have a wide variety of beneficial activities. For example, anti-CD18 adhesion of neutrophils to endothelial cells and restenosis in primate model with stents and balloon angioplasty. Anti-CD18 protein product refers to and includes anti-CD18 antibodies that comprise CDRs derived from the YFC 5.1.1.1 antibody (*see, e.g.*, U.S. Patent No. 5,985,279). Anti-CD18 antibody product refers to and includes an antibody heavy and/or light chain protein comprising at least an antibody variable region wherein the heavy and/or light chain variable region binds to CD18, wherein the CDRs are those of the YFC 5.1.1.1 antibody as shown in SEQ ID NOS: 3-8 and 11-16 of U.S. Patent No. 5,985,279, and wherein the heavy and/or light chain variable region shares at least 80% (including, for example, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the murine heavy and/or light chain variable regions of the YFC 5.1.1.1 antibody.

[0109] Exemplary vectors have been developed according to the invention, with multiple transcription units encoding additional exemplary immunoglobulin polypeptides of interest, including humanized anti-CD18 immunoglobulin. Using such vectors, exemplary clones and cell lines have been developed. For example, a CHO-K1 cell line, Clone 128D12, has been developed, as detailed in the following examples, which secretes ~200 µg/ml of immunoglobulin in shake flasks. This cell line was developed using a novel expression vector pING2052 containing two genes, one copy each of the anti-CD18 light and heavy chain genes and a *neo* (G418-resistance) gene, each under the control of a human cytomegalovirus (hCMV) promoter and mouse light chain 3' untranslated region, and a *neo* gene for selection of G418-resistant transfectants, respectively.

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[0110] The current invention provides for increasing expression and production of anti-CD18 immunoglobulin through a second transfection of an exemplary cell line with a second multiple transcription unit vector. For example, Clone 264E2 was developed by sequential transfection of Clone 128D12, with the expression vector pING2057 which is similar to pING2052 except that it contains a *his* selective marker gene. Clone 264E2 in several shake flask tests in Ex-Cell 301 medium produced approximately 1.5 to greater than two times as much immunoglobulin polypeptides as Clone 128D12 as measured by Protein A-HPLC.

[0111] An additional series of exemplary vectors comprising multiple transcription units encoding complement inhibitory polypeptides were developed, including vectors containing multiple copies of the complement inhibitory peptide designated CAB2.1 (see, e.g., U.S. Patent Nos. 5,866,402; 6,316,253; and 6,451,539). For example, a vector was constructed containing two copies of a gene encoding a CAB2.1 polypeptide each under the control of a hCMV promoter and mouse light chain 3' untranslated region and either a *neo* or *his* gene for selection of transformants or transfectants. Initial testing of these vectors indicated that a preferred configuration was represented by the vector, pING2055 with a *neo* gene for selection. This vector and its *his* version, pING2056 were selected for use in full-scale cell line development.

[0112] Complement Activation Blocker-2 (CAB2.1) is a chimeric or fusion protein consisting of two human complement inhibitory proteins, membrane cofactor protein (MCP, CD46) at its N-terminus and decay accelerating factor (DAF, CD55) at its C-terminus. CAB2.1 protein has been isolated from CHO-DuKX-B11 DHFR⁻ cells transfected with a vector containing the CAB2.1 gene along with the DHFR gene for selection in the presence of methotrexate either by affinity chromatography with MCP-specific Mab (GB24) or by employing acid precipitation, anion exchange chromatography, immobilized metal affinity chromatography, and hydrophobic interaction chromatography [Higgins et al., *J. Immunol.* 158: 2872-2881 (1989)]. CAB2.1 obtained in such a manner was initially shown by Higgins [Higgins et al., *J. Immunol.* 158: 2872-2881 (1989)] to possess both factor 1 cofactor activity and decay-accelerating activity, and to inactivate both the classical and alternative pathways of complement activation by inactivating C3/C5 convertases. The

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molecular weight of CAB2.1 is approximately 110,000 daltons (110 kD). The amino acid sequence of the entire CAB2.1 protein and the nucleic acid sequence of DNA encoding the protein have been reported (*see, e.g.*, U.S. Patent No. 6,316,253).

[0113] CAB2.1 protein products have therapeutic potential for the prevention of complement mediated lysis of normal tissue, for example in the treatment of acute human diseases in which excessive complement activation causes damage to normal tissues. Some diseases in which complement is known to be activated include systemic lupus erythematosus, acute myocardial infarction, burn, sepsis, and adult respiratory distress syndrome. CAB2.1 protein product refers to and includes recombinantly produced CAB2.1 protein; complement inhibitory polypeptide fragments of CAB2.1 protein; complement inhibitory polypeptide variants of CAB2.1 protein or fragments thereof, including hybrid fusion proteins and multimer of monomers; complement inhibitory analogs of CAB2.1 protein or fragments or variants thereof; and complement inhibitory CAB2.1-derived peptides. Complement inhibitory fragments of CAB2.1 include biologically active molecules that have the same or similar amino acid sequence as a full length CAB2.1, except that the fragment molecule lacks amino-terminal amino acids, internal amino acids, and/or carboxy-terminal amino acids of the full length CAB2.1. Complement inhibitory analogs of CAB2.1 include but are not limited to CAB2.1 protein products wherein one or more amino acid residues have been replaced by a different amino acid. Other CAB2.1 protein products useful according to the methods of the invention are complement inhibitory peptides derived from or based on CAB2.1 produced by synthetic or recombinant means (CAB2.1-derived peptides).

[0114] Using vectors encoding CAB2.1, exemplary clones and cell lines expressing CAB2.1 were developed. For example, a CHO-K1 cell line, Clone 217B4 was developed, as detailed in the following examples, which secreted up to about 200 µg/ml of CAB2.1 in shake flasks. This cell line was developed using novel expression vectors containing one and two copies of the CAB2.1 gene, each under the control of a human cytomegalovirus (hCMV) promoter and mouse light chain 3' untranslated region, and either a *neo* (pING2054) or *his* (pING2056) genes for selection of G418-resistant or histinol transfectants, respectively.

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[0115] Other aspects, versions, and advantages of the present invention will be understood upon consideration of the following illustrative examples, wherein Example 1 addresses the construction of vectors according to the present invention that comprise multiple copies of a given transcription unit encoding a polypeptide of interest; Example 2 addresses development of an rBPI₂₁-Producing CHO-K1 cell line, Clone 51, by transfection with a two-transcription unit vector, pING1737; Example 3 addresses transfection of Clone 51 with a second two-transcription unit vector, pING1733, to yield the exemplary Clone 689; Example 4 addresses a third sequential transfection with a two-transcription unit vector, pING1753, to yield the exemplary Clone 341 and subclones thereof; and Example 5 addresses sequential transfection with a two-transcription unit vectors lacking an Ig enhancer, pING1744 and pING1915, to yield the exemplary Clones 338 and 58, respectively. Example 6 addresses the construction of additional vectors according to the present invention that comprise multiple copies of a given transcription unit, including those encoding immunoglobulin polypeptides; Example 7 includes development of a mouse-human chimeric ING-1 producing CHO-K1 cell line, Clone 40, by transfection with a two-transcription unit vector, pING1932 and the development of Clone 146, by transfection with a two-transcription unit vector, pING1937; Example 8 addresses the development of Clones 259 and 373 by sequential transfection of Clone 146 with a two-transcription unit vector pING1957 and Clone 373 by sequential transfection of Subclone 146.3 with a two-transcription unit vector pING1959; Example 9 addresses the development Clone 132 by sequential transfection of Clone 373 with a two-transcription unit vector pING1957; Example 10 addresses the development of Clones 53 and 157 by transfection with a two-transcription unit vector pING1959, containing one copy of each of the light and heavy chain genes of a human engineered anti-EpCAM antibody, and the development of Clone 17 transfected with a four-transcription unit vector pING1964, containing two copies of each of the light and heavy chain genes of a human engineered anti-EpCAM antibody; Example 11 addressing the binding activity of exemplary immunoglobulin polypeptides, including those encoding anti-Ep-CAM immunoglobulin polypeptides; Example 12 addresses the cloning of soluble Ep-CAM and the development of a direct binding ELISA assay with soluble Ep-CAM; Example 13 addresses the construction of

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additional expression vectors according to the present invention that contain multiple copies of a given transcription unit, including those encoding additional immunoglobulin polypeptides; Example 14 addresses the development of an anti-CD18 antibody-producing clone 128D12 by transfection with a two-transcription unit vector pING2052; Example 15 addresses the development of an anti-CD18 antibody-producing clone 264E2 by sequential transfection of clone 128D12 with the two-transcription unit vector pING264E2; Example 16 addresses the use of a number of additional cell culture media for anti-CD18 antibody production in selected cell lines; Example 17 addresses the construction of expression vectors that contain multiple copies of a given transcription unit, including those encoding complement inhibitory polypeptides; Example 18 addresses the development of Clone 3G8 and its Subclone G5F1 transfected with CAB2.1-encoding vectors, including a single-transcription unit *neo* vector pING2054 and Clones 156B8 and 176C6 transfected with a two-transcription unit *neo* vector pING2055; Example 19 addresses the development of additional CAB2.1-producing clones by sequential transfection of Clone 3G8 with the two-transcription unit *his* vector pING2056 followed by subcloning of the top producing clones.

EXAMPLE 1

CONSTRUCTION OF VECTORS

[0116] This example describes the construction of vectors that contain (*i.e.*, comprise) multiple copies of an exemplary transcription unit. Exemplary vector constructs are also described containing multiple copies of exemplary gene sequences encoding a polypeptide of interest.

A. Construction Of Expression Vectors Containing Two rBPI₂₁ Genes And a Single Copy Of The Mouse Immunoglobulin Heavy Chain Enhancer

[0117] The expression vectors, pING1729, pING4144, pING4151 and pING4155 were used as the source of DNAs encoding rBPI₂₁ sequences (SEQ ID NO: 3 and 4). Plasmids pING4144 and pING4151 have been described in co-owned U.S. Patent No. 5,674,834 by Theofan *et al.* pING4155 is similar to pING4144 and pING4151 except that it contains the *neo* gene for selection of G418-resistant transfectants. pING1729

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is similar to pING4144 except that it lacks the mouse immunoglobulin heavy chain enhancer. This vector was constructed by deleting a ~700 bp *HindIII* restriction fragment containing the enhancer.

[0118] The plasmid pING1733 was constructed from pING4155 and pING1729 (Figure 1). pING4155 was first digested with *XbaI*, which cuts at a unique site adjacent to the Ig enhancer. The *XbaI*-digested DNA was treated with T4 DNA polymerase in the presence of deoxyribonucleotides, which fills in the 4 bp 5' extension to make the ends blunt, and then digested with *NotI*, which cuts at a unique site within the vector ~500 bp counterclockwise to *XbaI* on a circular map. The resulting ~8700 bp vector fragment was gel purified. pING1729 was digested with *NotI* and *HpaI* and a ~3700 bp restriction fragment containing the human cytomegalovirus (hCMV) promoter, the gene encoding rBPI₂₁ and the mouse kappa light chain 3' untranslated region was gel purified. Ligation of these restriction fragments re-created the *XbaI* site and retained the *NotI* site. The resulting vector, pING1733 (Figure 1), contains the *neo* gene for selection of G418 - resistant transfectants, two copies of the rBPI₂₁ gene each under the control of the CMV promoter and mouse kappa light chain 3' untranslated region and one IgG enhancer unit.

[0119] The plasmid pING1737 is similar to pING1733 except that it contains the *gpt* instead of the *neo* gene for selection of mycophenolic acid-resistant transfectants and was constructed from pING1733 and the *gpt* vector, pING4144 (Figure 2). pING1733 and pING4144 were each digested with *HpaI* and *NotI* and a 7600 bp restriction fragment from pING1733 (containing the two rBPI₂₁ transcription units) and a 4400 bp restriction fragment from pING4144 (containing the *gpt* gene) were ligated. As with pING1733, pING1737 contains two copies of the rBPI₂₁ gene each under control of the CMV promoter with one Ig enhancer and unique *XbaI* and *NotI* sites. Digestion of pING1737 with *NotI* (Figure 3A) or *XbaI* (Figure 3B) yields linear plasmids identical to pING1733 digested with the same restriction enzymes except that they contain the *gpt* instead of the *neo* gene.

[0120] The plasmid pING1753 was constructed in a similar manner to pING1737 except that the vector, pING4151, containing the *his* gene for selection of histidinol - resistant transfectants, was used instead of the *gpt* - containing vector, pING4144.

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Both plasmids were digested with *HpaI* and *NotI* and the fragment containing the two rBPI₂₁ transcription units from pING1733 and the fragment containing the *his* gene from pING4151 were gel purified and ligated (Figure 4). As with pING1737 and pING1733, pING1753 contains two copies of the rBPI₂₁ gene each under control of the CMV promoter with one Ig enhancer and unique *XbaI* and *NotI* sites. Digestion of pING1753 with *XbaI* or *NotI* yields linear plasmid maps similar to those of pING1733 and pING1737 digested with the same restriction enzymes except that pING1753 contain the *his* instead of the *neo* or *gpt* genes.

[0121] Digestion of pING1733, 1737 or 1753 at the unique *XbaI* site each yields a linear restriction fragment containing both copies of the rBPI₂₁ gene configured so that the selection marker gene, *neo gpt* or *his*, is positioned between the two identical rBPI₂₁ transcription units. For example, pING1733 viewed as linear *XbaI*-digested DNA, the order of elements within the vector is as follows: IgG enhancer, CMV promoter, rBPI₂₁ gene, light chain 3' untranslated region, *neo* gene, *bla* (Amp^r) gene, CMV promoter, rBPI₂₁ gene, light chain 3' untranslated region. Digestion of pING1733 at a unique *NotI* site yields a linear restriction fragment with the two rBPI₂₁ transcription units being adjacent to each other and the selective marker gene positioned outside of the these two recombinant rBPI₂₁ transcription units. Viewed as linear *NotI* - digested DNA, the order of elements within the vector is as follows: CMV promoter, rBPI₂₁ gene, light chain 3' untranslated region, IgG enhancer, CMV promoter, rBPI₂₁ gene, light chain 3' untranslated region, *neo* gene, *bla* (Amp^r) gene.

B. Construction of an Expression Vector Containing Two rBPI₂₁ Genes without the Immunoglobulin Heavy Chain Enhancer

[0122] The expression vector pING1744 (Figure 5) was constructed using pING1732 and pING1740 as the source of DNAs encoding rBPI₂₁. pING1732 is similar to pING1729 except that it contains the *neo* gene. pING1740 was constructed by first cloning a multilinker site between the *NotI* and *HindIII* sites of pING1732 to generate pING1736, which contains the *neo* gene encoding resistance to G418. This linker destroyed the original *NotI* site and introduced a new *NotI* site adjacent to a *XbaI* site. The *neo* gene from pING1736 was then replaced with the *gpt* gene from pING4144 by ligating a 2090 bp *XmnI-XhoI* fragment containing the multilinker, the CMV

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promoter and the rBPI₂₁ gene from pING1736 with a 5600 bp *XmnI-XhoI* fragment from pING4144 containing the *gpt* gene. To construct pING1744, a ~3700 bp *HpaI - NotI* rBPI₂₁ - containing fragment from pING1732 was ligated with a ~7700 bp fragment from pING1740 generated by digestion with *XbaI*, treatment with T-4 DNA polymerase and deoxyribonucleotides to blunt end, and then digestion with *NotI* (Figure 5). pING1744 contains the *gpt* gene for selection of mycophenolic acid - resistant transfectants and two copies of the rBPI₂₁ gene each under the control of the CMV promoter and mouse kappa light chain 3' untranslated region. This vector lacks the Ig enhancer. Digestion of pING1744 at a unique *XbaI* site yields a linear restriction fragment containing both copies of the rBPI₂₁ gene configured so that the *gpt* selective marker gene is positioned between the two identical rBPI₂₁ transcription units. Viewed as linear DNA, the order of elements within the expression vector is as follows: CMV promoter, rBPI₂₁ gene, light chain 3' untranslated region, *gpt* gene, *bla* (Amp^r) gene, CMV promoter, rBPI₂₁ gene, light chain 3' untranslated region (Figure 6).

EXAMPLE 2

DEVELOPMENT AND CHARACTERIZATION OF TRANSFECTED CLONES AND CELL LINES

[0123] This example describes the development and characterization of clones and cell lines transfected with an exemplary vector following an embodiment of the present invention. The development and characterization of an rBPI₂₁-producing CHO-K1 cell line, Clone 51, is described from a transfection with a two-gene vector as described in Example 1. The CHO-K1 cell line used for the development of Clone 51 was obtained from ATCC (CCL61). The cells were grown in Ham's F12 culture medium supplemented with 10% FBS.

[0124] Prior to transfection, 40 µg of pING1737 was linearized with either *NotI* or *XbaI*. CHO-K1 cells from Research Cell Bank C1754 were transfected with either *NotI*- or *XbaI*-digested pING1737 using electroporation by the procedure of [Andreason, et al., *BioTechniques* 6: 650 (1988)]. Following a 48-hour recovery period in Ham's F12 medium supplemented with 10% FBS, the cells were trypsinized, diluted in selective medium (Ham's F12 medium supplemented with 10%

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fetal bovine serum (FBS), 292 mg/L glutamine, 10^5 units/L penicillin, 100 mg/L streptomycin, and 0.01 M HEPES, 250 mg/L xanthine and 25 mg/L mycophenolic acid [MPA]) and $\sim 1 \times 10^4$ cells/well were transferred to 96-well plates. The cells were incubated at 37 °C in a CO₂ incubator.

[0125] Starting at about 2 weeks of incubation, culture supernatants from 96-well plate wells containing only one colony per well were screened using the BPI sandwich ELISA by the procedure of [White, *et al. J. Immunol. Methods* 167: 227-235 (1988)]. Approximately 500 clones each were screened for cells transfected with the *NotI*- and *XbaI*-digested pING1737. The 42 clones from the *NotI*-digested pING1737 (*NotI* clones) and the 26 clones from the *XbaI*-digested pING1737 (*XbaI* clones) secreting the highest levels of rBPI₂₁ were transferred to Ham's F12 selective medium in 24-well plates. The cells were grown to confluence, and the master and replica 24-well plate cultures were prepared. To assess productivity, the cells in the replica plate were grown to confluence in the Ham's F12 medium (48-72 hours), the medium removed and replaced with 1 ml/well of serum-free medium (HB-CHO, Irvine Scientific) plus 40 μ l of sterile S-Sepharose beads and the cells incubated for an additional seven days. The S-Sepharose beads were then removed, washed once with ~ 1 ml of Tris buffer (20 mM Tris, pH 7.4, 0.1 M NaCl), and the rBPI₂₁ was eluted from the beads with 1 ml 1.5 M NaCl in 20 mM sodium acetate pH 4.0. The level of secreted rBPI₂₁ eluted from the S-Sepharose beads was quantitated using the sandwich ELISA. The results demonstrated that the top transfectants from each group secreted up to ~ 3 μ g/ml. The top 10 *XbaI* clones (including, for example, clones 51 and 127) and top 11 *NotI* clones (including, for example, clones 255 and 266) were chosen for further study.

[0126] Cells from the 24-well plate cultures were transferred to 24-well plate wells containing Ex-Cell 301 medium (JRH Biosciences, Lenexa, Kansas) supplemented with 25 μ g/ml MPA and 250 μ g/ml xanthine (Selective Ex-Cell 301 medium) plus 10% FBS. At confluency, the cells from the 24-well plate wells were transferred to 125 ml Erlenmeyer flasks containing 20 ml selective Ex-Cell 301 medium supplemented with 2% FBS. The clones were maintained in Erlenmeyer flasks in the selective Ex-Cell 301 medium containing 2% FBS during the screening period.

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[0127] An initial productivity test was performed for the top 11 *NotI* and 10 *XbaI* clones. At this stage, the cells were growing in selective Ex-Cell 301 medium and supplemented with 2% FBS. They were transferred to a 250 ml Erlenmeyer (shake) flask containing a total volume of 50ml of Ex-Cell 301 medium plus 2% FBS (includes inoculum) and 2 ml of SP-Sepharose (Big Beads) and grown at 37 °C, 100 RPM for 12 days. As controls, Clone 180 (which contains a single copy of the rBPI₂₁ gene) and Clone 228 (which contains two copies of the rBPI₂₁ gene from two successive transfections of single copies of the rBPI₂₁ gene) were also included in this test. Following incubation, the beads were removed, washed with low salt buffer (20 mM sodium phosphate, pH 7.0, 0.15 M NaCl) and the rBPI₂₁ eluted in 20 mM sodium acetate (pH 4.0) buffer containing 1.5 M NaCl. The rBPI₂₁ level in the eluates was measured by ion exchange HPLC. The results indicated that three clones from the transfection with *NotI*-digested DNA and two from the transfection with *XbaI*-digested DNA expressed rBPI₂₁ at levels similar to those of Clone 228. The results further indicated that both rBPI₂₁ gene copies in pING1737 were being maximally expressed at least in some of the clones.

[0128] Upon continued growth of these clones as suspension cells in selective Ex-Cell 301 medium (supplemented with 2% FBS), those arising from transfection with the *NotI*-digested DNA eventually lost productivity gradually over the course of a ten week period from a range of ~37-60 µg/ml to ~5-35 µg/ml rBPI₂₁ even though selection was maintained. This outcome was not entirely unexpected as the two rBPI₂₁ genes were positioned in tandem and could be subject to recombination. By comparison, Clone 51, arising from transfection with the *XbaI*-digested pING1737, maintained or increased its productivity for the 10 week period of the study. These results indicated that the placement of the selective marker (*gpt*) gene between the two rBPI₂₁ genes within the integrated expression vector can help maintain both rBPI₂₁ gene copies in the transfectants.

[0129] During the course of additional passages of Clone 51, the FBS content was gradually reduced until a serum-free condition was achieved. Clone 51 growing in Ex-Cell 301 medium without FBS was redesignated as Clone 51b.

[0130] The stability of Clones 51 and 51b in Ex-Cell 301 medium with and without 2% FBS supplementation, respectively, was examined. For this test, the cells were

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maintained in a 125 ml Erlenmeyer flask containing 25 ml of Ex-Cell 301 medium with (Clone 51) or without (Clone 51b) 2% FBS and with or without full selection and passage twice each week. Once a week, a shake flask test was set up and performed as described above. The stability study for Clone 51 and Clone 51b lasted 16 weeks. For the Clone 51b study, the level of expression was compared to that of Clone 228 maintained in selective Ex-Cell 301 medium. The results of the Clone 51 stability study demonstrated that after an initial drop in expression levels, the cells in selective and non-selective medium maintained similar rBPI₂₁ expression levels at least out to week 11. Clone 51b maintained similar productivity in the presence and absence of selection during the entire 18 week course of the experiment. Throughout the course of the Clone 51b study, productivity was equal to or greater than that of Clone 228, which also contains two rBPI₂₁ genes as a consequence of sequential transfection with vectors containing one rBPI₂₁ gene.

EXAMPLE 3

INCREASING EXPRESSION THROUGH A SECOND SEQUENTIAL VECTOR TRANSFECTION: DEVELOPMENT AND CHARACTERIZATION OF TRANSFECTED CLONES AND CELL LINES

[0131] This example describes the further increase in expression and production of polypeptides through a second transfection of an exemplary cell line with a second multi-transcription unit vector. The development and characterization of an rBPI₂₁-producing CHO-K1 cell line, Clone 689, is described. Clone 689 was developed by transfecting Clone 51 cells as described in Example 2 adapted to Ex-Cell 301 medium supplemented with 2% FBS with the *neo* expression vector, pING1733 (Figure 1) digested with *Xba*I prior to transfection. Clone 51 cells still growing in selective Ex-Cell 301 medium supplemented with 2% FBS (rather than completely adapted to serum-free growth) were used to allow prompt initiation of the second transfection.

A. Transfection of Clone 51 with pING1733

[0132] Clone 51 cells growing in selective Ex-Cell 301 medium supplemented with 2% FBS, MPA and xanthine were electroporated with 40 µg of *Xba*I-digested pING1733. Following a 48-hour recovery period in non-selective Ex-Cell 301 medium supplemented with 2% FBS, the cells were placed in selective medium (Ex-

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Cell 301 medium supplemented 0.8 mg/ml G418 and 2% FBS) and transferred to 96-well plates at $\sim 1 \times 10^4$ cells/well. The MPA and xanthine were not included in the medium at this time. The cells were placed in a CO₂ incubator at 37 °C. Two additional transfections were performed as above.

B. Screening and Selection of Clone 689

[0133] A total of 1,253 clones were screened at the 96-well level from the three transfections. The 125 clones secreting the highest rBPI₂₁ levels were transferred to selective Ex-Cell 301 medium (250 µg/ml xanthine, 25 µg/ml mycophenolic acid and 0.8 mg/ml G418) supplemented with 2% FBS in 24-well plates. Although the transfectants had been selected in 96-well plates in Ex-Cell 301 with 2% FBS, they initially grew poorly when transferred to the 24-well plates with this medium. This problem was solved by temporarily increasing the FBS concentration to 4%. The cells were grown to confluence, and then master and replica 24-well plate cultures were prepared as described above for Clone 51. To screen for productivity in the 24-well plate cultures, the cells were transferred to 1 ml of selective Ex-Cell 301 medium containing 4% FBS plus 40 µl of sterile S-Sepharose beads and they were incubated until extinct (7-10 days). The S-Sepharose beads were removed, washed once with ~ 1 ml of Tris buffer (20mM Tris, pH 7.4, 0.1 M NaCl), and the rBPI₂₁ was eluted from the beads into 1 ml of the same buffer containing 1.5 M NaCl. The level of secreted rBPI₂₁ eluted from the S-Sepharose beads was quantitated using the sandwich ELISA. The top 46 clones were transferred from the 24-well plate to a 125 ml Erlenmeyer (shake) flask containing 25 ml of Ex-Cell 301 supplemented with 4% FBS and selective agents at one-half the concentration used for selection of transfectants. Shake flask tests were performed with these clones in the Ex-Cell 301 medium with 4% FBS. From these and subsequent tests, a number of high producing clones were identified (including, for example, clones 357, 548, 689, and 815). The results of these tests indicated that Clone 689 was the top producer at ~ 100 µg/ml. Subsequent shake flask tests confirmed that Clone 689 was the best producer with an average of 104.3 ± 1.99 µg/ml in six tests in Ex-Cell 301 medium with 4% FBS. Vials of Clone 689 were frozen (designated Research Cell Bank C3043). Clone 689 was maintained

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in Ex-Cell 301 medium supplemented with 4% FBS and used for the initial stability study set forth below.

C. Adaptation of Clone 689 to Ex-Cell 301 Medium without FBS

[0134] Clone 689 was adapted to growth in Ex-Cell 301 medium without FBS by reducing the serum level by one half at each passage. The adaptation to serum-free growth was completed and vials were frozen (designated Research Cell Bank C3056).

[0135] A second, more aggressive adaptation of Clone 689 to Ex-Cell 301 medium without FBS was undertaken. Prior to this adaptation, a vial of research cell bank C3043 had been thawed and placed in culture. This Ex-Cell 301-adapted clone was designated as Clone 689b and a research cell bank, (designated C3071), was prepared. Additional research cell banks (designated C3131 and C3287) were also prepared.

[0136] Although Clone 689 produced ~100 µg/ml in the initial shake flask tests, including one test immediately following its adaptation to growth in serum-free medium, its productivity slowly dropped over the course of 6 weeks on continued passage until it leveled out to ~75-80 µg/ml. As with Clone 51, it is not clear why a decline was observed.

D. Stability of Clone 689

[0137] Three stability studies were performed with Clone 689. As controls, the expression levels of Clones 228 and 180 (both in selective Ex-Cell 301 medium) were also monitored as a part of these studies. The first study was initiated with Clone 689 adapted to growth in Ex-Cell 301 medium supplemented with 4% FBS with and without selection (mycophenolic acid, xanthine, G418) in 125 ml Erlenmeyer flasks (25 ml cells/flask). The cultures were incubated at 37°C, 100 RPM and passaged twice each week. Once each week, the cells were also inoculated into 50 ml of non-selective Ex-Cell 301 medium supplemented with 2% FBS and 2.5 ml sterile SP-Sepharose beads (Big Beads). These cultures were then grown at 37 °C for 12 days after which the beads were collected, washed and the rBPI₂₁ eluted and analyzed by HPLC as above. This study was completed after a total of 14 weeks. The results demonstrated that after an initial decline through week 6 for cells grown both in the presence and absence of selection (similar to that observed with Clone 51), thereafter

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Clone 689 maintained similar productivity (~75-80 µg/ml) in the presence and absence of selection at least through the eleventh week, after which productivity of cells grown without selection seemed to decline very slightly through week 14 to a level of ~65-70 µg/ml. The second study was performed with Clone 689b, resulting from the second adaptation of Clone 689 to completely FBS-free medium. This study was conducted for a total of 14 weeks. For both studies, the cultures were incubated in Ex-Cell 301 medium with and without selection and evaluated for productivity as described above. The results of the Clone 689b stability study demonstrated that there was no significant decrease in expression levels in the absence of selection out to 14 weeks. A similar result was obtained with the Clone 689 cells that had been initially adapted to Ex-Cell 301.

E. Growth and Productivity

[0138] The average productivity of Clone 689 was at least ~1.5 fold higher than Clone 228 and Clone 51. Since productivity is a function of both specific productivity and cell density, tests were conducted to determine if relative expression levels for various clones were reflected in specific productivity differences. Therefore, growth and productivities of Clone 180 (1 copy), Clones 228 and 51 (2 copies), and Clone 689 (~4 copies) were compared in shake flask tests. For each clone, cells were grown to logarithmic phase and inoculated at $1-2 \times 10^5$ cells/ml into seven 250 ml Erlenmeyer flasks each containing 50 ml of Ex-Cell 301 medium supplemented with 2% FBS and 2% S-Sepharose (big beads). One flask was dedicated for each time point. Previous studies had indicated that a high concentration (>100 µg/ml) of rBPI₂₁ was inhibitory for CHO-K1 cell growth. Therefore, a flask without SP-Sepharose was also inoculated for each clone to compare the growth with and without beads. The cells were incubated at 37 °C and SP-Sepharose was harvested from flasks at Days 2, 3, 4, 5, 8 and 12.

[0139] The cell growth results for cultures with and without SP-Sepharose indicated that there were no significant growth differences with and without SP-Sepharose beads even for the highest producers. These results suggested that the secreted rBPI₂₁ did not significantly influence cell growth under conditions where it was not absorbed by the SP-Sepharose beads. The greatest growth was observed with Clone 180

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(maximum cell density of $\sim 3.5 \times 10^6$ cells/ml) followed by Clone 228 (maximum cell density of $\sim 3 \times 10^6$ cells/ml). The next highest density was Clone 689 (maximum cell density of $\sim 2.5 \times 10^6$ cells/ml) and Clone 51 displayed the lowest maximum cell density although this result may actually be a consequence of cell clumping.

[0140] The results of productivity tests for cultures supplemented with SP-Sepharose demonstrated that as previously observed, the maximum productivities of Clones 228 and 51 were ~ 2 times that of Clone 180 (~ 50 vs. $25 \mu\text{g/ml}$) while the productivity of Clone 689 was ~ 1.5 times that of Clone 228, at least when measured at Day 5 (the day Clone 228 achieved its maximum productivity). However, Clone 689 slowly accumulated up to $\sim 100 \mu\text{g/ml}$ by Day 12 in this test.

[0141] The above results indicated that the relative expression levels in the shake flasks for Clones 180, 228, 51 and 689 were directly proportional to gene dosage, assuming that as with Clones 180 and 228, Clones 51 and 689 received only one copy of the plasmids from each transfection. To confirm that this increased yield resulted from a higher specific productivity (pg/cell/day), we estimated this value from Days 2 to 4 for Clone 180 and Days 3 to 5 for the other cell lines at which time the cell viabilities were high ($>90\%$) and the cultures were at or approaching their maximum cell densities. This calculation was made by dividing the average daily production during these 2 days by the average cell density during this period. The results demonstrated that the specific productivity for Clone 689 (~ 9 pg/cell/day) was about twice that of Clone 228 (~ 4 pg/cell/day) which, in turn, was about twice that of Clone 180 (~ 2 pg/cell/day). Clone 51, which was from the initial transfection with the 2-gene vector and served as the parent for Clone 689, had almost the same specific productivity as Clone 689, although this value is probably inflated due to the abnormally low cell counts as a result of cell clumping.

F. Effect of Culture Conditions on rBPI₂₁ Expression by Clone 689

[0142] Typically, shake flask tests were performed in Erlenmeyer flasks that were sealed after inoculation and only re-opened after the 12-day incubation period. Under these conditions, Clone 689, on average has produced ~ 75 - $80 \mu\text{g/ml}$ compared to ~ 45 - $50 \mu\text{g/ml}$ for Clone 228.

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[0143] As part of the evaluation of the growth characteristics of Clone 689 in the presence of S-Sepharose, an experiment was performed in which cells were inoculated with $\sim 1 \times 10^5$ cells/ml into three flasks containing Ex-Cell 301 with 2% FBS and S-Sepharose. One of the flasks remained sealed during the course of the experiment, as is usually the case, while the other two were used to perform cell counts at days 2-7, 9, 11. After day 11, the S-Sepharose was removed, washed, eluted and the levels of rBPI₂₁ determined by HPLC. Surprisingly, while cells in the sealed flask expressed rBPI₂₁ at 75 $\mu\text{g/ml}$ as expected, cells in the flasks from which samples were taken for cell counts expressed an average of $\sim 130 \mu\text{g/ml}$ (for two tests), representing a $\sim 70\%$ increase. As previously observed, the Clone 689 cells achieved a maximum cell density of $\sim 2.5 \times 10^6$ cells/ml at days 4-5 indicating that the increased rBPI₂₁ expression in the "opened" flasks was not due to significantly higher cell densities.

[0144] To further examine this phenomenon with Clone 689 and determine if it occurred with other clones, a similar experiment was performed with Clones 180, 228 and 689. The results demonstrated that a similar phenomenon occurred to some extent with all of the tested clones. As before, Clone 689 produced 79 $\mu\text{g/ml}$ in the closed flask and up to 128 $\mu\text{g/ml}$ in the open flask, representing a 62% increase. Clone 228 produced up to 47 and 62 $\mu\text{g/ml}$ in the "closed" and "open" flasks, respectively, representing a 32% increase. Clone 180 expression levels increased $\sim 20\%$ in the open vs. closed flask.

[0145] In the initial open flask experiment with Clone 689, we also observed that the media in the flasks that had been opened appeared to be less acidic. To determine if this were the case with the second experiment, the pH values of the culture supernatants from each flask were measured. The results demonstrated that the pH of the media from the flasks that had been opened was 0.2 to 0.5 units higher than that of the flasks which had remained sealed. The greatest pH difference between the closed and open flasks occurred with Clone 689. It is possible that the gas exchange that occurs when the flasks are opened allows the cells to more efficiently utilize glucose and perhaps produce less lactic acid, which may adversely affect both growth and production.

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[0146] The results of these experiments indicated that future shake flask tests could incorporate the gas exchange step because the highest productivities were observed under these conditions.

G. rBPI₂₁ Expression Levels Without S-Sepharose

[0147] Previous experiments showed that the presence of S-Sepharose facilitates efficient detection and recovery of rBPI₂₁ from the culture supernatants of transfected cells [Horwitz, A. H. et al., *Protein Expr Purif.* 18: 77-85 (2000)]. The loss of rBPI₂₁ from the culture supernatants without beads may be due to its interaction with heparan proteoglycans on the surface of the CHO cells.

[0148] One potential consequence of achieving higher expression levels is that the surface receptors for rBPI₂₁ eventually might be saturated, resulting in elevated levels of rBPI₂₁ in the supernatant in the absence of S-Sepharose. To determine if this was the case, the levels of rBPI₂₁ expression were compared with and without SP-Sepharose for Clones 180, 228, and 689. The rBPI₂₁ levels in the supernatants from cultures without beads were measured by ELISA, while the rBPI₂₁ levels eluted from the beads were measured by both ELISA and HPLC. The results demonstrated that the level of rBPI₂₁ present both during the production phase and at the end of the incubation period increased with higher expression occurring with Clone 689. However, even for Clone 689, SP-Sepharose beads were still required for optimal detection and recovery of rBPI₂₁.

H. RNA Levels

[0149] Relative levels of rBPI₂₁ mRNA were compared for Clones 228 and 689. Clone 228, 689 and untransfected CHO-K1 cells were grown to logarithmic phase in Ex-Cell 301 medium and total cytoplasmic RNA was isolated as described by [Gough *Anal. Biochem.* 173: 93-95 (1988)]. The RNA samples were first checked for the level of ribosomal RNAs and then run on a formamide, 1% agarose gel, blotted to nitrocellulose and hybridized with a ³²P-labeled DNA probe for the light chain 3' untranslated region (Figure 7). The membrane was washed to remove non-specifically-bound counts and exposed to X-ray film. The results as shown in Figure 8 indicate that Clone 689 expressed rBPI₂₁ mRNA at an estimated ~1.6X higher level

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than Clone 228. However, a method such as RNAase protection or quantitative PCR will be needed to provide more accurate quantitation of the mRNA levels.

I. Summary

[0150] Thus, in accordance with the invention, vectors were designed that contain (*i.e.*, comprise) multiple copies of a cDNA encoding rBPI₂₁. Two of these two-transcription unit vectors were used in sequential transfections with CHO-K1 cells to produce a cell line, Clone 689, which produced approximately twice the rBPI₂₁ level as Clone 228 which contained two one-transcription unit vectors as a result of sequential transfection. This increased productivity appeared to result from a two-fold increase in specific productivity (compared to Clone 228), and not merely from an increase in cell density. Indeed, Clone 689 cells did not grow to as high a cell density as Clone 228 cells while producing higher rBPI₂₁ levels. In addition, Clone 689 maintained its productivity in the absence of selection for up to 14 weeks, an amount of time sufficient to account for scale-up into large fermenters. This example demonstrates that increased expression can be achieved through increased transcription unit dosage in a non-amplified expression system through the methods of the present invention in at least two different employed strategies with surprising effectiveness. First, by positioning the selective marker gene between multiple copies of transcription units encoding the rBPI₂₁ gene, the problem of homologous recombination can be avoided and high producing, stable cell lines can be developed which express multiple copies of the rBPI₂₁ gene. Second, by sequentially transfecting cells in the methods of the present invention, the number of multiple integrated copies of transcription units can be increased stepwise to increase expression levels. The present invention contemplates the use of any transcription unit encoding any polypeptide of interest and is in no way limited to BPI protein products, as exemplified by rBPI₂₁, as would be understood by one skilled in the art.

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EXAMPLE 4

FURTHER INCREASING EXPRESSION BY A
THIRD SEQUENTIAL VECTOR TRANSFECTION: DEVELOPMENT AND
CHARACTERIZATION OF TRANSFECTED CLONES AND CELL LINES

[0151] This example describes the further increase in expression and production by a third sequential transfection of an exemplary cell line with a third multi-transcription unit vector, resulting in clones and cell lines that express increased levels of polypeptide production.

A. Selection of Sequential Transfectants and Development Thereof

[0152] Further sequential transfections with multiple copies of transcription units separated by selective marker genes were carried out to test for still further increased expression of the transcription units. A third sequential transfection of Clone 689 with a vector containing two rBPI₂₁ genes and a third selective marker might yield expression levels at least 1.5 times those of Clone 689 based on increased gene dosage, assuming that gene dosage could be achieved. Clone 689 was thus sequentially transfected a third time. This cell line was transfected with the vector, pING1753, containing two copies of the rBPI₂₁ gene and the *his* gene as a selective marker for selection of histidinol resistance. Subsequent to transfection and selection, a total of ~500 triple-transfectant clones were screened at the 96 well level and 85 at the 24 well level with S-Sepharose. The top producers secreted up to 1.5 times higher levels (~50 µg/ml) of rBPI₂₁ as compared to Clone 689 when tested at the 24-well level, but only secreted ~20-30% higher than Clone 689 in the initial shake flask tests which were performed in closed flasks. A top producing clone (341), was chosen for further study.

B. Production Under Open Flask Conditions.

[0153] Subsequent to the surprising results as described in Example 3 above of increased expression in shake flasks when gas exchange was allowed, the shake flask tests were again performed, but this time with open flasks. Several triple transfectants were evaluated in open shake flask tests in Ex-Cell 301 medium supplemented with 2% FBS. The results indicated that the expression levels were considerably higher than those previously observed with closed flasks for Clone 341 (the triple

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transfectant obtained by transfecting Clone 689). In fact, in four separate tests, Clone 341 consistently secreted higher levels (ranging from 107.6 $\mu\text{g/ml}$ to 142.0 $\mu\text{g/ml}$) than Clone 689 (which ranged from 67.8 $\mu\text{g/ml}$ to 93.4 $\mu\text{g/ml}$). Thus, expression levels of the triple transfectant, Clone 341, did approximate a 1.5 fold increase in expression at both the 24-well level and in open shake flasks.

C. Adaptation of Clone 341 to 1% FBS

[0154] Clone 341 was subsequently tested after adaptation to growth in Ex-Cell 301 medium supplemented with 1% FBS. The triple sequential transfectant had initially been selected in 4% FBS and then adapted to 2% FBS in Ex-Cell 301 medium. Next, Clone 341 was adapted to 1% FBS and re-tested in shake flask tests. The results for eight shake flask tests indicated that this clone maintained its high productivity for at least 33 passages over the 17 weeks in which it was tested with concentrations of rBPI₂₁ ranging from 122.6 $\mu\text{g/ml}$ to 144.8 $\mu\text{g/ml}$.

D. Subcloning of Clone 341 and the Development of Clone 83

[0155] Attempts to adapt Clone 341 to completely serum-free conditions were not initially successful. In addition, although the above expression levels were higher than those for the initial closed flask experiments and were slightly higher than those achieved with some subclones of Clone 689, to further improve expression levels and potentially select for a faster growing, higher producing clone, Clone 341 was subcloned. The cells were plated in Ex-Cell 301 medium containing 1% FBS at ~30, 15, 7.5 and 3 cells/well. A total of 69 clones from wells with single colonies were transferred to 24 well plates and then to shake flasks where they were evaluated for productivity. Of these, the top 6 clones were ultimately kept for re-evaluation over 90 days where screening for rBPI₂₁ production was periodically conducted. The results of the screening indicated that Clone 83 was identified as the top subclone, secreting in the range of 150-165 $\mu\text{g/ml}$ (compared to ~135 $\mu\text{g/ml}$ for parent Clone 341) in shake flask tests.

E. Productivity and Stability of Clone 341 Subclone, Clone 83

[0156] The top 6 subclones were maintained and evaluated for productivity for an additional 8 weeks under full selection. The results demonstrated that Clone 83 had

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remained the best producer, secreting 180 and 195 $\mu\text{g/ml}$ on two occasions and always outperforming the Clone 341 parent.

[0157] The stability of Clone 83 was examined by growing the subclone in Ex-Cell 301 medium in the presence and absence of selection. The cells were passaged twice each week and shake flask tests were set up weekly. The results demonstrated that Clone 83 maintained productivity at a stable range of $\sim 160 \mu\text{g/ml}$ in the presence of selection. However, while Clone 83 maintained productivity in the absence of selection through at least week 5, by week 7 rBPI₂₁ levels had decreased in the absence of selection to $\sim 120 \mu\text{g/ml}$. These results suggest that this subclone is stable for at least 5 weeks (10 passages, ~ 30 -35 generations) in the absence of selection. Other Clone 689 subclones were shown to be stable for longer periods of time in the absence of selection. Lower productivity without selection may be due to instability of the integrated DNA encoding histidinol-resistance. If this is the case, high productivity may be maintained by keeping histidinol in the medium during the early scale-up stages.

EXAMPLE 5

SEQUENTIAL TRANSFECTION WITH A MULTIPLE TRANSCRIPTION UNIT VECTOR LACKING AN ENHANCER: DEVELOPMENT AND CHARACTERIZATION OF CLONES AND CELL LINES

[0158] This example demonstrates that sequential transfections with a multiple transcription unit vector, that effectively increases the expressed amounts of a given transcription unit, even when an enhancer has been removed from the vector. This is shown through the development and characterization of an exemplary clone, Clone 338, transfected with an exemplary vector, pING1744, which contained two rBPI₂₁ genes similar to Clone 51, but without the Ig enhancer. The resulting Clone, Clone 338, was then retransfected with a second two-gene (rBPI₂₁) vector, pING1915, also without the Ig enhancer, and then characterized. Clone 58 resulted from the sequential transfection and when screened, demonstrated expression of rBPI₂₁ at concentrations markedly higher than that of Clone 338, the single transfectant.

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A. Transfection with pING1744: Clone 338

[0159] Clone 338 was developed by transfection of attached CHO-K1 cells with the vector pING1744, which contains two rBPI₂₁ genes each under the control of the CMV promoter and light chain 3' untranslated region with the *gpt* gene for selection of MPA-resistant transfectants, but without an Ig enhancer. pING1744 had been first digested at a unique *Xba*I site prior to transfection so as to place the selective marker gene between the two copies of the rBPI₂₁ gene (Figure 5).

[0160] Ex-Cell 301-adapted Clone 338 cells initially were grown in the presence of 2% FBS and produced up to ~100 µg/ml in shake flask tests. The cells were subsequently adapted to Ex-Cell 301 medium supplemented with 1% FBS, retaining productivity of 80-100 µg/ml.

B. Stability of Clone 338 with Selection

[0161] Selection for mycophenolic acid is relatively poor in Ex-Cell 301 medium and maintenance of the high expression levels suggests that this clone is relatively stable. To further examine stability, cultures were grown in Ex-Cell 301 medium supplemented with 1% FBS and with or without selection. Cells were passaged twice each week and shake flask tests were set up weekly. Results showed that rBPI₂₁ was expressed at ~100µg/ml in the presence and absence of selection through 7 weeks, although there was a slight decline in expression over this period of ~20µg/ml in the absence of selection. These results suggest that although the cells without selection expressed rBPI₂₁ at slightly lower levels, this clone was stable for at least 7 weeks in the absence of selection. The study was discontinued at week 7.

C. Transfection with pING1915: Clone 58

[0162] To further optimize expression, Clone 338 adapted to Ex-Cell 301 medium with 1% FBS was re-transfected with pING1915 which is similar to pING1744 except that it contains the *neo* gene for selection of G418-resistant transfectants, but also lacks an Ig enhancer. A total of ~600 transfectants were screened at the 96 well level and 250 were screened in 24 well plates with S-Sepharose. Results of this screen revealed a number of clones that secreted at 30-60 µg/ml. By comparison, the highest expression levels in 24 well plates for a single two-gene vector with an Ig enhancer

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generally was ~15 µg/ml, the levels from a sequential transfection with two such two-gene vectors) was ~30-35 µg/ml, and the levels from a third transfection with such two-gene vectors was up to ~45 µg/ml under similar conditions.

[0163] The top 76 clones from the ~600 transfectants screened, secreting from ~20 to 64 µg/ml, were transferred to shake flasks and grown in Ex-Cell 301 medium supplemented initially with 4% FBS (to ensure a smooth transition to shake flask growth). Results of an initial shake flask test for two series of transfectants indicated that there were several clones that secreted above the levels of subclones of Clone 689 and Clone 341 (subclones 689.47 and 341.83 from previous sequential transfectants) at the time of the assay.

[0164] The top clones were maintained and adapted to Ex-Cell with 2% FBS and re-screened several more times. The results indicated that Clone 58 consistently secreted at levels equal to or better than subclones of Clones 689 and 341 (subclones 689.47 and 341.83), and at markedly higher levels than Clone 338, from which the respective subclones were derived. For example, Clone 338 was screened at secretion levels over a 7 week period from 80.8 µg/ml to 101.4 µg/ml, while a subclone of Clone 689 (689.47) secreted at 112.0 µg/ml to 134.8 µg/ml and a subclone of Clone 341 (341.83) was at 95.2 µg/ml to 121.4 µg/ml over the same period. Comparatively, Clone 58 secreted at 119 µg/ml to 134.4 µg/ml during this period of testing. Although all of the above tested sequential transfectants of Clone 338 secreted at higher levels than Clone 338, they did not secrete at twice the level although the number of transcription unit copies were effectively doubled through the sequential transfections. This could be due to either growth related differences, limitations in the medium and/or to an inability of the plasmid, pING1915, to generate transfectants that express at the same levels as pING1744, which had been used to generate Clone 338, in these experiments. Nevertheless, this example clearly demonstrates that sequential transfections with vectors containing multiple copies of a transcription unit in accordance with the present invention effectively increases expression levels to substantially higher amounts, even when an enhancer has been removed from the vector.

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EXAMPLE 6 CONSTRUCTION OF ADDITIONAL EXPRESSION VECTORS

[0165] This example describes the construction of expression vectors that contain multiple copies of additional exemplary transcription units. Exemplary vector constructs are also described containing multiple copies of exemplary gene sequences encoding polypeptides of interest, for example, immunoglobulin gene sequences including light and/or heavy chain sequences.

A. Construction of Expression Vectors Comprising Mouse-Human Chimeric ING-1 Light Chain Gene

[0166] Vectors comprising sequences encoding mouse-human chimeric ING-1 light (SEQ ID NOS: 5 and 6) and heavy chains (SEQ ID NOS: 7 and 8) which incorporate the necessary elements for optimal expression in CHO-K1 cells have been constructed. These ING-1 vectors serve both as the starting point for construction of human engineered antibody genes and have been used to develop CHO-K1 cell lines expressing mouse-human chimeric ING-1. The expression vectors described below have a CMV promoter and a mouse kappa light chain 3' un-translated region and transcription units encoding selective gene markers, and light and/or heavy chain sequences.

[0167] A mouse-human chimeric ING-1 light chain vector, pING1928, was constructed by digesting pING2207 (*see, e.g.*, U.S. Patent No. 5,576,184), comprising a mouse-human chimeric ING-1 light chain gene (SEQ ID NO: 5) fused to a mouse light chain 3' untranslated region, with *SalI* plus *HpaI* and isolating the ~2200 bp fragment comprising a light chain (Figure 9). This fragment was ligated to a ~6300 bp *SalI*-*HpaI* vector fragment from pING1732 (described in Example 1 above), placing the mouse-human chimeric ING-1 light chain gene (SEQ ID NO: 5) under control of the CMV promoter and mouse light chain 3' untranslated region (Figure 9). The sequence of the chimeric mouse-human ING-1 light chain is shown as SEQ ID NO: 5. Alternative light chain variable region gene may be cloned into the *SalI* *HindIII* sites of pING1928, including human engineered antibody variable region genes sequences as described below.

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B. Construction Of Expression Vectors Comprising Mouse-Human Chimeric ING-1 Heavy Chain Gene

[0168] A mouse-human chimeric ING-1 heavy chain vector (SEQ ID NOS: 7 and 8), pING1931, was constructed by digesting pING2225 (*see, e.g.*, U.S. Patent No. 5,576,184), comprising a mouse-human chimeric ING-1 heavy chain gene (SEQ ID NO: 7) with *SalI* plus *NaeI* and isolating the ~1433 bp fragment comprising the heavy chain gene sequence (Figure 10). This fragment was ligated to the ~7352 bp vector fragment from pING1736 (described in Example 1 above, similar to pING1740 except that it contains the *neo* instead of the *gpt* gene) which had been digested with *XhoI*, treated with T4 DNA polymerase in the presence of deoxyribonucleotides to blunt end, and then with *SalI* placing the mouse-human chimeric ING-1 heavy chain gene (SEQ ID NO: 7) under control of the human CMV promoter and the mouse light chain 3' untranslated region (Figure 10). The sequence of the chimeric mouse-human ING-1 heavy chain is shown as SEQ ID NO: 7. Alternative heavy chain variable region genes may be cloned into the *SalI*-*ApaI* sites of pING1931, including human engineered antibody variable region genes as described below.

C. Construction of Mouse-Human Chimeric Light Plus Heavy Chain Expression Vectors Mouse-Human Chimeric (Two Gene Vectors).

[0169] Vectors comprising mouse-human chimeric ING-1 light chain plus heavy chain gene sequences (SEQ ID NOS: 5 and 7) were constructed using pING1928 and pING1931 (Figure 11). pING1931 was digested with *EcoRV* and treated with calf intestinal alkaline phosphatase (CIAP). *EcoRV* cuts at a unique site adjacent (and counterclockwise on a circular map) to a unique *NotI* site. pING1928 was digested with *NotI* and *HpaI*, and then treated with T4 DNA polymerase in the presence of deoxyribonucleotides to blunt end. The ~3720 bp fragment comprising a mouse-human chimeric light chain gene (SEQ ID NO: 5) was purified and ligated with *EcoRV*-digested pING1931, comprising a mouse-human chimeric heavy chain gene (SEQ ID NO: 7). Both possible orientations, represented by pING1932 and pING1932R, were obtained as shown in Figure 11.

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D. Construction of Expression Vectors Comprising Human Engineered ING-1 Light Chain Gene

[0170] Human engineering of antibody variable domains has been described by Studnicka [see, e.g., Studnicka *et al.* U.S. Patent No. 5,766,886; Studnicka *et al.*, *Protein Engineering* 7: 805-814 (1994)] as a method for reducing immunogenicity while maintaining binding activity of antibody molecules. According to the method, each variable region amino acid has been assigned a risk of substitution. Amino acid substitutions are distinguished by one of three risk categories: (1) low risk changes are those that have the greatest potential for reducing immunogenicity with the least chance of disrupting antigen binding or protein folding (2) moderate risk changes are those that would further reduce immunogenicity, but have a greater chance of affecting antigen binding or protein folding; (3) high risk residues are those that are important for binding (e.g., CDR loops) or for maintaining antibody structure and carry the highest risk that antigen binding or protein folding will be affected.

[0171] A human engineered ING-1 light chain vector, pING1933 (Figure 12), was constructed by digesting pING1928 (Figure 9), containing a mouse-human chimeric ING-1 light chain gene, with *SalI* plus *NotI* and isolating the ~1518 bp fragment with a CMV promoter and separately digesting pING1928 with *HindIII* plus *NotI* and isolating the ~6566 bp fragment comprising a human light chain constant region, a mouse light chain 3' untranslated region and a *neo* gene for selection of G418-resistant transfectants. These fragments were ligated to a ~400 bp PCR-generated *SalI-HindIII* fragment comprising an ING-1 light chain variable region human engineered with a total of 6 low risk amino acid substitutions (Figure 13; SEQ ID NO: 9), placing the low risk human engineered ING-1 light chain gene under control of a CMV promoter and mouse light chain 3' untranslated region. Low risk changes as well as low plus moderate risk changes in an ING-1 light chain variable region are shown in Figure 13. For the light chain, a total of 6 low risk changes were made for a low risk variable region (SEQ ID NO: 10) as described, and separately a total of 10 low plus moderate risk changes were made for a low plus moderate risk variable region in the light chain (SEQ ID NO: 11 and 12). The vector pING1933 comprises a PCR-generated human engineered ING-1 light chain variable region with 6 low risk changes incorporated. A DNA fragment encoding a low risk modified light chain

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variable region was constructed using 6 overlapping oligonucleotides KL1 (SEQ ID NO: 13), KL2 (SEQ ID NO: 14), KL3 (SEQ ID NO: 15), KL4 (SEQ ID NO: 16), KL5 (SEQ ID NO: 17), and KL6 (SEQ ID NO: 18). These segments were annealed to each other, extended with DNA polymerase and then the assembled variable region amplified by PCR using 5' forward primer KF (SEQ ID NO: 19) and 3' reverse primer KR (SEQ ID NO: 20), digested with *SaII* and *HindIII* to yield a restriction fragment that was cloned directly into expression vector pING1928 to generate pING1933 as shown in Figure 12. Another expression vector, pING1939, was constructed using a similar method and is like pING1933 except that pING1939 comprises an ING-1 light chain variable region human engineered with the low plus moderate risk changes as shown in Figure 13 (SEQ ID NO: 11 and 12). The low plus moderate risk modified light chain variable region was constructed using 6 overlapping oligonucleotides, including 5 used in the construction of the low risk modified variable region described above: KL1 (SEQ ID NO: 13), KM2 (SEQ ID NO: 21), KL3 (SEQ ID NO: 15), KL4 (SEQ ID NO: 16), KL5 (SEQ ID NO: 17) and KL6 (SEQ ID NO: 18).

E. Construction of Expression Vectors Comprising Human Engineered ING-1 Heavy Chain Gene

[0172] A human engineered ING-1 heavy chain vector, pING1936 (Figure 14), was constructed by digesting pING1931, containing mouse-human chimeric ING-1 heavy chain with *SaII* plus *ApaI* and isolating the ~8344 bp fragment comprising a CMV promoter, heavy chain constant region, light chain 3' untranslated region and a *neo* gene for selection of G418-resistant transfectants. This fragment was ligated to the ~450 bp PCR-generated *SaII-ApaI* fragment comprising an ING-1 heavy chain variable region human engineered with a total of 13 low risk amino acid substitutions (Figure 15; SEQ ID NO: 22), placing the low risk human engineered ING-1 heavy chain gene under control of a CMV promoter and mouse light chain 3' untranslated region. Low risk changes as well as low plus moderate risk changes in an ING-1 heavy chain variable region are shown in Figure 15. For the heavy chain, a total of 13 low risk changes were made for a low risk heavy chain variable region (SEQ ID NO: 23) as described, and separately a total of 20 low plus moderate risk changes were made for a low plus moderate risk variable region (SEQ ID NO: 24 and 25). The vector pING1936 separately attached contains a PCR-generated human engineered

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ING-1 heavy chain variable region with 13 low risk changes incorporated. A DNA fragment encoding the heavy chain variable region was constructed using 6 overlapping oligonucleotides GL1 (SEQ ID NO: 26), GL2 (SEQ ID NO: 27), GL3 (SEQ ID NO: 28), GL4 (SEQ ID NO: 29), GL5 (SEQ ID NO: 30), and GL6 (SEQ ID NO: 31). These segments were annealed to each other, extended with DNA polymerase and then the assembled variable region amplified by PCR using a 5' forward primer GF (SEQ ID NO: 32) and a 3' reverse primer GR (SEQ ID NO: 33), digested with *SalI* and *ApaI* to yield a restriction fragment that was cloned directly into expression vector pING1931 to generate pING1936 as shown in Figure 14. Another expression vector, pING1942, was constructed using a similar method and is like pING1936 except that pING1942 comprises an ING-1 heavy chain variable region human engineered with the low plus moderate risk changes as shown in Figure 15 (SEQ ID NO: 24 and 25). The low plus moderate risk modified heavy chain variable region was constructed using 6 overlapping oligonucleotides, including 2 used in the construction of the low risk modified variable region described above: GL1 (SEQ ID NO: 26), GM2 (SEQ ID NO: 34), GM3 (SEQ ID NO: 35), GM4 (SEQ ID NO: 36), GM5 (SEQ ID NO: 37), and GL6 (SEQ ID NO: 31).

F. Construction of Expression Vectors Comprising Human Engineered ING-1 Light Plus Heavy Chain Genes (Two Gene Vectors)

[0173] A vector pING1937, comprising human engineered ING-1 light plus heavy chain genes, was constructed using pING1933 and pING1936 (Figure 16). pING1936 was digested with *XbaI*, treated with T4 polymerase and then digested with *NotI*. The ~8780 bp restriction fragment was purified. pING1933 was digested with *NheI*, then *NotI* and *HpaI*, and the ~3716 bp fragment comprising a human engineered light chain gene was purified and ligated with the *XbaI*-*NotI*-digested pING1936 to generate pING1937, which has a *neo* gene for selection of G418-resistant transfectants. The variable region DNAs were re-sequenced before being used to construct light plus heavy chain expression vectors. The features of pING1937 are summarized in Table 1.

Table 1. Description of pING1937 vector.

Plasmid region	Start nt	End nt	Description
<i>NotI</i> - <i>HindIII</i>	1	479	= pUC12 2616-399 (includes pBR322 4291-4361, 2069-2354 and part of <i>lac</i> gene)
<i>HindIII</i> - <i>1/2BamHI</i>	479	643	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., 1983 sequence; ref 8)
<i>1/2HincII</i> - <i>BamHI</i>	643	1414	hCMV promoter (= -598 to 174 of Boshart et al., ref 1; includes splice donor)
<i>BamHI</i> - <i>SalI</i>	1414	1517	SV40 16S splice acceptor (1654-1741= SV40 1410-1497)
<i>SalI</i> - <i>HindIII</i>	1517	1925	ING-1(heMab) light chain V region
<i>HindIII</i> -NS ^a	1925	2263	ING-1 light chain(heMab) C region (kappa)
NS- <i>BamHI</i>	2263	3582	LC genomic DNA including poly A site
<i>BamHI</i> - <i>1/2BamHI</i>	3582	3889	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter
<i>1/2HincII</i> - <i>BamHI</i>	3889	4660	hCMV promoter, including splice donor
<i>BamHI</i> - <i>SalI</i>	4660	4763	SV40 16S splice acceptor
<i>SalI</i> - <i>ApaI</i>	4763	5206	ING-1 (heMab) heavy chain V region
<i>ApaI</i> - <i>XhoI</i>	5206	6198	ING-1 heavy chain(heMab) C region (gamma-1)
<i>XhoI</i> - <i>BamHI</i>	6198	7562	LC genomic DNA including poly A site
<i>BamHI</i> - <i>1/2BclI</i>	7562	7802	SV40 polyadenylation (=SV40 2532-2774)
<i>1/2BstXI</i> -NS ^a	7802	8409	SV40 small T intron (=SV40 4769-4099)
<i>1/2 SalI</i> - <i>NheI</i>	8409	9898	bacterial neomycin phosphotransferase (neo) gene from pSV2neo (coding region =9545-8753)
<i>NheI</i> - <i>PvuII</i>	9898	10242	SV40 promoter (=SV40 5172-272)
<i>PvuII</i> - <i>NotI</i>	10242	12496	bacterial origin of replication and beta-lactamase (ampicillin resistance) gene (= pBR322 2069-4290)

^a NS - restriction site not identified.

[0174] The vector, pING1959, which is similar to pING1937 except that it has a *gpt* gene for selection of mycophenolic acid-resistant transfectants, was constructed by ligating the ~7696 bp *HpaI*-*NotI* fragment from pING1937 (comprising human engineered ING-1 light and heavy chain genes each fused to a CMV promoter and

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light chain 3' untranslated region) with a ~4441 bp *HpaI-NotI* fragment from pING4144 (described in Example 1 above) comprising a gene encoding *gpt* as shown in Figure 17.

[0175] The vector, pING1957, which is similar to pING1937 and pING1959 except that it has a *his* gene for selection of histidinol-resistant transfectants, was constructed by ligating the ~7696 bp *HpaI-NotI* fragment from pING1937 (as described above) with a ~4639 bp *HpaI-NotI* fragment from pING4152 (described in Example 1 above) comprising a *his* gene as shown in Figure 18.

[0176] Another vector pING1944 was constructed by similar methods used in the construction of pING1937 described above, and is similar to pING1937 except that pING1944 was constructed using pING1939 in place of pING1933 and using pING1942 in place of pING1936. The resulting vector, pING1944 comprises light chain and heavy chain variable regions (SEQ ID NOS: 11, 12, 24, 25) with both the low plus moderate risk substitutions as shown in Figures 13 and 15. Thus, expression vectors for both low risk ING-1 (pING1937) and low plus moderate risk ING-1 (pING1944) were prepared.

G. Construction of Expression Vectors Comprising Two Copies of Human Engineered ING-1 Light and Heavy Chain Genes (Four Gene Vectors)

[0177] A human engineered ING-1 heavy plus light chain vector, pING1937, was treated with *NotI*, T4 DNA polymerase in the presence of deoxyribonucleotides to blunt end and allowed to self-close, destroying the *NotI* site and generating the vector pING1963 lacking a *NotI* site as shown in Figure 19. The vector pING1937 was then digested with *NheI* and *EcoRV* and the ~9905 bp fragment was purified and ligated with the ~10,298 bp *NheI-HpaI* fragment from pING1963 to generate the vector pING1964 as shown in Figure 20 which comprises four ING-1 genes (a four gene vector). pING1964 has two copies of human engineered ING-1 light chain genes and two copies of ING-1 heavy chain genes, with each of the four genes under control of a CMV promoter and light chain 3' untranslated region and a *neo* gene for selection of G418-resistant transfectants. A vector, pING1965, which is similar to pING1964 except that it contains a *gpt* gene for selection of mycophenolic acid-resistant transfectants was constructed by ligating the 1933 bp *HpaI-SfiI* fragment from

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pING1959 with the ~17,935 bp *HpaI-SfiI* fragment from pING1964 as shown in Figure 21.

[0178] Digestion of pING1964 or pING1965 at the unique *NotI* site yields a linear restriction fragment containing four transcription units: two copies of human engineered ING-1 light plus heavy chain genes configured so that a selective marker gene, *neo*, or *gpt*, respectively, is positioned between the two identical light and heavy chain transcription units. Viewed as linear *NotI*-digested DNA, the order of elements within the vector(s) is as follows: CMV promoter, light chain gene, light chain 3' untranslated region, CMV promoter, heavy chain gene, light chain 3' untranslated region, *neo* (pING1964) or *gpt* (pING1965) genes, *bla* (Amp^r) gene, CMV promoter, light chain gene, light chain 3' untranslated region, CMV promoter, heavy chain gene, light chain 3' untranslated region, (Figure 22).

EXAMPLE 7

DEVELOPMENT AND CHARACTERIZATION OF ADDITIONAL TRANSFECTED CLONES AND CELL LINES

[0179] This example describes the development and characterization of additional clones and cell lines transfected with additional exemplary vectors according to the present invention. The development and characterization of immunoglobulin producing cell lines is described from transfections, for example, with two gene vectors as described in Example 6.

A. pING1932 and pING1932R

[0180] The expression vectors, pING1932 and pING1932R described in Example 6 were transfected into Ex-Cell 301-adapted CHO-K1 cells. CHO-K1 cells adapted to suspension growth in Ex-Cell 301 medium were typically electroporated with 40 µg of linearized vector. Both pING1932 and pING1932R contain a unique *NotI* site. In preparation of DNA for transfection, digestion at *NotI* results in linear DNA such that light and heavy chain genes, under the control of a CMV promoter and light chain 3' untranslated region, are separated by the selective marker gene when inserted into the CHO chromosome. With pING1932, the heavy and light chains are oriented in the same direction, whereas in pING1932R, they are oriented in opposite directions.

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[0181] The cells were plated in 96 well plates containing Ex-Cell 301 medium supplemented with 2% FBS and G418. A total of 155 and 168 clones were screened in 96 well plates for pING1932 and pING1932R, respectively. The top 22 clones for each transfection were transferred to 24 well plates containing Ex-Cell 301 medium without FBS.

[0182] A productivity test was performed in 24 well plates in Ex-Cell 301 medium with or without 2% FBS. Cells were grown to extinction and culture supernatants tested for levels of secreted antibody by an immunoglobulin ELISA assay for IgG. The results demonstrated that the pING1932 transfectants generally secreted higher levels of immunoglobulin polypeptide than the pING1932R transfectants. Interestingly, in some cases, the levels of secreted immunoglobulin polypeptides were higher in the medium without FBS than in those supplemented with FBS. The top transfectants from each group secreted in the range from about 7 $\mu\text{g/ml}$ IgG to more than about 30 $\mu\text{g/ml}$ IgG.

[0183] The top 7 clones from the pING1932 transfection (including, for example, Clones 27, 40 and 82) and the top clone from the pING1932R transfection (Clone 168R) were transferred to shake flasks containing Ex-Cell 301 medium. As soon as the cells were adapted to suspension growth, a shake flask test was performed with these clones in Ex-Cell 301 medium with and without 2% FBS. The cells were grown for up to 10 days in 125 ml Erlenmeyer flasks containing 25 ml media. The flasks were sealed for the most of the incubation period and the levels of immunoglobulin polypeptide in the culture medium were determined by IgG ELISA at the end of the incubation period. The results of the initial shake flask test demonstrated that the top clone (Clone 40) secreted up to $\sim 66 \mu\text{g/ml}$. In many cases, there was little difference in productivity between cultures grown with and without FBS.

[0184] The initial shake flask test was performed with flasks that were not opened regularly during the incubation period. Because introducing a gas exchange step at least every other day was found to significantly influence the productivity of certain rBPI₂₁-producing CHO-K1 clones as described in Examples 3 and 4 above, this approach was evaluated with Clones 27, 40, 82 and 168R. Cells were seeded at 1.5×10^5 cells/ml into duplicate 125 ml Erlenmeyer flasks in 25 ml Ex-Cell 301 medium supplemented with 1% FBS and incubated at 37°C, 100 RPM. One set of flasks

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remained sealed for the duration of the incubation, while the other set was opened every day for cell counts and aeration. The results demonstrated that cells grown in flasks that were periodically opened expressed immunoglobulin polypeptide at a higher level (for example, from about 50 $\mu\text{g/ml}$ to about 116 $\mu\text{g/ml}$) than those in which the flasks remained closed (for example, from about 35 $\mu\text{g/ml}$ to about 81 $\mu\text{g/ml}$). These results also corresponded to those obtained in the first shake flask test (for example, from about 45 $\mu\text{g/ml}$ to about 66 $\mu\text{g/ml}$), although the conditions were slightly different (1% FBS in the second test vs. 2% FBS in the first test).

[0185] The cultures that were opened periodically were also examined for growth and productivity at various times. The results of this analysis for Clones 27 and 40 indicated that the cells produced mouse-human chimeric antibody during both the log and the stationary phases.

B. pING1937

[0186] The expression vector pING1937, comprising one copy each of the human engineered ING-1 low risk light and heavy chain genes and the *neo* (G418-resistant) gene, was linearized by digested with *Xba*I followed by transfection into serum-free adapted CHO-K1 cells in Ex-Cell 301 medium. G418-resistant transfectants were selected and screened for immunoglobulin polypeptide expression. Clone 146 was selected as one of the top transfectants and produced up to about 60 mg/ml in shake flasks and about 200 mg/L in a fermentor.

EXAMPLE 8

INCREASING EXPRESSION THROUGH A SECOND SEQUENTIAL VECTOR TRANSFECTION: DEVELOPMENT AND CHARACTERIZATION OF ADDITIONAL TRANSFECTED CLONES AND CELL LINES

[0187] This example describes a further increase in expression and production of polypeptides, for example, immunoglobulins, through a second transfection of an exemplary cell line with a second multi-transcription unit vector.

[0188] Two additional vectors (as described in Example 6) were employed that were identical to pING1937 each comprising two transcription units, with a low risk human engineered ING-1 light chain gene and a low risk human engineered heavy chain gene, except that they have either a *his* gene encoding histidinol resistance

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(pING1957) or a *gpt* gene encoding mycophenolic acid resistance (pING1959). The development of an ING-1 immunoglobulin producing CHO cell line, Clone 259, is described. Clone 259 was developed by transfecting Clone 146 cells (as described in Example 7) with the *his* expression vector pING1957. The development of another ING-1 immunoglobulin producing CHO cell line, Clone 373, is also described. Clone 373 was developed by transfecting a subclone of Clone 146, Clone 146.3 cells, with the *gpt* expression vector pING1959.

A. Transfection of Clone 146 with pING1957 and Development of Clone 259

[0189] Clone 146 was transfected with pING1957 in serum-free medium (Ex-Cell 301). First, 650 clones were screened from 2 transfections in 96 well plates. Then, 142 clones were selected from the 96 well plates and then screened from 24 well plates. Finally, 31 clones were selected from the 24 well plates and screened in shake flasks. The results for top producers in Ex-Cell 301 media without FBS of antibody as measured by HPLC demonstrated that the top producers expressed antibody at greater than 2 times higher levels than Clone 146. For example, the top producer, Clone 146.2-259, expressed 172 $\mu\text{g/ml}$ and 192 $\mu\text{g/ml}$ in two different tests.

[0190] Clone 146.2 - 259 was subcloned in Ex-Cell 301 medium and screened in a 24 well format. The top subclones were further selected based on shake flask productivity in Ex-Cell 301 serum-free medium. Shake flask results for top producers in Ex-Cell 301 without FBS of expression of immunoglobulin polypeptide as measured by HPLC demonstrated that the top Clone 259 subclones expressed antibody at about 1.5 to 2 times higher levels (e.g., from about 229 $\mu\text{g/ml}$ to about 271 $\mu\text{g/ml}$) than the parent Clone 259 (e.g., about 116 $\mu\text{g/ml}$).

B. Transfection of Subclone of Clone 146, Clone 146.3, with pING1959 and Development of Clone 373

[0191] Clone 146, the initial pING1937 G418-resistant transfectant was also subjected to subcloning in Ex-Cell medium and one subclone, 146.3, secreted ~121 $\mu\text{g/ml}$ compared to ~65 $\mu\text{g/ml}$ for Clone 146 in Ex-Cell medium.

[0192] Since Clone 146.3 secreted at a relatively high level for a single transfection, it was therefore subjected to transfection with pING1959. Serum-free medium (Ex-Cell 301) adapted Clone 146.3 cells were transfected with pING1959 (same as pING1937

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except for mycophenolic acid resistance as described in Example 6), plated in Ham's F12 with 5% FBS/mycophenolic acid and xanthene for selection. First, 520 clones were screened from 2 transfections in 96 well plates. Then 106 clones were selected from 24 well plates and screened. Finally, 26 clones were selected from the 24 well plates and screened in shake flasks. The sequential transfection of Clone 146.3 with pING1959 resulted in the selection of Clone 373 which expressed ~225 and ~257 µg/ml immunoglobulin polypeptide as determined by the shake flask results in Ex-Cell 301 medium.

EXAMPLE 9

FURTHER INCREASING EXPRESSION BY A THIRD SEQUENTIAL VECTOR TRANSFECTION: DEVELOPMENT AND CHARACTERIZATION OF ADDITIONAL TRANSFECTED CLONES AND CELL LINES

[0193] This example describes a further increase in expression and production by a third sequential transfection of an exemplary cell line with a third multi-transcription unit vector, resulting in clones and cell lines that express increased levels of polypeptide production, for example, immunoglobulin production.

[0194] Clone 373 as described in Example 8 was chosen for additional studies and was further subjected to another sequential transfection using pING1957 (same as pING1937 except for histidinol resistance) in serum-free medium (Ex-Cell 301) and plated in Ex-Cell 301 supplemented with FBS and histidinol. Once the clones were selected, they were maintained with G418, MPA/xanthine/histidinol.

[0195] First, 160 clones were screened from 2 transfections in 96 well plates. Then, 48 clones were selected from the 96 well plates and screened in 24 well plates. Finally, 12 clones were selected from the 24 well plates and screened in shake flasks. Results for shake flask tests in Ex-Cell 301 yielded 8 top producing clones.

[0196] The top producing clones displayed an expression level ranging from about 310 to about 370 µg/ml, including Clone 132 which had an expression level of about 317 µg/ml.

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EXAMPLE 10

TRANSFECTION WITH ADDITIONAL MULTIPLE TRANSCRIPTION
UNIT VECTORS: DEVELOPMENT AND CHARACTERIZATION OF
ADDITIONAL TRANSFECTED CLONES AND CELL LINES

[0197] The expression vectors, pING1959 (Figure 17) and pING1965 (Figure 21) containing one copy of each of the human engineered ING-1 light and heavy chain genes (pING1959, two gene vector) or two copies of each of the human engineered ING-1 light and heavy chain genes (pING1965, four gene vector) were transfected into CHO-K1 cells. CHO-K1 cells adapted to suspension growth in Ex-Cell 301 medium were electroporated with 40 µg of each linearized vector. After a recovery period of 2 days without selective agent, cells were plated in 96 well plates containing Ham's F12 medium supplemented with 5% FBS, mycophenolic acid and xanthine. A total of 300 and 255 clones were screened in 96 well plates for transfections with pING1959 and pING1965, respectively. For the pING1959 transfections, the top 18 clones were transferred to 24 well plates containing Ex-Cell 301 medium supplemented with 1% FBS. For the pING1965 transfections, the top 40 clones, were transferred to 24 well plates containing Ex-Cell 301 medium supplemented with 1% FBS. All 18 clones from the pING1959 transfection were next transferred to shake flasks containing Ex-Cell 301 medium supplemented with 1% FBS and evaluated for productivity. The top two producers, Clones 53 and 157 secreted ~116 and ~133 µg/ml, respectively in the presence of 1% FBS. In ExCell 301 medium without FBS supplementation Clones 53 and 157 secreted ~117 and ~121 µg/ml, respectively. For the pING1965 transfection, the top 8 clones were transferred to shake flasks and evaluated for productivity. The top producer, Clone 17, secreted ~216 µg/ml in ExCell 301 medium supplemented with 1% FBS and ~214 µg/ml in ExCell 301 medium without FBS supplementation. Accordingly, a cell line transfected with a four gene vector (pING1965) with two copies of each of the human engineered light and heavy chain genes did produce approximately twice as much immunoglobulin polypeptides as cell lines transfected with a two gene vector (pING1959) with one copy of each of the human engineered ING-1 light and heavy chain genes.

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EXAMPLE 11

EVALUATION OF BINDING ACTIVITY OF IMMUNOGLOBULIN
POLYPEPTIDES

[0198] Vectors constructed according to Example 6 encoding human engineered ING-1 light and heavy chain genes, for example, pING1937 (low risk human engineered ING-1) and pING1944 (low plus moderate risk human engineered ING-1) were linearized by *Xba*I and used to transfect serum-free adapted CHO-K1 followed by selection of G418-resistant transfectants. Protein was purified from shake flask culture supernatants by passage over a protein A column. To evaluate the binding activity of the produced immunoglobulin polypeptides, competition binding assays with the human carcinoma cell line HT-29 were performed. This colorectal carcinoma cell line expresses a molecule known as Ep-CAM on its surface. Ep-CAM is recognized by immunoglobulin polypeptides having the antigen binding specificity of the mouse-human chimeric ING-1 antibody produced by cell line HB 9812 as deposited with the ATCC.

[0199] HTG29 cells were grown to confluency ($\sim 2 \times 10^5$ cells/well) in 96 well plates. Mouse-human chimeric ING-1 was labeled with Na^{125}I (Iodo-gen®, Pierce). The competition conditions included in a 100 μl assay volume, 0.1 nM labeled mouse-human chimeric ING-1, 2-fold serial dilutions of unlabeled immunoglobulin polypeptides, for example, as produced by cells transfected with pING1937 and pING1944. The labeled and unlabeled immunoglobulin polypeptides were incubated with HT-29 cells at 4°C for 5 hours followed by washing. Labeled cells were removed with NaOH and counted. Data analysis was performed using Ligand [Munson and Redbard, *Analytical Biochem.* 107:220-239 (1980)].

[0200] Results for competition binding assays using immunoglobulin polypeptides obtained from transfection with the pING1937 (low risk) vector are shown in Figure 23. The affinities for the mouse-human chimeric ING-1, containing an un-modified ING-1 murine variable domain, and the human engineered ING-1, with its variable domain modified at low risk positions, showed very similar affinities (2-5nM) (Figure 23).

[0201] Human engineered ING-1 modified at the low risk plus moderate risk positions was also evaluated using competition binding assays. Results with the

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human engineered ING-1 purified from pING1944 transfected cell culture supernatant are shown in Figure 24. No differences in binding between the mouse-human chimeric and the human engineered (low risk) ING-1 were observed. The human engineered (low plus moderate risk) ING-1 obtained from transfection with the vector pING1944 showed a reduced competition binding activity as shown in Figure 24.

[0202] To examine the contribution of the light and heavy chain moderate risk changes on ING-1 binding activity, immunoglobulin polypeptides were expressed from vectors constructed with either the combination of a low risk light chain with a low plus moderate risk heavy chain or a low risk heavy chain with a low plus moderate risk light chain. Vectors containing both modified ING-1 light chain plus heavy chain were used to transfect serum-free medium-adapted CHO-K1 cells. Approximately 100 clones were screened in microtiter plates to select the top 8 to 10 clones for shake flask evaluation. For production purposes, the best producers were grown in shake flasks and modified ING-1 IgG's were purified on a protein A column followed by concentration determination by A_{280} .

[0203] Competition binding assays, employing iodinated human engineered (low risk) ING-1 and Ep-CAM-expressing HT-29 cells, were used to characterize modified ING-1 immunoglobulin polypeptides. Exemplary results are shown in Figure 25. Moderate risk changes made to the light chain appeared to have the greatest impact on binding of the modified ING-1 antibodies tested. Moderate risk changes made to the heavy chain also appeared to affect binding, but to a lesser extent than the light chain changes. The results suggested that the effects observed with individual chains were additive.

[0204] Moderate risk changes include changes involving prolines (see, e.g., Figures 13 and 15). The moderate risk ING-1 light chain has 3 prolines introduced within Framework 1 (amino acids 1-59 (SEQ ID NO: 12)), and the moderate risk ING-1 heavy chain has 1 proline removed within framework 1 (amino acids 1-57 (SEQ ID NO: 25)). To examine the effects of proline changes in greater detail, low risk human engineered ING-1 light chain variable regions were constructed with prolines substituted in the low risk human engineered variable regions either one at a time (e.g. Proline1 (P1) (SEQ ID NOS: 38 and 39), Proline 2 (P2) (SEQ ID NOS: 40 and 41), Proline 3 (P3) (SEQ ID NOS: 42 and 43) or combinations of prolines P1P2 (SEQ ID

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NOS: 44 and 45), P1P3 (SEQ ID NOS: 46 and 47), P2P3 (SEQ ID NOS: 48 and 49) of the light chain. As is shown in Figure 13, the total of 3 amino acid positions in the low risk light chain that were changed to proline are within Framework 1. Therefore, all combinations of prolines were incorporated by using two overlapping oligonucleotides. KL1 (SEQ ID NO: 13) remained unchanged, and was as described in Example 6 for the construction of the low risk ING-1 light chain vector pING1933. The second oligonucleotide used was one of 6 variations of the oligonucleotide KM2 (SEQ ID NO: 21) described in Example 6 for the construction of the low plus moderate risk ING-1 light chain vector pING1939. The variation of KM2 chosen depended upon which combinations of prolines were to be introduced into the low risk ING-1 light chain sequence. A low risk light chain variable region was further with the first moderate risk proline (P1) substituted for the alanine at position 8 of the low risk ING-1 light chain (SEQ ID NO: 39). A low risk variable region was modified with the second moderate risk proline (P2) substituted for the leucine at position 15 of the low risk ING-1 light chain [SEQ ID NO: 41]. A low risk variable region was modified with the third moderate risk proline (P3) substituted for the serine at position 18 (SEQ ID NO: 43). By employing one of 6 variations of the KM2 oligonucleotide (SEQ ID NO: 21), each proline residue was first changed separately using the oligonucleotide P1 (SEQ ID NO: 50), P2 (SEQ ID NO: 51), P3 (SEQ ID NO: 52), and then in pairs using the oligonucleotide P1P2 (SEQ ID NO: 53), P1P3 (SEQ ID NO: 54) or P2P3 (SEQ ID NO: 55). The cloning strategy employed to construct expression vectors encoding various ING-1 light chains with different combinations of moderate risk proline residues incorporated into the low risk human engineered ING-1 light chain is shown in Figure 26A. Subsequent to annealing the modified KM2 variant with the unmodified KL1 oligonucleotide, the annealing reaction was extended with DNA polymerase followed by amplification by PCR employing ING-1 light chain forward primer KF and reverse primer KBsr (SEQ ID NO: 56). The resultant product was then digested with *SalI* and *BsrFI*, followed by purification of the resultant 130 base pair fragment corresponding to low risk ING-1 light chain framework 1 region modified by the introduction of proline residues at one of three positions or in various combinations. The vector pING1939 was then digested with *SalI* and *HpaI* followed by purification of the large linear vector

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fragment. The vector pING1939 in a separate reaction was digested with *Bsr*F1 and *Hpa*I followed by purification of the 2 kb fragment.

[0205] A three way ligation was performed in which the *Hpa*I end of the pING1939 linear vector first ligates with the *Hpa*I end of the 2 kb fragment comprising the ING-1 light chain minus the framework 1 region. The full length ING-1 light chain is reconstructed when the *Bsr*F1 end of the 130 bp proline modified fragment ligates to the *Bsr*F1 end of the 2 kb fragment comprising the ING-1 light chain minus the framework 1 region. The vector was then closed when the *Sal*I end of the 130 bp proline modified framework 1 region fragment ligates to the *Sal*I end of the pING1939 linear vector. These light chains, modified at various proline positions, were then expressed with the low risk heavy chain.

[0206] Competition binding assays, again employing iodinated human engineered (low risk) ING-1 and Ep-CAM-expressing HT-29 cells, were used to determine the effect of prolines on binding activity. Results for modified ING-1 with a low risk heavy chain in combination with a light chain modified at P1, P2, P1P2, or P1P3 are shown in Figure 26B. When compared with each other, changes at any one or a combination of two, of the tested prolines, have a similar effect. Changing all three positions to prolines has the greatest effect.

EXAMPLE 12

CLONING AND EXPRESSION OF SOLUBLE HUMAN

EP-CAM AND EP-CAM BINDING ASSAYS

[0207] Competition binding assays with Ep-CAM-expressing cells such as HT-29 cells as described in Example 11 above require the use of isotopes and the maintenance and growth of cells for each assay, with the potential variability as with any cell-based assay. A direct binding ELISA assay with soluble Ep-CAM was also developed and used to evaluate the binding activity of immunoglobulin polypeptides produced by transfected cells according to the invention. For these assays, soluble human Ep-CAM (SEQ ID NOS: 57 and 58) was cloned and expressed. Immunoglobulin polypeptides used included human engineered (low risk) ING-1 from either 2L or 500L fermentor runs and soluble sEp-CAM from shake flasks or 2L fermentors purified by ING-1 affinity chromatography.

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[0208] Soluble Ep-CAM has previously been expressed in insect cells for expression in and secretion from CHO-K1 cells, truncated Ep-CAM (sEp-CAM) was cloned into an expression vector with the CMV promoter and the *neo* gene encoding for G418-resistance. The cloning strategy is outlined in Figure 27. The vector pING1736 (described in Example 6 above) was digested with *XhoI*, followed by treatment with T4 DNA polymerase to blunt end. The blunt-ended linear vector was then digested with *SalI* and the large fragment was isolated. The Ep-CAM gene was obtained from HT-29 mRNA using a RT PCR reaction which incorporated a 5' *SalI* site, a 3' *SmaI* site, and truncated the Ep-CAM gene by introducing a stop codon at amino acid position 266 (SEQ ID NO: 57 or 58). Without that stop codon the Ep-CAM sequence comprises 314 amino acids as shown in (SEQ ID NOS: 59 and 60). For the RT PCR, two primers were used, an Ep-CAM forward primer (SEQ ID NO: 61) and an Ep-CAM reverse primer (SEQ ID NO: 62). The RT PCR reaction was digested with *SmaI* and *SalI* and the resultant ~800 base pair fragment was purified and ligated with the digested pING1736 large fragment to produce vector pING1954.

[0209] Serum-free medium adapted CHO-K1 cells were then transfected with linearized pING1954. Transfectants were then selected in Ex-Cell 301 with 2% FBS. Screening was performed in 24 well plates, and shake flask formats by direct ELISA. Detection was performed with ING-1 followed by peroxidase-labeled goat anti-human IgG. 150 clones, adapted to grow without FBS were transferred to 24 well plates. The cells were grown to extinction and 50 µl supernatant was used to pre-coat the Immulon II plates. The top 6 clones were then transferred to shake flasks. The productivity for Clone 51 was subsequently estimated to be about 20 to 35 mg/L in both shake flasks and 2 liter fermentors.

[0210] Western blot analysis was employed to confirm that ELISA signal corresponded to specific proteins. Multiple bands were subsequently observed with the Ep-CAM supernatant. These multimers are also observed with purified Ep-CAM and were not an artifact of running crude culture supernatant, and therefore did not adversely impact the use of supernatants in an ELISA-based direct binding assay. Moreover, the lack of detection on a reduced gel is consistent with the non-linear nature of the known Ep-CAM epitope structure recognized by immunoglobulin polypeptides with antigen binding like ING-1.

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[0211] Immulon II plates were precoated with the sEp-CAM antigen to immobilize it to the microplate. In preparation for pre-coating, the sEp-CAM antigen was first diluted in PBS coating. sEp-CAM test concentrations ranging from 0.25 to 20 µg/ml were added at 50 µl/well and incubated at 4°C overnight. The plates were then washed 3 times with PBS-0.05% Tween. The plates were blocked by adding in PBS-0.05% Tween 1% BSA followed by a 30 minute incubation at 37°C.

[0212] Dilutions of immunoglobulin polypeptides were prepared in PBS-0.05% Tween, 1% BSA solution. two- or three-fold serial dilutions were prepared and added (100 µl/well) in duplicate or triplicate. After a 90 minute incubation at 37°C, the microplate was washed 3 times with PBS-0.05% Tween. For signal development, goat anti-human IgG (gamma- or Fc-specific) secondary antibody conjugated to peroxidase was added to each well and incubated for 60 minutes at 37°C followed by addition of OPD at 0.4 mg/ml in citrate buffer plus 0.012% H₂O₂. After 5 – 10 minutes at room temperature, the assay was stopped by the addition of 100 µl 1M H₂SO₄ and the plates were read at 490nm. Both goat anti-human IgG (gamma-specific) and goat anti-human IgG (Fc-specific) antibodies have been employed. Results of the direct binding ELISA for human engineered (low risk) ING-1 on soluble Ep-CAM is shown in Figure 28.

EXAMPLE 13

CONSTRUCTION OF ADDITIONAL EXPRESSION VECTORS

[0213] This example describes the construction of expression vectors that comprise multiple copies of additional exemplary transcription units. Exemplary vector constructs are also described comprising multiple copies of exemplary gene sequences encoding polypeptides of interest, for example additional immunoglobulin gene sequences including light and/or heavy chain sequences.

[0214] To facilitate cloning of additional genes into exemplary expression vectors useful according to the invention, modular expression vectors containing a multicloning site between a CMV promoter and a light chain 3' untranslated region were constructed. A polylinker, assembled from two complementary DNA oligonucleotides (pING1736 polylinker 5'TCGACGAATTCATCGATGGTACC3' (SEQ ID NO. 63) and 3'GCTTAAGTAGCTACCATGG5' (SEQ ID NO: 64))

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(pING1732 polylinker 5'TCGACGAATTCATCGATGATATC3' (SEQ ID NO 65)) and 3'GCTTAAGTAGCTACTATAG5' (SEQ ID NO: 66)) which when annealed form a duplex insert containing several unique sites (*SalI*, *EcoRI*, *ClaI*, *EcoRV* for pING1732 and *SalI*, *EcoRI*, *ClaI*, *KpnI* for pING1736) and overhangs for cloning into two separate vectors, pING1732 and pING1736, each containing a *neo* gene for selection of G418-resistant clones. Both pING1732 and pING1736 were first digested with *XhoI*, treated with T4 polymerase and then the linear vectors digested a second time with *SalI*. The purified linear vectors and the polylinker inserts were purified and ligated. As shown in Figures 29 and 30, the two resultant vectors, pING1732-RIClaRV and pING1736-RIClaKpnI, are designed with several unique restriction sites between the 3' end of the promoter/splice region and the 5' end of the polyadenylation region and useful for expression of any inserted gene encoding a polypeptide of interest.

[0215] Light and heavy chains from a humanized antibody against CD18 (*see*, U.S. Patent Nos. 5,985,279 and 5,997,867) were cloned as shown in Figures 31 and 32 into pING1732-RIClaRV and pING1736-RIClaKpnI, respectively, by PCR from a vector having light and heavy chain genes. A new light chain vector, designated pING2050, was prepared as outlined in Figure 3, and was constructed by first digesting the vector pING1732-RIClaRV with *XhoI* and *EcoRI* followed by purification of the linear vector. A gene encoding an anti-CD18 light chain (designated LDP-1 LC in Figure 31; SEQ ID NOS: 67 and 68), was then amplified by PCR from a vector having that gene employing a forward primer LDP5PrFwd (5'TGTATTGAATTCACCATGGGATGGAGCTG 3'; SEQ ID NO: 69) and a reverse primer LDPLC3PrXhoRev (sequence = 5' GATAACTCGAGCTAACACTCTCCCCTGTTG 3' (SEQ ID NO: 70)). The resultant PCR product was then purified, digested with the restriction enzymes *EcoRI* and *XhoI*, and ligated with the pING1732-RIClaRV made linear by digestion with *EcoRI* and *XhoI*. The resulting light chain vector was designated pING2050 as shown in Figure 31.

[0216] A new heavy chain vector, designated pING2051, was prepared as outlined in Figure 32. The vector pING1736-RIClaKpnI was first digested with *XhoI* and *EcoRI* followed by purification of the linear vector. A gene encoding an anti-CD18 heavy chain (designated LDP-1 HC in Figure 32; SEQ ID NOS. 71 and 72) was then

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amplified by PCR from a vector having that gene employing a forward primer LDP 5PrFwd (5'TGTATTGAATTCACCATGGGATGGAGCTG3'; SEQ ID NO. 73) and a reverse primer LDPLX3PrXhoRev (5'CTTATCTCGAGTCATTTACCCGGAGACAGG3' (SEQ ID NO: 74)). The resultant PCR product was then purified, digested with the restriction enzymes *EcoRI* and *XhoI*, and ligated with the pING1736-RIClaKpnI made linear by digestion with *EcoRI* and *XhoI*. The resulting heavy chain vector was designated pING2051 as shown in Figure 32. The DNA sequences of the light and heavy chain genes in pING2050 and pING2051, respectively, were verified by DNA sequence analysis. The light and heavy chain DNA and amino acid sequences are shown in Figures 33 (SEQ ID NOS: 64 and 70) and 34 (SEQ ID NOS: 68 and 72), respectively.

[0217] The ping2052 a vector comprising anti-CD18 light plus heavy chain genes and a *neo* gene encoding resistance to G418 was constructed as shown in Figure 35. The vector pING2050 was first digested with the restriction enzymes *HpaI* and *NheI*, and the large linear vector fragment purified. The vector pING2051 was digested with *XbaI*, blunt ended by treatment with T4 polymerase, digested with *NheI*, followed by purification of the large linear vector fragment. Both the pING2050 and pING2051 digested and purified vectors were then ligated to form a light plus heavy chain expression vector pING2052, which contains a *neo* gene encoding resistance to G418. As shown in Figure 35, the pING2052 vector contains several unique restriction sites for generation of linear DNA in preparation for transfection. The features of pING2052 are summarized in Table 2.

Table 2. Description of pING2052 vector.

Plasmid region	Startnt	End nt	Description
NotI-HindIII	1	479	= pUC12 2616-399 (includes pBR322 4291-4361, 2069-2354 and part of <i>lac</i> gene)
HindIII-1/2BamHI (Sau3AI)	479	643	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., 1983 sequence; ref 5)
1/2HincII-BamHI	643	1414	hCMV promoter (= -598 to 174 of Boshart et al., ref 1; includes splice donor)
BamHI-EcoRI	1414	1523	SV40 16S splice acceptor (1421-1509=SV40 1410-1497)

Plasmid region	Start nt	End nt	Description
<i>EcoRI-XhoI</i>	1523	2234	LDP-01 light chain gene
<i>XhoI-BamHI</i>	2234	3598	LC genomic DNA including poly A site
<i>BamHI-1/2HpaI</i> (<i>XbaI-HindIII</i>)	3598	3741	portion of SV40 polyadenylation (=SV40 2532-2668)
<i>HindIII-1/2BamHI</i> (<i>Sau3AI</i>)	3741	3905	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., 1983 sequence; ref 5)
<i>1/2HincII-BamHI</i>	3905	4676	hCMV promoter, including splice donor
<i>BamHI-EcoRI</i>	4676	4785	SV40 16S splice acceptor
<i>EcoRI-XhoI</i>	4785	6206	LDP-01 heavy chain gene
<i>XhoI-BamHI</i>	6206	7570	LC genomic DNA including poly A site
<i>BamHI-1/2BclI</i> (<i>Sau3AI</i>)	7570	7808	SV40 polyadenylation (=SV40 2532-2774)
<i>1/2BstYI(Sau3AI)-NS^a</i>	7808	8415	SV40 small T intron (=SV40 4769-4099)
<i>1/2 SalI-NheI</i>	8415	9927	bacterial neomycin phosphotransferase (neo) gene from pSV2neo (coding region =9583-8761)
<i>NheI-PvuII</i>	9927	10250	SV40 promoter (=SV40 5172-272)
<i>PvuII-NotI</i>	10250	12504	bacterial origin of replication and beta-lactamase (ampicillin resistance) gene (= pBR322 2069-4290)

^a NS - restriction site not identified.

[0218] A second anti-CD18 light plus heavy chain expression vector, pING2057, containing a *his* gene for selection of histidinol-resistant transfectants, was also constructed by exchanging the *neo* gene in pING2052 with a *his* gene from pING1957 (described in Example 6 and Figure 18). The vector pING2052 was first digested with the restriction enzymes *HpaI* and *NotI* followed by purification of the large linear vector fragment. The vector pING1957 was then digested with *HpaI* and *NotI* followed by purification of the smaller *his* gene fragment. The linear vector pING2052 and *his* gene fragments were then ligated to form a light plus heavy chain expression vector pING2057 as shown in Figure 36, which contains a *his* gene for selection of histidinol-resistant transfectants. The features of pING2057 are summarized in Table 3.

Table 3. Description of pING2057 vector.

Plasmid region	Start nt	End nt	Description
<i>NotI</i> - <i>HindIII</i>	1	479	= pUC12 2616-399 (includes pBR322 4291-4361, 2069-2354 and part of <i>lac</i> gene)
<i>HindIII</i> - <i>1/2BamHI</i> (<i>Sau3AI</i>)	479	643	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., 1983 sequence; ref 5)
<i>1/2HincII</i> - <i>BamHI</i>	643	1414	hCMV promoter (= -598 to 174 of Boshart et al., ref 1; includes splice donor)
<i>BamHI</i> - <i>EcoRI</i>	1414	1523	SV40 16S splice acceptor (1421-1509=SV40 1410-1497)
<i>EcoRI</i> - <i>XhoI</i>	1523	2234	LDP-01 light chain gene
<i>XhoI</i> - <i>BamHI</i>	2234	3598	LC genomic DNA including poly A site
<i>BamHI</i> - <i>1/2HpaI</i> (<i>XbaI</i> - <i>HindIII</i>)	3598	3741	Part of SV40 polyadenylation (=SV40 2532-2668)
<i>HindIII</i> - <i>1/2BamHI</i> (<i>Sau3AI</i>)	3741	3905	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., 1983 sequence; ref 5)
<i>1/2HincII</i> - <i>BamHI</i>	3905	4676	hCMV promoter, including splice donor
<i>BamHI</i> - <i>EcoRI</i>	4676	4785	SV40 16S splice acceptor
<i>EcoRI</i> - <i>XhoI</i>	4785	6206	LDP-01 heavy chain gene
<i>XhoI</i> - <i>BamHI</i>	6206	7570	LC genomic DNA including poly A site
<i>BamHI</i> - <i>1/2BclI</i> (<i>Sau3AI</i>)	7570	7808	SV40 polyadenylation (=SV40 2532-2774)
<i>1/2BstYI</i> (<i>Sau3AI</i>)-NS ^a	7808	8415	SV40 small T intron (=SV40 4769-4099)
<i>1/2 SalI</i> - <i>StuI</i>	8415	9790	bacterial histidine (<i>his</i>) gene from pSV2his (coding region =9760-8458)
<i>StuI</i> - <i>PvuII</i>	9790	10113	SV40 promoter (=SV40 5172-272)
<i>PvuII</i> - <i>NotI</i>	10113	12342	bacterial origin of replication and beta-lactamase (ampicillin resistance) gene (= pBR322 2069-4290)

^a NS - restriction site not identified.

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EXAMPLE 14

DEVELOPMENT AND CHARACTERIZATION OF ADDITIONAL
TRANSFECTED CLONES AND CELL LINES

[0219] This example describes the development and characterization of additional clones and cell lines transfected with additional exemplary vectors according to the present invention, the development and characterization of additional immunoglobulin producing cell lines is described from transfections with vectors, including multigene vectors, as described in Example 13.

[0220] Cell lines were developed by electroporation of Ex-Cell 301 – adapted CHO-K1 cells. Prior to transfection, the anti-CD18 light plus heavy chain *neo* vector, pING2052, was linearized with *Xba*I as shown in Figure 37. Forty µg of linearized DNA typically was used in conjunction with $\sim 1 \times 10^7$ cells for transfection by electroporation. At least 3 electroporations were performed on any given day (for a total of ~ 120 µg DNA used with $\sim 3 \times 10^7$ cells). Following a 2 day recovery period in non-selective Ex-Cell 301 medium supplemented with 2% FBS and 50 µg/ml gentamicin, the cells were plated at ~ 4000 cells/well (200 µL/well) in 96 well plates in Ex-Cell 301 medium supplemented with Glutamine/Penicillin/Streptomycin, 1% FBS and with 0.8 mg/mL G418 sulfate; Geneticin® (Invitrogen, Carlsbad, CA ____) for transfectants with *neo* vectors. The plates were incubated at 37°C in a CO₂ incubator. Starting at days 10-12, the 96 well plates were scanned for wells containing single clones and the supernatants were sampled and screened by ELISA.

[0221] For the ELISA, culture supernatants from 96 or 24 well plates or from shake flasks were pipetted into 96 well dilution plates containing PBS, 1% BSA and 0.05% Tween 20 and stored overnight at 4°C. The supernatants were assayed for levels of immunoglobulin polypeptides by ELISA using Immulon 4 plates precoated overnight at 4°C with an anti-Human IgG gamma coating antibody (Anti-Human IgG, Fd Fragment, Catalog No. 411411 or Anti-Human IgG, J Chain, Catalog No. 401441, Calbiochem, San Diego, CA). Following a blocking step with PBS containing 1% BSA and 0.05% Tween 20, the diluted supernatants were added to the plates. After incubation at room temperature for 1 hour on a plate shaker, the plates were washed three times with PBS supplemented with 0.05% Tween. A biotinylated anti-Human

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Kappa - specific secondary antibody (Pierce, Rockford, IL, Product No. 31780) was added and the plates were incubated for 30 minutes at room temperature on a plate shaker. After additional washes, Extravidin (Sigma Chemical Co., St. Louis, MO) was added and the plates incubated for 30 minutes. Detection was performed with a KPL/ABTS system (KPL, Gaithersburg, MD). After color development, the reaction was stopped by adding an equal volume of KPL stop buffer. Plates were read at 405nm and concentrations were calculated using a program with a 4 parameter curve fit.

[0222] A total of 3-4 ELISAs were performed over ~7 days for any given transfection. Clones secreting immunoglobulin polypeptides above an established level were transferred to 24 well plates and when confluent, replica 24 well plates were prepared. Screening was performed by ELISA on the original 24 well plate cultures once they reached extinction at ~14 days post plating. The top – producing clones in 24 well plates were transferred to 6 well plates and then to shake flasks. CHO-K1 transfectants were inoculated into 25 mL Ex-Cell 301 medium in 125 mL shake flasks at $\sim 1 - 2 \times 10^5$ cells per mL, depending on the assay. Ex-Cell 301 media was supplemented with Glutamine/Penicillin/Streptomycin as described above. Selective agents generally were not incorporated into media used for the shake flask tests except in cases in which maintenance cultures were allowed to incubate to extinction. The cells were incubated at 37°C, 100 RPM until viability was 20% (usually ~14 days). For growth and productivity experiments, viable cell density was determined either by hemocytometer and/or with the Guava flow cytometer (Guava Technologies, Inc., Burlingame, CA 94010) every 2 to 4 days. For studies in which the viable cell density was not determined, the flasks were opened and swirled in the biosafety hood every 2 to 3 days to provide some gas exchange. At the end of the incubation period, cells were removed by centrifugation and supernatants were assayed for immunoglobulin polypeptide concentration by ELISA. The highest producers identified by ELISA also were assayed by Protein A HPLC. For the Protein A HPLC, concentrations of immunoglobulin polypeptides in samples from transfectants were determined using a Shimadzu LC-10A HPLC System (Torrance, CA) and a Perceptive Biosystems' Protein A column (Albertville, MN; Poros A affinity, 20 μ m, A/M, 4.6 x 100mm, Catalog number 1-5022-26). HPLC Buffer A

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was phosphate buffered saline (PBS) at pH 7.2 and HPLC Buffer B was 0.1 M glycine / 2% acetic acid, pH 3.0. The flow rate of the column was 4.0 ml/minute and the gradient was as follows: 100% A at 0-3 minutes; 100% B at 3.1 – 5 minutes; and 100% A at 5.1 – 8 minutes, with detection at 280 nm.

[0223] Three independent sets of transfections each consisting of three electroporations were performed using the *neo* vector, pING2052. A total of 3564 clones were screened from the 96 well plates. Of these clones, 471 were transferred from 96 well to 24 well plates and extinct cultures were screened by ELISA. The top 80 clones were transferred to shake flasks and extinct cultures were screened by ELISA and for some of the top clones, by Protein A HPLC. The results for the top 8 clones demonstrated that Clone 128D12 appeared to be the highest producer in Ex-Cell 301 medium with 14 day (extinct) shake flask productivities (determined by HPLC) of 134, 89, 157, and 127 µg/mL. Productivities determined by ELISA appeared to be up to ~2-fold higher than those obtained by HPLC.

[0224] Six of the top clones were evaluated in a growth and productivity test in 125 mL shake flasks. The cultures were inoculated at 1×10^5 cells/mL in Ex-Cell 301 medium and incubated for 13 days. The results as shown in Figure 38 demonstrated that Clone 128D12 was an optimal clone with respect to both growth and productivity. Cells containing only the *neo* selective marker were grown in the presence of a maintenance level of G418.

EXAMPLE 15

INCREASING EXPRESSION THROUGH A SECOND SEQUENTIAL VECTOR TRANSFECTION: DEVELOPMENT AND CHARACTERIZATION OF ADDITIONAL TRANSFECTED CLONES AND CELL LINES

[0225] This example describes a further increase in expression and production of polypeptides, including additional immunoglobulins, through a second transfection of an exemplary cell line with a second multi-transcription unit vector.

[0226] The top-producing clone, Clone 128D12, selected from transfections with pING2052, the anti-CD18 light plus heavy chain *neo* vector as described in Example 13 above, were re-transfected with the pING2057 *his* vector also described in Example 13 above. For example, Clone 128D12 was re-transfected with pING2057

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in two sets of transfections (3 electroporations per set). In preparation for transfection, vector pING2057 was made linear by digestion with *Xba*I. A total of 1101 clones from 96 well plates were screened by ELISA. The top 218 clones were transferred to 24 well plates and extinct replica cultures were screened by ELISA. The top 8 clones expressed immunoglobulin polypeptides at levels up to ~3-fold higher than Clone 128D12 at the 24 well stage of screening. The top 120 clones were transferred to shake flasks. The top 10 clones secreted up to ~290 µg/mL in extinct shake flask cultures in Ex-Cell 301 medium. Cells containing both *neo* and *his* selective markers were grown in the presence of a maintenance levels of geneticin and histidinol.

[0227] Clones 264E2, 254G12, 257G8, 262E8, 259B6, and 253E10 secreted from 250 - 290 µg/ml in shake flasks, with Clone 264E2 selected as a top producer. Clone 264E2, in several shake flash tests in Ex-Cell 301 medium produced ~1.5 to greater than 2 times as much immunoglobulin POLYPEPTIDE as Clone 128D12 as measured by Protein A-HPLC.

EXAMPLE 16 POLYPEPTIDE EXPRESSION IN SEVERAL COMMERCIALY AVAILABLE MEDIA

[0228] This example describes the use of a number of additional cell culture media. A number of chemically-defined and/or animal-product free media have been designed for high protein production in CHO cells and are commercially available. Four such media were utilized for culture of transfectants, including ProCHO3, ProCHO4 and ProCHO5, produced by BioWhittaker, now known as Cambrex (Catalog No. 12-762Q, 12-029Q, and 12-766Q, respectively) and HyQ-PFCHO, produced by HyClone (Catalog No. SH30359.02). Each of these media was supplemented with Glutamine/Penicillin/Streptomycin and selective agents (e.g., G418 Geneticin® and/or histidinol). Shake flask tests were performed in the absence of selective agents. Titers were determined by Protein A HPLC on Day 14 or when culture viability dropped below 20%.

[0229] Clones 264E2 and 254G12, both Clone 128D12 re-transfectants, were passaged directly into these media and after a period of adaptation, production was

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assessed in shake flask tests. The cells readily adapted to all of these media where they grew quickly and to high cell densities (3-4 million cells/mL). The results of four Clone 254G12 or five Clone 264E2 sequential shake flask tests in ProCHO4 and ProCHO5 as shown in Figure 39 demonstrate that Clone 264E2 expressed at up to ~400 µg/ml in ProCHO4 and ProCHO5 while Clone 254G12 expressed up to ~340 µg/ml in each of these media. The titers in HyQ and ProCHO3 media were lower than those in the other commercial media. For instance, in ProCHO3, Clone 264E2 produced 321 µg/mL and Clone 254G12 produced 252 µg/mL whereas in HyQ, Clone 264E2 produced 146 µg/mL and Clone 254G12 produced 140 µg/mL. A variety of cell culture media is useful for transfectants, including clones and cell lines obtained as described herein.

EXAMPLE 17

CONSTRUCTION OF ADDITIONAL EXPRESSION VECTORS

[0230] This example describes the construction of expression vectors that contain multiple copies of additional exemplary transcription units. Exemplary vector constructs are also described containing multiple copies of exemplary gene sequences encoding polypeptides of interest, for example, polypeptides with complement inhibitory activities including chimeric or fusion proteins with such activities. Several modular expression vectors that contain a multicloning site between a CMV promoter and a light chain 3' untranslated region were constructed as described in Example 13 and utilized for the construction of additional expression vectors encoding complement inhibitory proteins. These expression vectors, pING1732-RIClaRV and pING1736-RIClaKpnI, are shown in Figures 29 and 30. A vector containing a CAB2.1 gene (see Figure 1 of U.S. Patent No. 6,316,253; SEQ ID NOS: 76 and 77) was cloned by PCR into the multiple cloning site of the *neo* (G418-resistance) expression vectors pING1732-RIClaRV and pING1736-RIClaKpnI as shown in Figures 40 and 41 respectively. The resulting CAB2.1 – containing vectors were designated pING2053 as shown in Figure 40 and pING2054 as shown in Figure 41. As shown in Figure 40, pING2053 was constructed by first digesting the vector pING1732-RIClaRV with the restriction enzyme *Cla*I followed by digestion with the restriction enzyme *Eco*RI. Next, the gene encoding CAB2.1 was then amplified by

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PCR employing a forward primer CAB2RI5PrFwd (5'GTTAAGAATTCCACCATG GAGCCTCCCGG3' (SEQ ID NO: 78) and a reverse primer CAB2Cla3PrRev 3'GAAGTCCATGATGGGCAACTTAGCTAAATCT5' (SEQ ID NO: 79). The resultant PCR product was then purified, digested with the restriction enzymes *EcoRI* and *ClaI*, and ligated with the pING1732-RIClaRV made linear by digestion with *EcoRI* and *ClaI* to form a CAB2.1 vector, pING2053, comprising a CAB2.1 gene (SEQ ID NO: 76).

[0231] An additional CAB2.1 single gene expression vector, designated pING2054, was prepared as shown in Figure 41. The vector pING1736-RIClaKpnI was first digested with *KpnI* and *EcoRI* followed by purification of the linear vector. A gene encoding CAB2.1 was then amplified by PCR employing the forward primer CAB2RI5PrFwd as described above (SEQ ID NO: 78) and the reverse primer CAB2.1Kpn3PrRev (3'GAAGTCCATCATGGGCAACTCCATGGAATCT5' SEQ ID NO: 80). The resultant PCR product was then purified, digested with the restriction enzymes *EcoRI* and *KpnI*, and ligated with the pING1736-RIClaKpnI made linear by digestion with *EcoRI* and *KpnI*. This additional vector comprising CAB2.1 gene (SEQ ID NO: 76) was designated pING2054 as shown in Figure 41. The features of pING2054 are summarized in Table 4.

Table 4. Description of pING2054.

Plasmid region	Start nt	End nt	Description
NotI-HindIII	1	16	Part of multilinker site
<i>HindIII</i> - <i>1/2BamHI</i> (<i>Sau3AI</i>)	16	179	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., (1983) sequence)
<i>1/2HincII-BamHI</i>	179	951	hCMV promoter (= -598 to 174 of Boshart et al., <i>Cell</i> 41:521-530 (1985) includes splice donor)
<i>BamHI-EcoRI</i>	951	1060	SV40 16S splice acceptor (=SV40 1410-1497)
<i>EcoRI-KpnI</i>	1060	2910	CAB2.1 gene
<i>XhoI-BamHI</i>	2910	4275	LC genomic DNA including poly A site
<i>BamHI-1/2BclI</i> (<i>Sau3AI</i>)	4275	4512	SV40 polyadenylation (=SV40 2532-2774)

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Plasmid region	Start nt	End nt	Description
1/2 <i>Bst</i> YI(<i>Sau</i> 3AI)-NS ^a	4512	5123	SV40 small T intron (=SV40 4769-4099)
1/2 <i>Sal</i> I- <i>Nhe</i> I	5123	6611	bacterial neomycin phosphotransferase (neo) gene from pSV2neo (coding region =9583-8761)
<i>Nhe</i> I- <i>Pvu</i> II	6611	6951	SV40 promoter (=SV40 5172-272)
<i>Pvu</i> II- <i>Not</i> I	6951	9910	bacterial origin of replication and beta-lactamase (ampicillin resistance) gene (= pBR322 2069-4290)

^a NS - restriction site not identified.

[0232] An additional vector with two copies of a CAB 2.1 gene, designated pING2055, with a *neo* gene for selection of G418-resistant transfectants was constructed as shown in Figure 42. The vector pING2053 was first digested with the restriction enzyme *Hpa*I blunt-end ligated to *Xba*I linkers and the linkers were trimmed with *Xba*I. The DNA was then digested with *Bgl*II and the large linear vector fragment comprising a CAB2.1 gene was purified. The vector pING2054 was digested with *Xba*I and *Bgl*II followed by purification of the large linear vector fragment with a CAB2.1 gene. Both the pING2053 and pING2054 digested and purified fragments were then ligated to form the two-gene expression vector pING2055 as shown in Figure 42. The features of pING2055 are summarized in Table 5.

Table 5. Description of pING2055.

Plasmid region	Start nt	End nt	Description
<i>Not</i> I- <i>Hind</i> III	1	479	= pUC12 2616-399 (includes pBR322 4291-4361, 2069-2354 and part of <i>lac</i> gene)
<i>Hind</i> III-1/2 <i>Bam</i> HI (<i>Sau</i> 3AI)	479	643	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., Proc Natl. Acad. Sci (USA) 80: 3623-3627 (1983))
1/2 <i>Hinc</i> II- <i>Bam</i> HI	643	1414	hCMV promoter (= -598 to 174 of Boshart et al., Cell 41:521-530 (1985) includes splice donor)
<i>Bam</i> HI- <i>Eco</i> RI	1414	1523	SV40 16S splice acceptor (1421-1509=SV40 1410-1497)
<i>Eco</i> RI- <i>Cla</i> I	1523	3369	CAB2.1 gene
<i>Cla</i> I- <i>Bam</i> HI	3369	4744	LC genomic DNA including poly A site

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Plasmid region	Start nt	End nt	Description
<i>Bam</i> HI-1/2 <i>Hpa</i> I (<i>Xba</i> I- <i>Hind</i> III)	4745	4889	portion of SV40 polyadenylation (=SV40 2532-2668)
<i>Hind</i> III- 1/2 <i>Bam</i> HI (<i>Sau</i> 3AI)	4889	5053	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., Proc Natl. Acad. Sci (USA) 80: 3623-3627 (1983)
1/2 <i>Hinc</i> II- <i>Bam</i> HI	5053	5825	hCMV promoter, including splice donor
<i>Bam</i> HI- <i>Eco</i> RI	5825	5934	SV40 16S splice acceptor
<i>Eco</i> RI- <i>Xho</i> I	5933	7779	CAB2.1 gene
<i>Xho</i> I- <i>Bam</i> HI	7779	9149	LC genomic DNA including poly A site
<i>Bam</i> HI-1/2 <i>Bcl</i> II (<i>Sau</i> 3AI)	9149	9386	SV40 polyadenylation (=SV40 2532-2774)
1/2 <i>Bst</i> YI(<i>Sau</i> 3AI)-NS ^a	9386	9997	SV40 small T intron (=SV40 4769-4099)
1/2 <i>Sal</i> I- <i>Nhe</i> I	9997	11485	bacterial neomycin phosphotransferase (neo) gene from pSV2neo (coding region =9583-8761)
<i>Nhe</i> I- <i>Pvu</i> II	11485	11831	SV40 promoter (=SV40 5172-272)
<i>Pvu</i> II- <i>Not</i> I	11831	14059	bacterial origin of replication and beta-lactamase (ampicillin resistance) gene (= pBR322 2069-4290)

^a NS - restriction site not identified.

[0233] Another vector with two copies of a CAB2.1 gene, designated pING2056 was also constructed as shown in Figure 43. ping 2056 which has the same structure as the *neo* two-gene vector pING2055, except that it encodes a *his* gene for histinol-resistance to facilitate multiple transfections. The features of pING2056 are summarized in Table 6.

Table 6. Description of pING2056.

Plasmid region	Start nt	End nt	Description
<i>Not</i> I- <i>Hind</i> III	1	479	= pUC12 2616-399 (includes pBR322 4291-4361, 2069-2354 and part of <i>lac</i> gene)
<i>Hind</i> III- 1/2 <i>Bam</i> HI (<i>Sau</i> 3AI)	479	643	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., Proc Natl. Acad. Sci (USA) 80: 3623-3627 (1983)

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Plasmid region	Start nt	End nt	Description
<i>1/2HincII-BamHI</i>	643	1414	hCMV promoter (= -598 to 174 of Boshart et al., cell 41:521-530 (1985); includes splice donor)
<i>BamHI-EcoRI</i>	1414	1523	SV40 16S splice acceptor (1421-1509=SV40 1410-1497)
<i>EcoRI-XhoI</i>	1523	3369	CAB2.1 gene
<i>XhoI-BamHI</i>	3369	4745	LC genomic DNA including poly A site
<i>BamHI-1/2HpaI</i> (<i>XbaI-HindIII</i>)	4745	4890	Part of SV40 polyadenylation (=SV40 2532-2668)
<i>HindIII-1/2BamHI</i> (<i>Sau3AI</i>)	4890	5053	upstream region of Abelson murine leukemia virus 3' LTR enhancer/promoter (= 4627-4804 of Reddy et al., Proc Natl. Acad. Sci (USA) 80: 3623-3627 (1983))
<i>1/2HincII-BamHI</i>	5053	5825	hCMV promoter, including splice donor
<i>BamHI-EcoRI</i>	5825	5934	SV40 16S splice acceptor
<i>EcoRI-XhoI</i>	5933	7779	CAB2.1 gene
<i>XhoI-BamHI</i>	7779	9149	LC genomic DNA including poly A site
<i>BamHI-1/2BclI</i> (<i>Sau3AI</i>)	9149	9386	SV40 polyadenylation (=SV40 2532-2774)
<i>1/2BstYI</i> (<i>Sau3AI</i>)-NS ^a	9386	9997	SV40 small T intron (=SV40 4769-4099)
<i>1/2 SalI-StuI</i>	9997	11368	bacterial histidine (his) gene from pSV2his (coding region =9760-8458)
<i>StuI-PvuII</i>	11368	11694	SV40 promoter (=SV40 5172-272)
<i>PvuII-NotI</i>	11694	13920	bacterial origin of replication and beta-lactamase (ampicillin resistance) gene (= pBR322 2069-4290)

^a NS - restriction site not identified.

EXAMPLE 18

DEVELOPMENT AND CHARACTERIZATION OF ADDITIONAL
TRANSFECTED CLONES AND CELL LINES

[0234] This example describes the development and characterization of clones and cell lines transfected with additional exemplary vectors according to the present invention. The development and characterization of polypeptide – producing cell lines is described from transfections with vectors, including multigene vectors, as described in Example 17.

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[0235] Transfections were performed first with a single gene (pING2054) and a two gene (pING2055) *neo* vector. Cell lines were developed by electroporation of Ex-Cell 301 – adapted CHO-K1 cells by the procedure of [Andreason, et al., *BioTechniques* 6: 650 (1988)]. CHO-K1 cells were obtained from ATCC (CCL61).

[0236] Prior to transfection, 40 µg of either the single-gene *neo* vector pING2054 or the two-gene *neo* vector pING2055 was digested with *Xba*I yielding a linearized DNA structure shown in Figure 44A and B respectively. Forty µg of linearized DNA typically was used in conjunction with $\sim 1 \times 10^7$ cells for each transfection. Generally, one to three electroporations were performed on any given day. Following a 2 day recovery period in non-selective Ex-Cell 301 medium supplemented with 2% FBS, the cells were plated at ~ 4000 cells/well in 96 well plates in Ex-Cell 301 medium supplemented with Glutamine-Pen-Strep, 1% FBS and with 0.8 mg/ml G418 (Geneticin®, Invitrogen). The plates were incubated at 37 °C in a CO₂ incubator.

[0237] Starting at days 10-12, the 96 well plates for either the single gene *neo* vector pING2054 or the two gene *neo* vector pING2055 transfections, were scanned for single clone colonies that were then sampled and screened by ELISA. A total of 3-4 ELISAs was performed over ~ 7 days. ELISAs were performed by removing culture supernatants from the 96 well plates (same for 24 well plates and shake flasks) and pipetting into 96 well dilution plates containing PBS and 1% BSA and stored overnight at 4 °C. The supernatants were assayed for expressed polypeptide levels by ELISA using Immulon 4 plates precoated overnight at 4 °C with GB24, a mouse anti-DAF monoclonal antibody, diluted to 2 µg/ml in PBS. Following two washes with wash buffer (PBS + 0.1% Tween 20) and a blocking step with PBS containing 0.05% Tween and 1% BSA, the diluted supernatants were added to the PBS - 1% BSA buffer in the plates and incubated at 37 °C for 1 hour. The plates then were washed three times with PBS supplemented with 0.05% Tween and 0.5 µg/mL of a rabbit anti-Human CAB2.1 secondary antibody was added and incubated for 1 hour at 37 °C. After three washes, a 1:15,000 dilution of horse radish peroxidase – conjugated goat anti-rabbit IgG (Pierce) was added and incubated for 1 hour. Detection was performed with the TMB system (KPL). After color development, the reaction was stopped by adding an equal volume of 1N H₂SO₄ and plates read at 450nm.

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[0238] Clones secreting polypeptides above an established baseline level, as determined by ELISA, were transferred from the 96 well plates to 24 well plates and replica plates prepared. Screening was performed by ELISA on extinct 24 well plate cultures as described above for 96 well plates. The top – producing clones in 24 well plates were then transferred to shake flasks. Shake flask experiments were performed by inoculating into 25 ml Ex-Cell 301 at $\sim 1 - 2 \times 10^5$ cells per ml, depending on the assay. Ex-Cell 301 medium was supplemented as described above. Selective agents generally were not incorporated into media used for the shake flask tests except in cases in which maintenance cultures were allowed to incubate to extinction. The cells were incubated at 37 °C, 100 RPM until viability was 20% (usually ~14 days). For growth and productivity experiments, viable cell density was determined either by hemocytometer and/or with the Guava Personal Flow Cytometer every 2 to 4 days. For studies in which the viable cell density was not determined, the flasks were opened for a few seconds in the biosafety hood every 2 to 3 days to provide some gas exchange. At the end of the experiment, cells were removed by centrifugation and filtration and the supernatants were assayed for CAB2.1 concentration either by ELISA as described above or by reverse-phase HPLC.

[0239] Specifically, for the transfections with pING2054, two independent sets of transfections were performed, one consisting of a single electroporation and the other consisting of two. A total of thirty eight 96 well plates were prepared from each of these transfections. Multiple colonies were observed in a number of wells following the 10-12 day incubation. 528 wells containing single colonies were screened by ELISA as described above. Wells with multiple colonies were triturated, pooled, labeled with a FITC conjugated mouse anti-human CD46 monoclonal antibody and subjected to Fluorescence activated cell sorting (FACS) analysis using a FACS Vantage SE-Diva (BD Biosciences, _____). FACS sorted clones were re-plated in 96 well plates and 223 individual clones were screened by ELISA.

[0240] The top 125 pING2054 transfectants from the 96 well format, of which 29 were from the FACS sorted pool, were transferred to 24 well plates and extinct cultures were screened by ELISA as described above. The results demonstrated that the top transfectants produced between ~ 9 to ~ 16 $\mu\text{g/ml}$ in 24 well plates (including, for example, Clones 3G8, 13A5, and 19A6). The top 30 of these clones, of which 4

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were from the FACS sorted pool, were transferred to shake flasks and grown in ExCell 301 (without FBS) to ~20% viability as described above. Extinct cultures were screened by ELISA. The top three producing clones, based on shake flask tests, included Clones 3G8, 13A5, and 19A6. Clone 3G8 appeared to be the highest producer in Ex-Cell 301 medium with 14 day (extinct) shake flask productivities (determined by HPLC) of 107, 86, and 116 $\mu\text{g/mL}$.

[0241] To select further for high expression, Clone 3G8 was subcloned. Cells were plated at in 96 well plates 5, 3, and 1 cells/well in Excell 301 medium. A total of 417 subclones were screened at the 96 well plate level as described above, with the top subclones secreting up to ~14 $\mu\text{g/ml}$ (including, for example, 3G8 – G5-F1, 3G8-G13-C12, and 3G8-G10-H4). The top 60 subclones were next screened in 24 well plates, with the top producers secreting ~48 $\mu\text{g/ml}$ (including, for example, 3G8-G5-F1, 3G8-G13-C-12, 3G8-G5E8, and 3G8-GA11). Twelve clones were selected and transferred to shake flask in ExCell medium without FBS. The cells were grown to extinction and the culture supernatant was assayed by reverse phase HPLC. Clone G5F1 appeared to be the highest producing clone in Ex-Cell 301 medium with 14 day (extinct) shake flask productivities (determined by HPLC) of 184, 194 and 182 $\mu\text{g/ml}$. This level was almost 2 - fold higher than observed with the parent, Clone 3G8. Clone G5F1 was evaluated in a growth and productivity test in a 125 mL shake flask. The culture was inoculated at 1×10^5 cells/mL and incubated for 12-13 days. The results as shown in Figure 45 demonstrate that Clone G5F1 was satisfactory with respect to both growth and productivity, maintaining a viability of 49% at Day 12 and ~175 $\mu\text{g/ml}$ at Day 13.

[0242] Transfections and clonal development using pING2055, were performed as described for Clone 3G8, transfected with pING2054. Specifically, two independent sets of transfections each consisting of three electroporations were performed using pING2055 prepared as described above. A total of 3374 individual clones were screened from the 96 well plates. The top 263 clones were transferred to 24 well plates and extinct cultures were screened by ELISA. The results demonstrated that the top transfectants produced between ~9 to ~37 $\mu\text{g/ml}$ in 24 well plates (including, for example, 44C8, 11C8, 122B10, 176C6, 156B8, and 134G3). The top 85 of these clones were transferred to shake flasks and extinct cultures were screened by ELISA.

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or RP-HPLC as described above. Clones 156B8 and 176C6 appeared to be the highest producers in Ex-Cell 301 medium with 14 day (extinct) shake flask productivities (determined by HPLC) up to ~140 µg/ml for both.

EXAMPLE 19

INCREASING EXPRESSION THROUGH A SECOND SEQUENTIAL VECTOR TRANSFECTION: DEVELOPMENT AND CHARACTERIZATION OF ADDITIONAL TRANSFECTED CLONES AND CELL LINES

[0243] This example describes the further increase in expression and production of polypeptides, including polypeptides with complement inhibitory activities, through a second transfection of an exemplary cell line with a multi-transcription unit vector.

[0244] The top CAB2.1 – producing clones developed with the single-gene *neo* vector (Clone 3G8) and the two-gene *neo* vector (Clones 156B8 and 176C6) were re-transfected with the CAB2.1 two-gene histidinol vector, pING2056. Specifically, Clone 3G8, transfected with a *neo* single gene vector pING2054, was re-transfected with a *his* two gene vector pING2056 linearized to position the histidinol-resistant selective marker gene between the two CAB2.1 genes as shown in Figure 44 for the *neo* vector. Cells were transfected as described above. A total of 387 clones were screened in 96 well plates in ExCell 301 medium and 120 clones were selected for screening in 24 well plates. Twenty of the top producing clones, secreting up to a ~68 µg/ml, were selected to be transferred to shake flasks in ExCell medium without FBS. Cells were grown to extinction and culture supernatants assayed by reverse phase HPLC. The top producing clones secreted in the range of ~142 to ~218 µg/ml (including, for example, 3G8-217B4, 3G8-206C3, 3G8-196E3, 3G8-190C7, 3G8-185E9, 3G8-178G2, 3G8-190E5). For example, for these top producing clones, Clone 217B4 consistently secreted more than ~200 µg/ml, and Clone 206C3 secreted up to ~199 µg/ml, Clone 185E9 secreted up to ~177 µg/ml.

[0245] The top re-transfectants of Clone 3G8, clones 217B4, 206C3, and 185E9, were subcloned. Specifically, Clones 217B4, 206C3 and 185E9 were subcloned by plating at 1, 3 or 5 cells/well in Ex-Cell 301 medium. A total of 282 individual subclones of Clone 217B4, 301 of Clone 206C3 and 308 of Clone 185E9 were screened in 96 well plates as described above for the development of clone 3G8. Of these, 59 subclones

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of Clone 217B4, 55 subclones of Clone 206C3 and 52 subclones of Clone 185E9 were transferred to 24 well plates and extinct cultures screened by ELISA as described above for the development of Clone 3G8. The top producing 217B4 subclones secreted in the range of ~ 35 to ~ 57 $\mu\text{g/ml}$. The top producing 206C3 subclones secreted in the range of ~ 31 to ~ 40 $\mu\text{g/ml}$ and the top producing 185E9 subclones secreted from ~ 17 to ~ 61 $\mu\text{g/ml}$. Of these, the top 20, 16 and 16 clones for 217B4, 206C3 and 185E9 respectively, were transferred to shake flasks. Extinct cultures were screened by ELISA as previously described and in some cases by RP-HPLC. The top 217B4 subclones, including clones B3B8, B5B10, B4E11, B6E3, B7C4, B7B7, and B9B4, secreted between ~ 170 to ~ 216 $\mu\text{g/ml}$ in shake flasks. The top 206C3 subclones including clones C2B7, C2D10, C4F1, C7H7, and C9A2, secreted in the range ~ 147 to ~ 188 $\mu\text{g/ml}$ in shake flasks. The top 185E9 subclones, including clones E7E9, E3C11, E10H5, and E2D5, secreted in the range of ~ 163 to ~ 210 $\mu\text{g/ml}$ in shake flasks. These subclones did not produce higher levels of CAB2.1 than their parent clones in Ex-Cell 301 medium and their titers remained similar to those of their parental clones.

[0246] To further evaluate expression in Ex-Cell 301 media, Clone 217B4, Clone 206C3, Subclones C2B7 and C7H7 of Clone 206C3 and Subclones B6E3 and B5B10 of Clone 217B4 were evaluated in shake flask test in which growth and productivity were measured over the course of the study. For this study, cells grown in Ex-Cell 301 were inoculated at 1×10^5 cells/ml. The cells were grown as described for the development of Clone 3G8. The growth and productivity for the re-transfectants of Clone 3G8, Clones 217B4 and 206C3, were measured. At 2 weeks the cells were still viable (1×10^6 cells/ml for Clone 206C3 and 4×10^5 cells/ml for Clone 217B4) and supported a titer of ~ 150 $\mu\text{g/ml}$. The growth and productivity results for the Clone 206C3 Subclones C2B7 and C7H7 were also measured. The results are similar to those for the parent Clone 206C3 with a measurable decrease in cell viability at 2 weeks and productivity at or just below ~ 150 $\mu\text{g/ml}$. Growth and productivity results for Clone 217B4 Subclones B6E3 and B5B10 were also measured. When compared to Clone 206C3 and its subclones C2B7 and C7H7, these results demonstrate in productivities at two weeks, of ~ 200 $\mu\text{g/ml}$ for B6E3 and above ~ 150 $\mu\text{g/ml}$ for B5B10.

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[0247] In view of the current problems and limitations in the art, there are many advantages of the present invention over the art, increased polypeptide production, production efficiency, greater control and regulation over the quantities of polypeptide expressed, increased stability of cell lines, and decreased costs for materials, reagents, and other resources. Thus, the present invention solves many of the problems previously existing in the art. One way in which the present invention solves these problems and is advantageous compared to the prior art is through the disclosed methods of vector construction that, when linearized, avoid homologous recombination of multiple copies of transcription units by separating the transcription unit DNA sequences with at least one selective marker gene. While the above examples demonstrate the use of two and four transcription unit vectors, the present invention contemplates use of all multiple transcription unit vectors, including three, four, five, six, and so on copies of transcription unit DNA sequences. Such vectors are constructed with at least one selective marker gene separating the transcription units in a manner which results in the avoidance or nullification of homologous recombination. All such permutations of multiple transcription unit copies are contemplated by this invention as one skilled in the art would recognize through this disclosure. A second way in which the present invention solves the problems of the prior art is through the disclosed methods to further increase expression through sequential transfections with multiple-transcription unit constructs. This leads to clones which can produce substantially increased levels of proteins and polypeptides, while still maintaining viability and stability in growth medium, thus, solving many problems in the art.

[0248] All patents, patent applications, literature publications and test methods cited herein are hereby incorporated by reference. The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

[0249] All the features disclosed in this specification (including any accompanying claims, abstract, and drawings) may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless

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expressly stated otherwise, each feature disclosed is only one example of a generic series of equivalent or similar features.

[0250] Although the present invention has been described in considerable detail with reference to certain preferred versions thereof, other versions are possible. Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed disclosure. All such modifications are within the full intended scope of the appended claims. Therefore, the spirit and scope of the appended claims should not be limited to the description of the preferred embodiments contained herein.

-1-

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aaa gtc ggg tgg ctg atc caa ctc ttc cac aaa aaa att gag tct gcg Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys Ile Glu Ser Ala 150 155 160 165			576
ctt cga aac aag atg aac agc cag gtc tgc gag aaa gtg acc aat tct Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys Val Thr Asn Ser 170 175 180			624
gta tcc tcc aag ctg caa cct tat ttc cag act ctg tag Val Ser Ser Lys Leu Gln Pro Tyr Phe Gln Thr Leu 185 190			663

<210> 4
 <211> 220
 <212> PRT
 <213> Homo Sapiens

<400> 4

Met Ala Arg Gly Pro Cys Asn Ala Pro Arg Trp Val Ser Leu Met Val
 -25 -20 -15

Leu Val Ala Ile Gly Thr Ala Val Thr Ala Ala Val Asn Pro Gly Val
 -10 -5 -1 1 5

Val Val Arg Ile Ser Gln Lys Gly Leu Asp Tyr Ala Ser Gln Gln Gly
 10 15 20

Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro Asp Tyr
 25 30 35

Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly His Tyr Ser Phe
 40 45 50

Tyr Ser Met Asp Ile Arg Glu Phe Gln Leu Pro Ser Ser Gln Ile Ser
 55 60 65

-8-

Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile Ser Asn Ala Asn Ile
70 75 80 85

Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Met Ser
90 95 100

Gly Asn Phe Asp Leu Ser Ile Glu Gly Met Ser Ile Ser Ala Asp Leu
105 110 115

Lys Leu Gly Ser Asn Pro Thr Ser Gly Lys Pro Thr Ile Thr Ala Ser
120 125 130

Ser Cys Ser Ser His Ile Asn Ser Val His Val His Ile Ser Lys Ser
135 140 145

Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys Ile Glu Ser Ala
150 155 160 165

Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys Val Thr Asn Ser
170 175 180

Val Ser Ser Lys Leu Gln Pro Tyr Phe Gln Thr Leu
185 190

<210> 5
<211> 720
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<223> Mouse Human Chimeric Light Chain DNA and Protein

<220>
<221> CDS
<222> (1)..(717)

<220>
<221> mat_peptide
<222> (61)..()

<400> 5
atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct 48
Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
-20 -15 -10 -5

gga tcc act gca gat att gtg atg acg cag gct gca ttc tcc aat cca 96
Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro
-1 1 5 10

-9-

gtc act ctt gga aca tca ggt tcc atc tcc tgc agg tct agt aag agt Val Thr Leu Gly Thr Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser 15 20 25	144
ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aag Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys 30 35 40	192
cca ggc cag tct cct cag ctc ctg att tat cag atg tcc aac ctt gcc Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala 45 50 55 60	240
tca gga gtc cca gac agg ttc agt agc agt ggg tca gga act gat ttc Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe 65 70 75	288
aca ctg aga atc agc aga gtg gag gct gag gat gtg ggt gtt tat tac Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr 80 85 90	336
tgt gct caa aat cta gaa ctt cct cgg acg ttc ggt gga ggc acc aag Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys 95 100 105	384
ctt gag atg aaa cga act gtg gct gca cca tct gtc ttc atc ttc ccg Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro 110 115 120	432
cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu 125 130 135 140	480
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp 145 150 155	528
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp 160 165 170	576
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys 175 180 185	624
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln 190 195 200	672
ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 205 210 215	720

<210> 6

<211> 239

<212> PRT

<213> Homo Sapiens

-10-

<400> 6

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro
 -1 1 5 10

Val Thr Leu Gly Thr Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala
 45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

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Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 7
 <211> 1398
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Mouse-Human chimeric Heavy Chain DNA and Protein Sequence

<220>
 <221> CDS
 <222> (1)..(1395)

<220>
 <221> mat_peptide
 <222> (58)..()

<400> 7
 atg gct tgg gtg tcc acc ttg cta ttc ctg atg gca gct gcc caa agt 48
 Met Ala Trp Val Ser Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
 -15 -10 -5

gcc caa gca cag atc cag ttg gtg cag tct gga cct gag ctg aag aag 96
 Ala Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys
 -1 1 5 10

cct gga gag aca gtc aag atc tcc tgc aag gct tct gga tat acc ttc 144
 Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 15 20 25

aca aaa tat gga atg aac tgg gtg aag cag gct cca gga aag ggt tta 192
 Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu
 30 35 40 45

aag tgg atg ggc tgg ata aac acc tac act gaa gag cca aca tat ggt 240
 Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Glu Glu Pro Thr Tyr Gly
 50 55 60

gat gac ttc aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc 288
 Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser
 65 70 75

act gcc aat ttg cag atc aac aac ctc aaa agt gag gac acg gct aca 336
 Thr Ala Asn Leu Gln Ile Asn Asn Leu Lys Ser Glu Asp Thr Ala Thr
 80 85 90

tat ttc tgt gca aga ttt ggc tct gct gtg gac tac tgg ggt caa gga 384
 Tyr Phe Cys Ala Arg Phe Gly Ser Ala Val Asp Tyr Trp Gly Gln Gly
 95 100 105

acc tcg gtc acc gtc tcc tca gcc agc aca aag ggc cca tcg gtc ttc 432
 Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe

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110	115	120	125	
ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg				480
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu				
	130	135	140	
ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg				528
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp				
	145	150	155	
aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta				576
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu				
	160	165	170	
cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc				624
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser				
	175	180	185	
agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc				672
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro				
	190	195	200	205
agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct tgt gac aaa				720
Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys				
	210	215	220	
act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg				768
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro				
	225	230	235	
tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc				816
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser				
	240	245	250	
cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac				864
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp				
	255	260	265	
cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat				912
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn				
	270	275	280	285
gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg				960
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val				
	290	295	300	
gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag				1008
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu				
	305	310	315	
tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa				1056
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys				
	320	325	330	
acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc				1104
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr				
	335	340	345	

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ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc 1152
 Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 350 355 360 365

tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag 1200
 Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg 1248
 Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395

gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag 1296
 Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 400 405 410

agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag 1344
 Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 415 420 425

gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt 1392
 Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 430 435 440 445

aaa tga 1398
 Lys

<210> 8
 <211> 465
 <212> PRT
 <213> Homo Sapiens

<400> 8

Met Ala Trp Val Ser Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
 -15 -10 -5

Ala Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys
 -1 1 5 10

Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 15 20 25

Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu
 30 35 40 45

Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Glu Glu Pro Thr Tyr Gly
 50 55 60

Asp Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser
 65 70 75

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Thr Ala Asn Leu Gln Ile Asn Asn Leu Lys Ser Glu Asp Thr Ala Thr
80 85 90

Tyr Phe Cys Ala Arg Phe Gly Ser Ala Val Asp Tyr Trp Gly Gln Gly
95 100 105

Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
110 115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
160 165 170

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
175 180 185

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
190 195 200 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
240 245 250

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
255 260 265

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
270 275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
290 295 300

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Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 305 310 315

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 320 325 330

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 335 340 345

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 350 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 400 405 410

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 415 420 425

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 430 435 440 445

Lys

<210> 9
 <211> 720
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Low Risk ING-1 Light Chain (LC)

<220>
 <221> misc_feature
 <223> Variable Region of the Light Chain is Amino Acids 1-112

<220>
 <221> CDS
 <222> (1)..(717)

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<220>

<221> mat_peptide

<222> (61)..()

<400> 9

atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct	48
Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro	
-20 -15 -10 -5	
gga tcc act gca gac atc gtg atg acc cag tct gca ctc tcc aat cca	96
Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Ala Leu Ser Asn Pro	
-1 1 5 10	
gtc act ctg gga gag tca ggt tcc atc tcc tgc cgg tct agt aag agt	144
Val Thr Leu Gly Glu Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser	
15 20 25	
ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aaa	192
Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys	
30 35 40	
cca ggg cag tct cct cag ctg ctc atc tat cag atg tct aac aga gcc	240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala	
45 50 55 60	
tca ggg gtc cca gac agg ttc agt agc agt gga tct ggg aca gat ttc	288
Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe	
65 70 75	
act ctc aag atc agc aga gtg gag gct gaa gat gtg gga gtt tat tac	336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr	
80 85 90	
tgt gct cag aac cta gag ctt ccg cgg acg ttc ggt cag ggc acc aag	384
Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys	
95 100 105	
ctt gag atg aaa cga act gtg gct gca cca tct gtc ttc atc ttc ccg	432
Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	
110 115 120	
cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg	480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	
125 130 135 140	
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat	528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	
145 150 155	
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac	576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	
160 165 170	
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa	624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	
175 180 185	
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag	672

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Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag 720
 Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 10
 <211> 239
 <212> PRT
 <213> Homo Sapiens

<400> 10

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Ala Leu Ser Asn Pro
 -1 1 5 10

Val Thr Leu Gly Glu Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

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Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 11
 <211> 720
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Low + Moderate Risk ING-1 Light Chain (LC)

<220>
 <221> CDS
 <222> (1)..(717)

<220>
 <221> mat_peptide
 <222> (61)..()

<400> 11
 atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct 48
 Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

gga tcc act gca gac atc gtg atg acc cag tct cca ctc tcc ctg cca 96
 Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro
 -1 1 5 10

gtc act cct gga gag ccg ggt tcc atc tcc tgc cgg tct agt aag agt 144
 Val Thr Pro Gly Glu Pro Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aaa 192
 Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

cca ggg cag tct cct cag ctg ctc atc tat cag atg tct aac aga gcc 240
 Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

tca ggg gtc cca gac agg ttc agt agc agt gga tct ggg aca gat ttc 288
 Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe

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65					70					75						
act	ctc	aag	atc	agc	aga	gtg	gag	gct	gaa	gat	gtg	gga	gtt	tat	tac	336
Thr	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	
			80					85					90			
tgt	gct	cag	aac	cta	gag	ctt	cca	cgg	acg	ttc	ggc	cag	ggc	acc	aag	384
Cys	Ala	Gln	Asn	Leu	Glu	Leu	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	
		95					100					105				
ctt	gag	atg	aaa	cga	act	gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	432
Leu	Glu	Met	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	
	110					115					120					
cca	tct	gat	gag	cag	ttg	aaa	tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	480
Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	
125					130				135						140	
ctg	aat	aac	ttc	tat	ccc	aga	gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	528
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	
			145					150						155		
aac	gcc	ctc	caa	tcg	ggc	aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	576
Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	
		160						165					170			
agc	aag	gac	agc	acc	tac	agc	ctc	agc	agc	acc	ctg	acg	ctg	agc	aaa	624
Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	
		175					180					185				
gca	gac	tac	gag	aaa	cac	aaa	gtc	tac	gcc	tgc	gaa	gtc	acc	cat	cag	672
Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	
	190					195					200					
ggc	ctg	agc	tcg	ccc	gtc	aca	aag	agc	ttc	aac	agg	gga	gag	tgt	tag	720
Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys		
205					210				215							

<210> 12

<211> 239

<212> PRT

<213> Homo Sapiens

<400> 12

Met	Arg	Phe	Ser	Ala	Gln	Leu	Leu	Gly	Leu	Leu	Val	Leu	Trp	Ile	Pro
-20					-15					-10					-5

Gly	Ser	Thr	Ala	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro
			-1	1				5					10		

Val	Thr	Pro	Gly	Glu	Pro	Gly	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser
		15					20					25			

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Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 13
 <211> 88
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> KL1 V Region Oligios ING-1 Light Chain (Kappa low)

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<400> 13
tgtcgacacc atgaggttct ctgctcagct tctggggctg cttgtgctct ggatccctgg 60
atccactgca gacatcgtga tgacccag 88

<210> 14
<211> 85
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<223> KL2 V Region Oligios ING-1 Light Chain (Kappa low)

<400> 14
actcttacta gaccggcagg agatggaacc tgactctccc agagtgactg gattggagag 60
tgcagactgg gtcatcacga tgtct 85

<210> 15
<211> 88
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<223> KL3 V Region Oligios ING-1 Light Chain (Kappa low)

<400> 15
ctgccggtct agtaagagtc tcctacatag taatggcatc acttatttgt attggtatct 60
gcagaaacca gggcagtctc ctcagctg 88

<210> 16
<211> 86
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<223> KL4 V Region Oligios ING-1 Light Chain (Kappa low)

<400> 16
tgtcccagat ccactgctac tgaacctgtc tgggaccctt gaggctctgt tagacatctg 60
atagatgagc agctgaggag actgcc 86

<210> 17
<211> 77
<212> DNA
<213> Homo Sapiens

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<220>
 <221> misc_feature
 <223> KL5 V Region Oligios ING-1 Light Chain (Kappa low)

 <400> 17
 agcagtggat ctgggacaga ttctactctc aagatcagca gagtggaggc tgaagatgtg 60
 ggagtttatt actgtgc 77

<210> 18
 <211> 75
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> KL6 V Region Oligios ING-1 Light Chain (Kappa low)

 <400> 18
 tttgatttca agcttggtgc cctgaccgaa cgtccgtgga agctctaggt tctgagcaca 60
 gtaataaact cccac 75

<210> 19
 <211> 22
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Low Risk Primers Forward Primer: KF Ing-1 Light Chain Oligos

 <400> 19
 ttatgtcgac accatgaggt tc 22

<210> 20
 <211> 21
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Low risk Primers Reverse Primer: KR Ing-1 Light Chain Oligos

 <400> 20
 tttgatttca agcttggtgc c 21

<210> 21
 <211> 85

-23-

<212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Moderate Risk Primer: KM2 Ing-1 Light Chain Oligos

<400> 21
 actcttacta gaccggcagg agatggaacc cggctctcca ggagtgactg gcagggagag 60
 tggagactgg gtcattcacga tgtct. 85

<210> 22
 <211> 1398
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Low Risk ING-1 Risk Heavy Chain (HC)

<220>
 <221> misc_feature
 <223> Variable region of HC is Amino Acids is 1-116

<220>
 <221> CDS
 <222> (1)..(1395)

<220>
 <221> mat_peptide
 <222> (58)..()

<400> 22
 atg gct tgg gtg tcc acc ttg cta ttc ctg atg gca gct gcc caa agt 48
 Met Ala Trp Val Ser Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
 -15 -10 -5

gcc caa gca cag atc cag ttg gtg cag tct gga cct gag gtg aag aag 96
 Ala Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
 -1 1 5 10

cct gga gag tcc gtc aag atc tcc tgc aag gct tct gga tat acc ttc 144
 Pro Gly Glu Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 15 20 25

aca aaa tat gga atg aac tgg gtg aag cag gct cca gga cag ggt tta 192
 Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Gln Gly Leu
 30 35 40 45

aag tgg atg ggc tgg ata aac acc tac act gaa gag cca aca tat ggt 240
 Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Glu Glu Pro Thr Tyr Gly
 50 55 60

gat gac ttc aag gga cgg ttt acc ttc acc ttg gac acc tct act agc 288

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Asp	Asp	Phe	Lys	Gly	Arg	Phe	Thr	Phe	Thr	Leu	Asp	Thr	Ser	Thr	Ser	
			65					70					75			
act	gcc	tat	ttg	gaa	atc	tct	tct	ctc	cgg	agt	gag	gac	acg	gct	aca	336
Thr	Ala	Tyr	Leu	Glu	Ile	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Thr	
		80					85				90					
tat	ttc	tgt	gca	aga	ttt	ggc	tct	gct	gtg	gac	tac	tgg	ggg	caa	gga	384
Tyr	Phe	Cys	Ala	Arg	Phe	Gly	Ser	Ala	Val	Asp	Tyr	Trp	Gly	Gln	Gly	
	95					100					105					
acc	ttg	gtc	acc	gtc	tcc	tca	gcc	agc	aca	aag	ggc	cca	tcg	gtc	ttc	432
Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	
110					115					120					125	
ccc	ctg	gca	ccc	tcc	tcc	aag	agc	acc	tct	ggg	ggc	aca	gcg	gcc	ctg	480
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	
			130						135					140		
ggc	tgc	ctg	gtc	aag	gac	tac	ttc	ccc	gaa	ccg	gtg	acg	gtg	tcg	tgg	528
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	
			145				150						155			
aac	tca	ggc	gcc	ctg	acc	agc	ggc	gtg	cac	acc	ttc	ccg	gct	gtc	cta	576
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	
		160					165					170				
cag	tcc	tca	gga	ctc	tac	tcc	ctc	agc	agc	gtg	gtg	acc	gtg	ccc	tcc	624
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	
	175					180					185					
agc	agc	ttg	ggc	acc	cag	acc	tac	atc	tgc	aac	gtg	aat	cac	aag	ccc	672
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	
190					195					200					205	
agc	aac	acc	aag	gtg	gac	aag	aga	gtt	gag	ccc	aaa	tct	tgt	gac	aaa	720
Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	
			210						215				220			
act	cac	aca	tgc	cca	ccg	tgc	cca	gca	cct	gaa	ctc	ctg	ggg	gga	ccg	768
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	
			225					230					235			
tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	816
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	
		240					245					250				
cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	864
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	
	255					260					265					
cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	912
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	
270					275					280					285	
gcc	aag	aca	aag	ccg	cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgg	gtg	960
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	
				290					295					300		

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gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag	1008
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu	
305 310 315	
tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa	1056
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys	
320 325 330	
acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc	1104
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr	
335 340 345	
ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc	1152
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr	
350 355 360 365	
tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag	1200
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu	
370 375 380	
agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg	1248
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu	
385 390 395	
gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag	1296
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys	
400 405 410	
agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag	1344
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu	
415 420 425	
gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt	1392
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly	
430 435 440 445	
aaa tga	1398
Lys	

<210> 23
 <211> 465
 <212> PRT
 <213> Homo Sapiens

<400> 23

Met Ala Trp Val Ser Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
-15 -10 -5

Ala Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
-1 1 5 10

Pro Gly Glu Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe

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15		20		25
Thr Lys Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Gln Gly Leu				
30		35	40	45
Lys Trp Met Gly Trp Ile Asn Thr Tyr Thr Glu Glu Pro Thr Tyr Gly				
	50		55	60
Asp Asp Phe Lys Gly Arg Phe Thr Phe Thr Leu Asp Thr Ser Thr Ser				
	65		70	75
Thr Ala Tyr Leu Glu Ile Ser Ser Leu Arg Ser Glu Asp Thr Ala Thr				
	80		85	90
Tyr Phe Cys Ala Arg Phe Gly Ser Ala Val Asp Tyr Trp Gly Gln Gly				
95		100	105	
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe				
110		115	120	125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu				
	130		135	140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp				
	145		150	155
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu				
	160		165	170
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser				
175		180	185	
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro				
190		195	200	205
Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys				
	210		215	220
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro				
	225		230	235
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser				
	240		245	250

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Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 255 260 265

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 270 275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 305 310 315

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 320 325 330

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 335 340 345

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 350 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 400 405 410

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 415 420 425

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 430 435 440 445

Lys

<210> 24

<211> 1398

<212> DNA

<213> Homo Sapiens

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<220>
 <221> misc_feature
 <223> Low + Moderate ING-1 Heavy Chain (HC)

<220>
 <221> CDS
 <222> (1)..(1395)

<220>
 <221> mat_peptide
 <222> (58)..()

<400> 24
 atg gct tgg gtg tcc acc ttg cta ttc ctg atg gca gct gcc caa agt 48
 Met Ala Trp Val Ser Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
 -15 -10 -5

gcc caa gca cag atc cag ttg gtg cag tct gga gct gag gtg aag aag 96
 Ala Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 -1 1 5 10

cct gga gag tca gtc aag atc tcc tgc aag gct tct gga tat acc ttc 144
 Pro Gly Glu Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 15 20 25

aca aaa tat gga atg aac tgg gtg cga cag gct cca gga caa ggt tta 192
 Thr Lys Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 30 35 40 45

gag tgg atg ggc tgg ata aac acc tac act gaa gag cca aca tat ggt 240
 Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Glu Glu Pro Thr Tyr Gly
 50 55 60

cag aag ttc cag gga cgg ttt acc ttc acc ttg gac acc tct act agc 288
 Gln Lys Phe Gln Gly Arg Phe Thr Phe Thr Leu Asp Thr Ser Thr Ser
 65 70 75

act gcc tat ttg gaa atc tct tcg ctc cgg agt gag gac acg gct gtg 336
 Thr Ala Tyr Leu Glu Ile Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 80 85 90

tat ttc tgt gca aga ttt ggc tct gct gtg gac tac tgg ggt caa gga 384
 Tyr Phe Cys Ala Arg Phe Gly Ser Ala Val Asp Tyr Trp Gly Gln Gly
 95 100 105

acc ttg gtc acc gtc tcc tca gcc agc aca aag ggc cca tcg gtc ttc 432
 Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 110 115 120 125

ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg 480
 Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
 130 135 140

ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg 528
 Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155

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aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta	576
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu	
160 165 170	
cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc	624
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser	
175 180 185	
agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc	672
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro	
190 195 200 205	
agc aac acc aag gtg gac aag aga gtt gag ccc aaa tct tgt gac aaa	720
Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys	
210 215 220	
act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg	768
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro	
225 230 235	
tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc	816
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser	
240 245 250	
cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac	864
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp	
255 260 265	
cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat	912
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn	
270 275 280 285	
gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg	960
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val	
290 295 300	
gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag	1008
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu	
305 310 315	
tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa	1056
Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys	
320 325 330	
acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc	1104
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr	
335 340 345	
ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc	1152
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr	
350 355 360 365	
tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag	1200
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu	
370 375 380	
agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg	1248
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu	

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385	390	395	
gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag			1296
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys			
400	405	410	
agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag			1344
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu			
415	420	425	
gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt			1392
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly			
430	435	440	445
aaa tga			1398
Lys			

<210> 25
 <211> 465
 <212> PRT
 <213> Homo Sapiens

 <400> 25

Met	Ala	Trp	Val	Ser	Thr	Leu	Leu	Phe	Leu	Met	Ala	Ala	Ala	Gln	Ser
				-15					-10					-5	

Ala	Gln	Ala	Gln	Ile	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys
	-1	1				5						10			

Pro	Gly	Glu	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
15					20					25					

Thr	Lys	Tyr	Gly	Met	Asn	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu
30				35					40					45	

Glu	Trp	Met	Gly	Trp	Ile	Asn	Thr	Tyr	Thr	Glu	Glu	Pro	Thr	Tyr	Gly
			50					55						60	

Gln	Lys	Phe	Gln	Gly	Arg	Phe	Thr	Phe	Thr	Leu	Asp	Thr	Ser	Thr	Ser
		65						70					75		

Thr	Ala	Tyr	Leu	Glu	Ile	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val
	80					85						90			

Tyr	Phe	Cys	Ala	Arg	Phe	Gly	Ser	Ala	Val	Asp	Tyr	Trp	Gly	Gln	Gly
95						100					105				

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Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe
110					115					120					125
Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu
				130					135					140	
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
			145					150					155		
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
		160					165					170			
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
	175					180					185				
Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
190					195					200					205
Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys
				210					215					220	
Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
			225					230					235		
Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
		240					245					250			
Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
	255					260					265				
Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
270					275					280					285
Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
				290					295					300	
Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
			305					310					315		
Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
		320					325					330			
Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
	335					340					345				

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Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 350 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 400 405 410

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 415 420 425

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 430 435 440 445

Lys

<210> 26
 <211> 91
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GL1 V Region Oligos: ING-1 Heavy Chain Oligos

<400> 26
 tgctgacacc atggcttggg tgtccacctt gctattcctg atggcagctg cccaaagtgc 60
 ccaagcacag atccagttgg tgcagtctgg a 91

<210> 27
 <211> 90
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GL2 V Region Oligos: ING-1 Heavy Chain Oligos

<400> 27
 atattttgtg aaggatatc cagaagcctt gcaggagatc ttgacggact ctccaggctt 60

-33-

cttcacctca ggtccagact gcaccaactg 90

<210> 28
 <211> 91
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GL3 V Region Oligos: ING-1 Heavy Chain Oligos

<400> 28
 tggatatacc ttcacaaaat atggaatgaa ctgggtgaag caggctccag gacagggttt 60

aaagtggatg ggctggataa acacctacac t 91

<210> 29
 <211> 90
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GL4 V Region Oligos: ING-1 Heavy Chain Oligos

<400> 29
 cagtgttagt agaggtgtcc aaggtgaagg taaaccgtcc cttgaagtca tcaccatatg 60

ttggctcttc agtgtaggtg tttatccagc 90

<210> 30
 <211> 90
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GL5 V Region Oligos: ING-1 Heavy Chain Oligos

<400> 30
 gacacctcta ctagcactgc ctatattggaa atctcttctc tccggagtga ggacacggct 60

acatatttct gtgcaagatt tggctctgct 90

<210> 31
 <211> 85
 <212> DNA
 <213> HomoSapiens

-34-

<220>
 <221> misc_feature
 <223> GL6 V Region Oligos: ING-1 Heavy Chain Oligos

 <400> 31
 gaccgatggg ccctttgtgc tggctgagga gacggtgacc aagggttcctt gaccccagta 60
 gtccacagca gagccaaatc ttgca. 85

<210> 32
 <211> 22
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> ING-1 Heavy Chain Oligos-Low Risk Primers Forward primer:GF

 <400> 32
 ttatgtcgac accatggcctt gg 22

<210> 33
 <211> 17
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> ING-1 Heavy Chain Oligos Low Risk Primers -Reverse Primer GR

<220>
 <221> misc_feature
 <223> ING-1 Heavy Chain Oligos Low Risk Primers Reverse Primer GR

<400> 33
 gaccgatggg ccctttg 17

<210> 34
 <211> 90
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GM2 V Region Oligos ING-1 Heavy Chain Oligos-Low + Moderate Risk Primers

<400> 34
 atattttgtg aaggatatc cagaagcctt gcaggagatc ttgactgact ctccaggcctt 60
 cttcacctca gctccagact gcaccaactg 90

-35-

<210> 35
 <211> 91
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GM3 V Region Oligos ING-1 Heavy Chain Oligos-Low + Moderate Risk
 Primers

<400> 35
 tggatataacc ttcacaaaat atggaatgaa ctgggtgcga caggctccag gacaaggttt 60
 agagtggatg ggctggataa acacctacac t 91

<210> 36
 <211> 90
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GM4 V Region Oligos ING-1 Heavy Chain Oligos-Low + Moderate Risk
 Primers

<400> 36
 cagtgttagt agaggtgtcc aaggtgaagg taaaccgtcc ctggaacttc tgaccatatg 60
 ttggctcttc agtgtaggtg tttatccagc 90

<210> 37
 <211> 90
 <212> DNA
 <213> HomoSapiens

<220>
 <221> misc_feature
 <223> GM5 V Region Oligos ING-1 Heavy Chain Oligos-Low + Moderate Risk
 Primers

<400> 37
 gacacctcta ctagcactgc ctatttggaa atctcttcgc tccggagtga ggacacggct 60
 gtgtatttct gtgcaagatt tggctctgct 90

<210> 38
 <211> 720
 <212> DNA
 <213> Homo sapiens

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<220>

<221> misc_feature

<223> P1=P Human Engineered (low risk) ING1 light Chain with one moderate risk proline change; proline at position 8 (P1)

<220>

<221> CDS

<222> (1)..(717)

<220>

<221> mat_peptide

<222> (61)..()

<400> 38

atg	agg	ttc	tct	gct	cag	ctt	ctg	ggg	ctg	ctt	gtg	ctc	tgg	atc	cct	48
Met	Arg	Phe	Ser	Ala	Gln	Leu	Leu	Gly	Leu	Leu	Val	Leu	Trp	Ile	Pro	
-20					-15					-10					-5	

gga	tcc	act	gca	gac	atc	gtg	atg	acc	cag	tct	cca	ctc	tcc	aat	cca	96
Gly	Ser	Thr	Ala	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Asn	Pro	
			-1	1				5					10			

gtc	act	ctg	gga	gag	tca	ggg	tcc	atc	tcc	tgc	cgg	tct	agt	aag	agt	144
Val	Thr	Leu	Gly	Glu	Ser	Gly	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	
		15					20					25				

ctc	cta	cat	agt	aat	ggc	atc	act	tat	ttg	tat	tgg	tat	ctg	cag	aaa	192
Leu	Leu	His	Ser	Asn	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Tyr	Leu	Gln	Lys	
	30					35					40					

cca	ggg	cag	tct	cct	cag	ctg	ctc	atc	tat	cag	atg	tct	aac	aga	gcc	240
Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Gln	Met	Ser	Asn	Arg	Ala	
45					50					55					60	

tca	ggg	gtc	cca	gac	agg	ttc	agt	agc	agt	gga	tct	ggg	aca	gat	ttc	288
Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Ser	Ser	Gly	Ser	Gly	Thr	Asp	Phe	
				65					70					75		

act	ctc	aag	atc	agc	aga	gtg	gag	gct	gaa	gat	gtg	gga	gtt	tat	tac	336
Thr	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	
			80					85					90			

tgt	gct	cag	aac	cta	gag	ctt	ccg	cgg	acg	ttc	ggg	cag	ggc	acc	aag	384
Cys	Ala	Gln	Asn	Leu	Glu	Leu	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	
		95					100					105				

ctt	gag	atg	aaa	cga	act	gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	432
Leu	Glu	Met	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	
	110					115					120					

cca	tct	gat	gag	cag	ttg	aaa	tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	480
Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	
125					130					135					140	

ctg	aat	aac	ttc	tat	ccc	aga	gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	528
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	
				145					150					155		

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aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac 576
 Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa 624
 Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag 672
 Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag 720
 Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 39
 <211> 239
 <212> PRT
 <213> Homo sapiens

<400> 39

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Pro Leu Ser Asn Pro
 -1 1 5 10

Val Thr Leu Gly Glu Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

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Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 40
 <211> 720
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> P2=P Human Engineered (low risk) ING1 light Chain with one
 moderate risk proline change; proline at position 15 (P2)

<220>
 <221> CDS
 <222> (1)..(717)

<220>
 <221> mat_peptide
 <222> (61)..()

<400> 40
 atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct 48
 Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

gga tcc act gca gac atc gtg atg acc cag tct gca ctc tcc aat cca 96
 Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Ala Leu Ser Asn Pro
 -1 1 5 10

gtc act cct gga gag tca ggt tcc atc tcc tgc cgg tct agt aag agt 144
 Val Thr Pro Gly Glu Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

-39-

ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aaa	192
Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys	
30 35 40	
cca ggg cag tct cct cag ctg ctc atc tat cag atg tct aac aga gcc	240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala	
45 50 55 60	
tca ggg gtc cca gac agg ttc agt agc agt gga tct ggg aca gat ttc	288
Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe	
65 70 75	
act ctc aag atc agc aga gtg gag gct gaa gat gtg gga gtt tat tac	336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr	
80 85 90	
tgt gct cag aac cta gag ctt ccg cgg acg ttc ggt cag ggc acc aag	384
Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys	
95 100 105	
ctt gag atg aaa cga act gtg gct gca cca tct gtc ttc atc ttc ccg	432
Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	
110 115 120	
cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg	480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	
125 130 135 140	
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat	528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	
145 150 155	
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac	576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	
160 165 170	
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa	624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	
175 180 185	
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag	672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln	
190 195 200	
ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag	720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	
205 210 215	

<210> 41
 <211> 239
 <212> PRT
 <213> Homo sapiens

<400> 41

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
-20 -15 -10 -5

-40-

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Ala Leu Ser Asn Pro
 -1 1 5 10

Val Thr Pro Gly Glu Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

-41-

<210> 42
 <211> 720
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> P3=P Human Engineered (low risk) ING1 light Chain with one moderate risk proline change; proline at position 18 (P3)

<220>
 <221> CDS
 <222> (1)..(717)

<220>
 <221> mat_peptide
 <222> (61)..()

<400> 42
 atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct 48
 Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

gga tcc act gca gac atc gtg atg acc cag tct gca ctc tcc aat cca 96
 Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Ala Leu Ser Asn Pro
 -1 1 5 10

gtc act ctg gga gag ccg ggt tcc atc tcc tgc cgg tct agt aag agt 144
 Val Thr Leu Gly Glu Pro Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aaa 192
 Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

cca ggg cag tct cct cag ctg ctc atc tat cag atg tct aac aga gcc 240
 Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

tca ggg gtc cca gac agg ttc agt agc agt gga tct ggg aca gat ttc 288
 Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

act ctc aag atc agc aga gtg gag gct gaa gat gtg gga gtt tat tac 336
 Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

tgt gct cag aac cta gag ctt ccg cgg acg ttc ggt cag ggc acc aag 384
 Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

ctt gag atg aaa cga act gtg gct gca cca tct gtc ttc atc ttc ccg 432
 Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg 480
 Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu

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125	130	135	140	
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat				528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp				
	145	150	155	
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac				576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp				
	160	165	170	
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa				624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys				
	175	180	185	
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag				672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln				
	190	195	200	
ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag				720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys				
	205	210	215	
<210> 43				
<211> 239				
<212> PRT				
<213> Homo sapiens				
<400> 43				
Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro				
-20		-15	-10	-5
Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Ala Leu Ser Asn Pro				
	-1 1	5	10	
Val Thr Leu Gly Glu Pro Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser				
	15	20	25	
Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys				
	30	35	40	
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala				
45		50	55	60
Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe				
	65	70	75	
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr				
	80	85	90	

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Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 44
 <211> 720
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> P1P2=Human Engineered (low risk) ING1 light Chain with one
 moderate risk proline change; proline at position 8 (P1) 15(P2)

<220>
 <221> CDS
 <222> (1)..(717)

<220>
 <221> mat_peptide
 <222> (61)..()

<400> 44
 atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct 48
 Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

gga tcc act gca gac atc gtg atg acc cag tct cca ctc tcc aat cca 96
 Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Pro Leu Ser Asn Pro

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	-1	1		5		10	
gtc act cct gga gag tca ggt tcc atc tcc tgc cgg tct agt aag agt							144
Val Thr Pro Gly Glu Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser							
	15			20		25	
ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aaa							192
Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys							
	30			35		40	
cca ggg cag tct cct cag ctg ctc atc tat cag atg tct aac aga gcc							240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala							
	45			50		55	60
tca ggg gtc cca gac agg ttc agt agc agt gga tct ggg aca gat ttc							288
Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe							
			65		70		75
act ctc aag atc agc aga gtg gag gct gaa gat gtg gga gtt tat tac							336
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr							
		80			85		90
tgt gct cag aac cta gag ctt ccg cgg acg ttc ggt cag ggc acc aag							384
Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys							
		95			100		105
ctt gag atg aaa cga act gtg gct gca cca tct gtc ttc atc ttc ccg							432
Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro							
	110			115		120	
cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg							480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu							
	125			130		135	140
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat							528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp							
			145		150		155
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac							576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp							
		160			165		170
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa							624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys							
		175			180		185
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag							672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln							
	190			195		200	
ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag							720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys							
	205			210		215	

<210> 45
 <211> 239
 <212> PRT

-45-

<213> Homo sapiens

<400> 45

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Pro Leu Ser Asn Pro
 -1 1 5 10

Val Thr Pro Gly Glu Ser Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

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Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 46
 <211> 720
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> P1P3= Human Engineered (low risk) ING1 light Chain with one
 moderate risk proline change; proline at position 8 (P1) 18 (P3)

<220>
 <221> CDS
 <222> (1)..(717)

<220>
 <221> mat_peptide
 <222> (61)..()

<400> 46
 atg agg ttc tct gct cag ctt ctg ggg ctg ctt gtg ctc tgg atc cct 48
 Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5
 gga tcc act gca gac atc gtg atg acc cag tct cca ctc tcc aat cca 96
 Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Pro Leu Ser Asn Pro
 -1 1 5 10
 gtc act ctg gga gag ccg ggt tcc atc tcc tgc cgg tct agt aag agt 144
 Val Thr Leu Gly Glu Pro Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25
 ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aaa 192
 Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40
 cca ggg cag tct cct cag ctg ctc atc tat cag atg tct aac aga gcc 240
 Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60
 tca ggg gtc cca gac agg ttc agt agc agt gga tct ggg aca gat ttc 288
 Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75
 act ctc aag atc agc aga gtg gag gct gaa gat gtg gga gtt tat tac 336
 Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90
 tgt gct cag aac cta gag ctt ccg cgg acg ttc ggt cag ggc acc aag 384
 Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

-47-

ctt gag atg aaa cga act gtg gct gca cca tct gtc ttc atc ttc ccg	432
Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro	
110 115 120	
cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg	480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu	
125 130 135 140	
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat	528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp	
145 150 155	
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac	576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp	
160 165 170	
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa	624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys	
175 180 185	
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag	672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln	
190 195 200	
ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tag	720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	
205 210 215	

<210> 47
 <211> 239
 <212> PRT
 <213> Homo sapiens

<400> 47

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
-20 -15 -10 -5

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Pro Leu Ser Asn Pro
-1 1 5 10

Val Thr Leu Gly Glu Pro Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
15 20 25

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
65 70 75

-48-

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
160 165 170

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
190 195 200

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
205 210 215

<210> 48
<211> 720
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> P2P3=Human Engineered (low risk) ING1 light Chain with one
moderate risk proline change; proline at position 8 (P1) 18 (P3)

<220>
<221> CDS
<222> (1)..(717)

<220>
<221> mat_peptide
<222> (61)..()

<400> 48

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atg	agg	ttc	tct	gct	cag	ctt	ctg	ggg	ctg	ctt	gtg	ctc	tgg	atc	cct	48
Met	Arg	Phe	Ser	Ala	Gln	Leu	Leu	Gly	Leu	Leu	Val	Leu	Trp	Ile	Pro	
-20					-15					-10					-5	
gga	tcc	act	gca	gac	atc	gtg	atg	acc	cag	tct	gca	ctc	tcc	aat	cca	96
Gly	Ser	Thr	Ala	Asp	Ile	Val	Met	Thr	Gln	Ser	Ala	Leu	Ser	Asn	Pro	
			-1	1			5						10			
gtc	act	cct	gga	gag	ccg	ggc	tcc	atc	tcc	tgc	cgg	tct	agt	aag	agt	144
Val	Thr	Pro	Gly	Glu	Pro	Gly	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	
		15					20					25				
ctc	cta	cat	agt	aat	ggc	atc	act	tat	ttg	tat	tgg	tat	ctg	cag	aaa	192
Leu	Leu	His	Ser	Asn	Gly	Ile	Thr	Tyr	Leu	Tyr	Trp	Tyr	Leu	Gln	Lys	
	30					35					40					
cca	ggg	cag	tct	cct	cag	ctg	ctc	atc	tat	cag	atg	tct	aac	aga	gcc	240
Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Gln	Met	Ser	Asn	Arg	Ala	
45					50					55					60	
tca	ggg	gtc	cca	gac	agg	ttc	agt	agc	agt	gga	tct	ggg	aca	gat	ttc	288
Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Ser	Ser	Gly	Ser	Gly	Thr	Asp	Phe	
				65					70					75		
act	ctc	aag	atc	agc	aga	gtg	gag	gct	gaa	gat	gtg	gga	gtt	tat	tac	336
Thr	Leu	Lys	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	
			80					85					90			
tgt	gct	cag	aac	cta	gag	ctt	ccg	cgg	acg	ttc	ggc	cag	ggc	acc	aag	384
Cys	Ala	Gln	Asn	Leu	Glu	Leu	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	
		95					100					105				
ctt	gag	atg	aaa	cga	act	gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	432
Leu	Glu	Met	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	
	110					115					120					
cca	tct	gat	gag	cag	ttg	aaa	tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	480
Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	
125					130					135					140	
ctg	aat	aac	ttc	tat	ccc	aga	gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	528
Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	
				145					150					155		
aac	gcc	ctc	caa	tcg	ggc	aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	576
Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	
			160					165					170			
agc	aag	gac	agc	acc	tac	agc	ctc	agc	agc	acc	ctg	acg	ctg	agc	aaa	624
Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	
		175					180					185				
gca	gac	tac	gag	aaa	cac	aaa	gtc	tac	gcc	tgc	gaa	gtc	acc	cat	cag	672
Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	
	190					195					200					
ggc	ctg	agc	tcg	ccc	gtc	aca	aag	agc	ttc	aac	agg	gga	gag	tgt	tag	720
Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys		

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205

210

215

<210> 49
 <211> 239
 <212> PRT
 <213> Homo sapiens
 <400> 49

Met Arg Phe Ser Ala Gln Leu Leu Gly Leu Leu Val Leu Trp Ile Pro
 -20 -15 -10 -5

Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ser Ala Leu Ser Asn Pro
 -1 1 5 10

Val Thr Pro Gly Glu Pro Gly Ser Ile Ser Cys Arg Ser Ser Lys Ser
 15 20 25

Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
 30 35 40

Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Arg Ala
 45 50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
 65 70 75

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
 80 85 90

Cys Ala Gln Asn Leu Glu Leu Pro Arg Thr Phe Gly Gln Gly Thr Lys
 95 100 105

Leu Glu Met Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
 110 115 120

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
 125 130 135 140

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
 145 150 155

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
 160 165 170

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Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
 175 180 185

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
 190 195 200

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 205 210 215

<210> 50
 <211> 85
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> P1 Oligo ING-1 with proline oligos

<400> 50
 actcttacta gaccggcagg agatggaacc tgactctccc agagtgactg gattggagag 60

tggagactgg gtcatcacga tgtct 85

<210> 51
 <211> 85
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> P2 Oligo ING-1 with proline oligos

<400> 51
 actcttacta gaccggcagg agatggaacc tgactctcca ggagtgactg gattggagag 60

tgcagactgg gtcatcacga tgtct 85

<210> 52
 <211> 85
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> P3 Oligo ING-1 with proline oligos

<400> 52
 actcttacta gaccggcagg agatggaacc cggctctccc agagtgactg gattggagag 60

tgcagactgg gtcatcacga tgtct 85

-52-

<210> 53
<211> 85
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<223> P1P2 Oligo ING-1 with proline oligos

<400> 53
actcttacta gaccggcagg agatggaacc cggctctcca ggagtgactg gattggagag 60
tgcagactgg gtcatcacga tgtct 85

<210> 54
<211> 85
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<223> P1P3 Oligo ING-1 with proline oligos

<400> 54
actcttacta gaccggcagg agatggaacc cggctctccc agagtgactg gattggagag 60
tggagactgg gtcatcacga tgtct 85

<210> 55
<211> 85
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature
<223> P2P3 Oligo ING-1 with proline oligos

<400> 55
actcttacta gaccggcagg agatggaacc cggctctcca ggagtgactg gattggagag 60
tgcagactgg gtcatcacga tgtct 85

<210> 56
<211> 19
<212> DNA
<213> Homo Sapiens

<220>
<221> misc_feature

-53-

<223> Reverse Primer KBsr ING-1 Light Chain

<400> 56

cttactagac cggcaggag

19

<210> 57

<211> 798

<212> DNA

<213> Homo sapiens

<220>

<221> misc_feature

<223> EpCam truncated sequence

<220>

<221> CDS

<222> (1)..(795)

<400> 57

atg	gcg	ccc	ccg	cag	gtc	ctc	gcg	ttc	ggg	ctt	ctg	ctt	gcc	gcg	gcg	48
Met	Ala	Pro	Pro	Gln	Val	Leu	Ala	Phe	Gly	Leu	Leu	Leu	Ala	Ala	Ala	
1				5					10				15			

acg	gcg	act	ttt	gcc	gca	gct	cag	gaa	gaa	tgt	gtc	tgt	gaa	aac	tac	96
Thr	Ala	Thr	Phe	Ala	Ala	Ala	Gln	Glu	Glu	Cys	Val	Cys	Glu	Asn	Tyr	
			20				25						30			

aag	ctg	gcc	gta	aac	tgc	ttt	gtg	aat	aat	aat	cgt	caa	tgc	cag	tgt	144
Lys	Leu	Ala	Val	Asn	Cys	Phe	Val	Asn	Asn	Asn	Arg	Gln	Cys	Gln	Cys	
		35					40					45				

act	tca	gtt	ggc	gca	caa	aat	act	gtc	att	tgc	tca	aag	ctg	gct	gcc	192
Thr	Ser	Val	Gly	Ala	Gln	Asn	Thr	Val	Ile	Cys	Ser	Lys	Leu	Ala	Ala	
		50				55					60					

aaa	tgt	ttg	gtg	atg	aag	gca	gaa	atg	aat	ggc	tca	aaa	ctt	ggg	aga	240
Lys	Cys	Leu	Val	Met	Lys	Ala	Glu	Met	Asn	Gly	Ser	Lys	Leu	Gly	Arg	
65					70				75					80		

aga	gca	aaa	cct	gaa	ggg	gcc	ctc	cag	aac	aat	gat	ggg	ctt	tat	gat	288
Arg	Ala	Lys	Pro	Glu	Gly	Ala	Leu	Gln	Asn	Asn	Asp	Gly	Leu	Tyr	Asp	
			85					90					95			

cct	gac	tgc	gat	gag	agc	ggg	ctc	ttt	aag	gcc	aag	cag	tgc	aac	ggc	336
Pro	Asp	Cys	Asp	Glu	Ser	Gly	Leu	Phe	Lys	Ala	Lys	Gln	Cys	Asn	Gly	
			100				105						110			

acc	tcc	acg	tgc	tgg	tgt	gtg	aac	act	gct	ggg	gtc	aga	aga	aca	gac	384
Thr	Ser	Thr	Cys	Trp	Cys	Val	Asn	Thr	Ala	Gly	Val	Arg	Arg	Thr	Asp	
		115				120					125					

aag	gac	act	gaa	ata	acc	tgc	tct	gag	cga	gtg	aga	acc	tac	tgg	atc	432
Lys	Asp	Thr	Glu	Ile	Thr	Cys	Ser	Glu	Arg	Val	Arg	Thr	Tyr	Trp	Ile	
	130					135				140						

atc	att	gaa	cta	aaa	cac	aaa	gca	aga	gaa	aaa	cct	tat	gat	agt	aaa	480
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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Ile Ile Glu Leu Lys His Lys Ala Arg Glu Lys Pro Tyr Asp Ser Lys	
145	150 155 160
agt ttg cgg act gca ctt cag aag gag atc aca acg cgt tat caa ctg	528
Ser Leu Arg Thr Ala Leu Gln Lys Glu Ile Thr Thr Arg Tyr Gln Leu	
	165 170 175
gat cca aaa ttt atc acg agt att ttg tat gag aat aat gtt atc act	576
Asp Pro Lys Phe Ile Thr Ser Ile Leu Tyr Glu Asn Asn Val Ile Thr	
	180 185 190
att gat ctg gtt caa aat tct tct caa aaa act cag aat gat gtg gac	624
Ile Asp Leu Val Gln Asn Ser Ser Gln Lys Thr Gln Asn Asp Val Asp	
	195 200 205
ata gct gat gtg gct tat tat ttt gaa aaa gat gtt aaa ggt gaa tcc	672
Ile Ala Asp Val Ala Tyr Tyr Phe Glu Lys Asp Val Lys Gly Glu Ser	
	210 215 220
ttg ttt cat tct aag aaa atg gac ctg aca gta aat ggg gaa caa ctg	720
Leu Phe His Ser Lys Lys Met Asp Leu Thr Val Asn Gly Glu Gln Leu	
	225 230 235 240
gat ctg gat cct ggt caa act tta att tat tat gtt gat gaa aaa gca	768
Asp Leu Asp Pro Gly Gln Thr Leu Ile Tyr Tyr Val Asp Glu Lys Ala	
	245 250 255
cct gaa ttc tca atg cag ggt cta aaa taa	798
Pro Glu Phe Ser Met Gln Gly Leu Lys	
	260 265

<210> 58
 <211> 265
 <212> PRT
 <213> Homo sapiens

<400> 58

Met Ala Pro Pro Gln Val Leu Ala Phe Gly Leu Leu Leu Ala Ala Ala	
1	5 10 15
Thr Ala Thr Phe Ala Ala Ala Gln Glu Glu Cys Val Cys Glu Asn Tyr	
	20 25 30
Lys Leu Ala Val Asn Cys Phe Val Asn Asn Asn Arg Gln Cys Gln Cys	
	35 40 45
Thr Ser Val Gly Ala Gln Asn Thr Val Ile Cys Ser Lys Leu Ala Ala	
	50 55 60
Lys Cys Leu Val Met Lys Ala Glu Met Asn Gly Ser Lys Leu Gly Arg	
65	70 75 80

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Arg Ala Lys Pro Glu Gly Ala Leu Gln Asn Asn Asp Gly Leu Tyr Asp
 85 90 95

Pro Asp Cys Asp Glu Ser Gly Leu Phe Lys Ala Lys Gln Cys Asn Gly
 100 105 110

Thr Ser Thr Cys Trp Cys Val Asn Thr Ala Gly Val Arg Arg Thr Asp
 115 120 125

Lys Asp Thr Glu Ile Thr Cys Ser Glu Arg Val Arg Thr Tyr Trp Ile
 130 135 140

Ile Ile Glu Leu Lys His Lys Ala Arg Glu Lys Pro Tyr Asp Ser Lys
 145 150 155 160

Ser Leu Arg Thr Ala Leu Gln Lys Glu Ile Thr Thr Arg Tyr Gln Leu
 165 170 175

Asp Pro Lys Phe Ile Thr Ser Ile Leu Tyr Glu Asn Asn Val Ile Thr
 180 185 190

Ile Asp Leu Val Gln Asn Ser Ser Gln Lys Thr Gln Asn Asp Val Asp
 195 200 205

Ile Ala Asp Val Ala Tyr Tyr Phe Glu Lys Asp Val Lys Gly Glu Ser
 210 215 220

Leu Phe His Ser Lys Lys Met Asp Leu Thr Val Asn Gly Glu Gln Leu
 225 230 235 240

Asp Leu Asp Pro Gly Gln Thr Leu Ile Tyr Tyr Val Asp Glu Lys Ala
 245 250 255

Pro Glu Phe Ser Met Gln Gly Leu Lys
 260 265

<210> 59
 <211> 945
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Full-Length EpCam

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<220>
 <221> CDS
 <222> (1)..(942)

<220>
 <221> mat_peptide
 <222> (70)..()

<400> 59

atg	gcg	ccc	ccg	cag	gtc	ctc	gcg	ttc	ggg	ctt	ctg	ctt	gcc	gcg	gcg	48
Met	Ala	Pro	Pro	Gln	Val	Leu	Ala	Phe	Gly	Leu	Leu	Leu	Ala	Ala	Ala	
			-20					-15					-10			

acg	gcg	act	ttt	gcc	gca	gct	cag	gaa	gaa	tgt	gtc	tgt	gaa	aac	tac	96
Thr	Ala	Thr	Phe	Ala	Ala	Ala	Gln	Glu	Glu	Cys	Val	Cys	Glu	Asn	Tyr	
		-5				-1	1				5					

aag	ctg	gcc	gta	aac	tgc	ttt	gtg	aat	aat	aat	cgt	caa	tgc	cag	tgt	144
Lys	Leu	Ala	Val	Asn	Cys	Phe	Val	Asn	Asn	Asn	Arg	Gln	Cys	Gln	Cys	
10					15					20					25	

act	tca	gtt	ggt	gca	caa	aat	act	gtc	att	tgc	tca	aag	ctg	gct	gcc	192
Thr	Ser	Val	Gly	Ala	Gln	Asn	Thr	Val	Ile	Cys	Ser	Lys	Leu	Ala	Ala	
			30						35					40		

aaa	tgt	ttg	gtg	atg	aag	gca	gaa	atg	aat	ggc	tca	aaa	ctt	ggg	aga	240
Lys	Cys	Leu	Val	Met	Lys	Ala	Glu	Met	Asn	Gly	Ser	Lys	Leu	Gly	Arg	
			45					50					55			

aga	gca	aaa	cct	gaa	ggg	gcc	ctc	cag	aac	aat	gat	ggg	ctt	tat	gat	288
Arg	Ala	Lys	Pro	Glu	Gly	Ala	Leu	Gln	Asn	Asn	Asp	Gly	Leu	Tyr	Asp	
		60					65					70				

cct	gac	tgc	gat	gag	agc	ggg	ctc	ttt	aag	gcc	aag	cag	tgc	aac	ggc	336
Pro	Asp	Cys	Asp	Glu	Ser	Gly	Leu	Phe	Lys	Ala	Lys	Gln	Cys	Asn	Gly	
	75					80					85					

acc	tcc	acg	tgc	tgg	tgt	gtg	aac	act	gct	ggg	gtc	aga	aga	aca	gac	384
Thr	Ser	Thr	Cys	Trp	Cys	Val	Asn	Thr	Ala	Gly	Val	Arg	Arg	Thr	Asp	
90					95					100					105	

aag	gac	act	gaa	ata	acc	tgc	tct	gag	cga	gtg	aga	acc	tac	tgg	atc	432
Lys	Asp	Thr	Glu	Ile	Thr	Cys	Ser	Glu	Arg	Val	Arg	Thr	Tyr	Trp	Ile	
				110					115					120		

atc	att	gaa	cta	aaa	cac	aaa	gca	aga	gaa	aaa	cct	tat	gat	agt	aaa	480
Ile	Ile	Glu	Leu	Lys	His	Lys	Ala	Arg	Glu	Lys	Pro	Tyr	Asp	Ser	Lys	
			125				130						135			

agt	ttg	cgg	act	gca	ctt	cag	aag	gag	atc	aca	acg	cgt	tat	caa	ctg	528
Ser	Leu	Arg	Thr	Ala	Leu	Gln	Lys	Glu	Ile	Thr	Thr	Arg	Tyr	Gln	Leu	
		140					145					150				

gat	cca	aaa	ttt	atc	acg	agt	att	ttg	tat	gag	aat	aat	gtt	atc	act	576
Asp	Pro	Lys	Phe	Ile	Thr	Ser	Ile	Leu	Tyr	Glu	Asn	Asn	Val	Ile	Thr	
	155					160					165					

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att gat ctg gtt caa aat tct tct caa aaa act cag aat gat gtg gac	624
Ile Asp Leu Val Gln Asn Ser Ser Gln Lys Thr Gln Asn Asp Val Asp	
170 175 180 185	
ata gct gat gtg gct tat tat ttt gaa aaa gat gtt aaa ggt gaa tcc	672
Ile Ala Asp Val Ala Tyr Tyr Phe Glu Lys Asp Val Lys Gly Glu Ser	
190 195 200	
ttg ttt cat tct aag aaa atg gac ctg aca gta aat ggg gaa caa ctg	720
Leu Phe His Ser Lys Lys Met Asp Leu Thr Val Asn Gly Glu Gln Leu	
205 210 215	
gat ctg gat cct ggt caa act tta att tat tat gtt gat gaa aaa gca	768
Asp Leu Asp Pro Gly Gln Thr Leu Ile Tyr Tyr Val Asp Glu Lys Ala	
220 225 230	
cct gaa ttc tca atg cag ggt cta aaa gct ggt gtt att gct gtt att	816
Pro Glu Phe Ser Met Gln Gly Leu Lys Ala Gly Val Ile Ala Val Ile	
235 240 245	
gtg gtt gtg gtg ata gca gtt gtt gct gga att gtt gtg ctg gtt att	864
Val Val Val Val Ile Ala Val Val Ala Gly Ile Val Val Leu Val Ile	
250 255 260 265	
tcc aga aag aag aga atg gca aag tat gag aag gct gag ata aag gag	912
Ser Arg Lys Lys Arg Met Ala Lys Tyr Glu Lys Ala Glu Ile Lys Glu	
270 275 280	
atg ggt gag atg cat agg gaa ctc aat gca taa	945
Met Gly Glu Met His Arg Glu Leu Asn Ala	
285 290	

<210> 60
 <211> 314
 <212> PRT
 <213> Homo sapiens

<400> 60

Met Ala Pro Pro Gln Val Leu Ala Phe Gly Leu Leu Leu Ala Ala Ala
-20 -15 -10

Thr Ala Thr Phe Ala Ala Ala Gln Glu Glu Cys Val Cys Glu Asn Tyr
-5 -1 1 5

Lys Leu Ala Val Asn Cys Phe Val Asn Asn Asn Arg Gln Cys Gln Cys
10 15 20 25

Thr Ser Val Gly Ala Gln Asn Thr Val Ile Cys Ser Lys Leu Ala Ala
30 35 40

Lys Cys Leu Val Met Lys Ala Glu Met Asn Gly Ser Lys Leu Gly Arg
45 50 55

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Arg Ala Lys Pro Glu Gly Ala Leu Gln Asn Asn Asp Gly Leu Tyr Asp
 60 65 70

Pro Asp Cys Asp Glu Ser Gly Leu Phe Lys Ala Lys Gln Cys Asn Gly
 75 80 85

Thr Ser Thr Cys Trp Cys Val Asn Thr Ala Gly Val Arg Arg Thr Asp
 90 95 100 105

Lys Asp Thr Glu Ile Thr Cys Ser Glu Arg Val Arg Thr Tyr Trp Ile
 110 115 120

Ile Ile Glu Leu Lys His Lys Ala Arg Glu Lys Pro Tyr Asp Ser Lys
 125 130 135

Ser Leu Arg Thr Ala Leu Gln Lys Glu Ile Thr Thr Arg Tyr Gln Leu
 140 145 150

Asp Pro Lys Phe Ile Thr Ser Ile Leu Tyr Glu Asn Asn Val Ile Thr
 155 160 165

Ile Asp Leu Val Gln Asn Ser Ser Gln Lys Thr Gln Asn Asp Val Asp
 170 175 180 185

Ile Ala Asp Val Ala Tyr Tyr Phe Glu Lys Asp Val Lys Gly Glu Ser
 190 195 200

Leu Phe His Ser Lys Lys Met Asp Leu Thr Val Asn Gly Glu Gln Leu
 205 210 215

Asp Leu Asp Pro Gly Gln Thr Leu Ile Tyr Tyr Val Asp Glu Lys Ala
 220 225 230

Pro Glu Phe Ser Met Gln Gly Leu Lys Ala Gly Val Ile Ala Val Ile
 235 240 245

Val Val Val Val Ile Ala Val Val Ala Gly Ile Val Val Leu Val Ile
 250 255 260 265

Ser Arg Lys Lys Arg Met Ala Lys Tyr Glu Lys Ala Glu Ile Lys Glu
 270 275 280

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Met Gly Glu Met His Arg Glu Leu Asn Ala
 285 290

<210> 61
 <211> 26
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Forward Primer (for both soluble and full length EpCam: EC-1

<400> 61
 ttatgtcgac agcatggcgc ccccgc 26

<210> 62
 <211> 31
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> EpCAM Reverse Primer

<400> 62
 gagttacgtc ccagatttta ttgggcccc t 31

<210> 63
 <211> 23
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Polylinker pING1736 oligonucleotides

<400> 63
 tcgacgaatt catcgatggt acc 23

<210> 64
 <211> 19
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Polylinker pING1736 oligonucleotides reverse 3' to 5'

<400> 64
 ggtaccatcg atgaattcg 19

-60-

<210> 65
 <211> 23
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Polylinker pING1732 oligonucleotides

<400> 65
 tcgacgaatt catcgatgat atc 23

<210> 66
 <211> 19
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> Polylinker pING1732 oligonucleotides reverse 3' to 5'

<400> 66
 gatatcatcg atgaattcg 19

<210> 67
 <211> 702
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> LDP-01 Light Chain

<220>
 <221> CDS
 <222> (1)..(699)

<220>
 <221> mat_peptide
 <222> (58)..()

<400> 67
 atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt 48
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 -15 -10 -5

gtc cac tcc gac atc cag atg acc cag agc cca agc agc ctg agc gcc 96
 Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
 -1 1 5 10

agc gtg ggt gac aga gtg acc atc acc tgt aaa gca agt aag agc att 144

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Ser	Val	Gly	Asp	Arg	Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Lys	Ser	Ile		
15						20					25						
agc	aat	tat	tta	gcc	tgg	tac	cag	cag	aag	cca	ggg	aag	gct	cca	aag	192	
Ser	Asn	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys		
30					35				40						45		
ctg	ctg	atc	tac	tat	ggg	tca	act	ttg	cga	tct	ggg	gtg	cca	agc	aga	240	
Leu	Leu	Ile	Tyr	Tyr	Gly	Ser	Thr	Leu	Arg	Ser	Gly	Val	Pro	Ser	Arg		
				50					55					60			
ttc	agc	ggg	agc	ggg	agc	ggg	acc	gac	ttc	acc	ttc	acc	atc	agc	agc	288	
Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Phe	Thr	Ile	Ser	Ser		
			65				70						75				
ctc	cag	cca	gag	gac	atc	gcc	acc	tac	tac	tgc	caa	cag	tat	tat	gaa	336	
Leu	Gln	Pro	Glu	Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Tyr	Tyr	Glu		
		80					85					90					
aga	ccg	ctc	acg	ttc	ggc	caa	ggg	acc	aag	gtg	gaa	atc	aaa	cga	act	384	
Arg	Pro	Leu	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr		
	95					100					105						
gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	cca	tct	gat	gag	cag	ttg	432	
Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu		
110					115					120					125		
aaa	tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	tat	ccc	480	
Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro		
				130					135					140			
aga	gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	aac	gcc	ctc	caa	tcg	ggg	528	
Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly		
			145					150					155				
aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	agc	aag	gac	agc	acc	tac	576	
Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr		
		160					165					170					
agc	ctc	agc	agc	acc	ctg	acg	ctg	agc	aaa	gca	gac	tac	gag	aaa	cac	624	
Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His		
	175					180					185						
aaa	gtc	tac	gcc	tgc	gaa	gtc	acc	cat	cag	ggc	ctg	agc	tcg	ccc	gtc	672	
Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val		
190					195					200					205		
aca	aag	agc	ttc	aac	agg	gga	gag	tgt	tag							702	
Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys									
				210													

<210> 68
 <211> 233
 <212> PRT
 <213> Homo Sapiens

<400> 68

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Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 -15 -10 -5

Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
 -1 1 5 10

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Lys Ser Ile
 15 20 25

Ser Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
 30 35 40 45

Leu Leu Ile Tyr Tyr Gly Ser Thr Leu Arg Ser Gly Val Pro Ser Arg
 50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser
 65 70 75

Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Tyr Glu
 80 85 90

Arg Pro Leu Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 95 100 105

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
 110 115 120 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
 130 135 140

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
 145 150 155

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
 160 165 170

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
 175 180 185

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
 190 195 200 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys

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210

<210> 69
 <211> 29
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> forward primer LDP5PrFwd

<400> 69
 tgtattgaat tcacccatggg atggagctg

29

<210> 70
 <211> 30
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> reverse primer LDPLC3PrXhoRev

<400> 70
 gataactcga gctaacactc tcccctgttg

30

<210> 71
 <211> 1410
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> LDP-01 Heavy Chain

<220>
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 <222> (1)..(1407)

<220>
 <221> mat_peptide
 <222> (58)..()

<400> 71
 atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 -15 -10 -5

48

gtc cac tcc cag gtc caa ctg cag gag agc ggt cca ggt ctt gtg aga
 Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
 -1 1 5 10

96

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cct agc cag acc ctg agc ctg acc tgc acc gtg tct ggc ttc acc ttc	144
Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe	
15 20 25	
acc gat tac ctt ctg cac tgg gtg aga cag cca cct gga cga ggt ctt	192
Thr Asp Tyr Leu Leu His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu	
30 35 40 45	
gag tgg att gga tgg att gat cct gag gat ggt gaa aca aag tat ggt	240
Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly	
50 55 60	
cag aag ttt caa agc aga gtg aca atg ctg gta gac acc agc aag aac	288
Gln Lys Phe Gln Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn	
65 70 75	
cag ttc agc ctg aga ctc agc agc gtg aca gcc gcc gac acc gcg gtc	336
Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val	
80 85 90	
tat tat tgt gca aga ggc gaa tat aga tac aac tcg tgg ttt gat tac	384
Tyr Tyr Cys Ala Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr	
95 100 105	
tgg ggt caa ggc tca cta gtc aca gtc tcc tca gcc tcc acc aag ggc	432
Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly	
110 115 120 125	
cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc	480
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly	
130 135 140	
aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg	528
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val	
145 150 155	
acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc	576
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe	
160 165 170	
ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg	624
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val	
175 180 185	
acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac gtg	672
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val	
190 195 200 205	
aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc aaa	720
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys	
210 215 220	
tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc	768
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu	
225 230 235	
gcg ggg gca ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag cac acc	816
Ala Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys His Thr	

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240	245	250	
ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val 255 260 265			864
agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val 270 275 280 285			912
gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser 290 295 300			960
acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu 305 310 315			1008
aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala 320 325 330			1056
ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro 335 340 345			1104
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 350 355 360 365			1152
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 370 375 380			1200
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 385 390 395			1248
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 400 405 410			1296
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 415 420 425			1344
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 430 435 440 445			1392
ctg tct ccg ggt aaa tga Leu Ser Pro Gly Lys 450			1410

<210> 72
 <211> 469
 <212> PRT

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<213> Homo Sapiens

<400> 72

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 -15 -10 -5

Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg
 -1 1 5 10

Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe
 15 20 25

Thr Asp Tyr Leu Leu His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu
 30 35 40 45

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asp Gly Glu Thr Lys Tyr Gly
 50 55 60

Gln Lys Phe Gln Ser Arg Val Thr Met Leu Val Asp Thr Ser Lys Asn
 65 70 75

Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val
 80 85 90

Tyr Tyr Cys Ala Arg Gly Glu Tyr Arg Tyr Asn Ser Trp Phe Asp Tyr
 95 100 105

Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 110 115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
 130 135 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 145 150 155

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 160 165 170

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 175 180 185

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
 190 195 200 205

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Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 430 435 440 445

Leu Ser Pro Gly Lys
 450

<210> 73
 <211> 30
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> reverse primer LDPLX3PrXhoRev

<400> 73
 cttatctcga gtcatttacc cggagacagg 30

<210> 74
 <211> 1836
 <212> DNA
 <213> Homo Sapiens

<220>
 <221> misc_feature
 <223> CAB2.1

<220>
 <221> CDS
 <222> (1)..(1833)

<220>
 <221> mat_peptide
 <222> (103)..()

<400> 74
 atg gag cct ccc ggc cgc cgc gag tgt ccc ttt cct tcc tgg cgc ttt 48
 Met Glu Pro Pro Gly Arg Arg Glu Cys Pro Phe Pro Ser Trp Arg Phe
 -30 -25 -20

cct ggg ttg ctt ctg gcg gcc atg gtg ttg ctg ctg tac tcc ttc tcc 96
 Pro Gly Leu Leu Leu Ala Ala Met Val Leu Leu Leu Tyr Ser Phe Ser
 -15 -10 -5

gat gcc tgt gag gag cca cca aca ttt gaa gct atg gag ctc atc ggg 144
 Asp Ala Cys Glu Glu Pro Pro Thr Phe Glu Ala Met Glu Leu Ile Gly
 -1 1 5 10

aaa cca aaa ccc tac tat gag att ggt gaa cga gta gat tat aag tgt 192
 Lys Pro Lys Pro Tyr Tyr Glu Ile Gly Glu Arg Val Asp Tyr Lys Cys
 15 20 25 30

aaa aaa gga tac ttc tat ata cct cct ctt gcc acc cat act att tgt 240

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Lys	Lys	Gly	Tyr	Phe	Tyr	Ile	Pro	Pro	Leu	Ala	Thr	His	Thr	Ile	Cys	
				35					40					45		
gat	cgg	aat	cat	aca	tgg	cta	cct	gtc	tca	gat	gac	gcc	tgt	tat	aga	288
Asp	Arg	Asn	His	Thr	Trp	Leu	Pro	Val	Ser	Asp	Asp	Ala	Cys	Tyr	Arg	
			50					55					60			
gaa	aca	tgt	cca	tat	ata	cgg	gat	cct	tta	aat	ggc	caa	gca	gtc	cct	336
Glu	Thr	Cys	Pro	Tyr	Ile	Arg	Asp	Pro	Leu	Asn	Gly	Gln	Ala	Val	Pro	
		65						70				75				
gca	aat	ggg	act	tac	gag	ttt	ggg	tat	cag	atg	cac	ttt	att	tgt	aat	384
Ala	Asn	Gly	Thr	Tyr	Glu	Phe	Gly	Tyr	Gln	Met	His	Phe	Ile	Cys	Asn	
	80					85					90					
gag	ggg	tat	tac	tta	att	ggg	gaa	gaa	att	cta	tat	tgt	gaa	ctt	aaa	432
Glu	Gly	Tyr	Tyr	Leu	Ile	Gly	Glu	Glu	Ile	Leu	Tyr	Cys	Glu	Leu	Lys	
95					100					105					110	
gga	tca	gta	gca	att	tgg	agc	gga	aaa	ccc	ccc	ata	tgt	gaa	aag	gtt	480
Gly	Ser	Val	Ala	Ile	Trp	Ser	Gly	Lys	Pro	Pro	Ile	Cys	Glu	Lys	Val	
				115					120					125		
ttg	tgt	aca	cca	cct	cca	aaa	ata	aaa	aat	gga	aaa	cac	acc	ttt	agt	528
Leu	Cys	Thr	Pro	Pro	Pro	Lys	Ile	Lys	Asn	Gly	Lys	His	Thr	Phe	Ser	
			130					135					140			
gaa	gta	gaa	gta	ttt	gag	tat	ctt	gat	gca	gta	act	tat	agt	tgt	gat	576
Glu	Val	Glu	Val	Phe	Glu	Tyr	Leu	Asp	Ala	Val	Thr	Tyr	Ser	Cys	Asp	
		145					150					155				
cct	gca	cct	gga	cca	gat	cca	ttt	tca	ctt	att	gga	gag	agc	acg	att	624
Pro	Ala	Pro	Gly	Pro	Asp	Pro	Phe	Ser	Leu	Ile	Gly	Glu	Ser	Thr	Ile	
	160					165					170					
tat	tgt	ggg	gac	aat	tca	gtg	tgg	agt	cgt	gct	gct	cca	gag	tgt	aaa	672
Tyr	Cys	Gly	Asp	Asn	Ser	Val	Trp	Ser	Arg	Ala	Ala	Pro	Glu	Cys	Lys	
175					180					185					190	
gtg	gtc	aaa	tgt	cga	ttt	cca	gta	gtc	gaa	aat	gga	aaa	cag	ata	tca	720
Val	Val	Lys	Cys	Arg	Phe	Pro	Val	Val	Glu	Asn	Gly	Lys	Gln	Ile	Ser	
				195					200					205		
gga	ttt	gga	aaa	aaa	ttt	tac	tac	aaa	gca	aca	gtt	atg	ttt	gaa	tgc	768
Gly	Phe	Gly	Lys	Lys	Phe	Tyr	Tyr	Lys	Ala	Thr	Val	Met	Phe	Glu	Cys	
			210					215					220			
gat	aag	ggg	ttt	tac	ctc	gat	ggc	agc	gac	aca	att	gtc	tgt	gac	agt	816
Asp	Lys	Gly	Phe	Tyr	Leu	Asp	Gly	Ser	Asp	Thr	Ile	Val	Cys	Asp	Ser	
		225					230					235				
aac	agt	act	tgg	gat	ccc	cca	gtt	cca	aag	tgt	ctt	aaa	gtg	tcg	act	864
Asn	Ser	Thr	Trp	Asp	Pro	Pro	Val	Pro	Lys	Cys	Leu	Lys	Val	Ser	Thr	
	240					245					250					
gac	tgt	ggc	ctt	ccc	cca	gat	gta	cct	aat	gcc	cag	cca	gct	ttg	gaa	912
Asp	Cys	Gly	Leu	Pro	Pro	Asp	Val	Pro	Asn	Ala	Gln	Pro	Ala	Leu	Glu	
255					260					265					270	

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ggc cgt aca agt ttt ccc gag gat act gta ata acg tac aaa tgt gaa	960
Gly Arg Thr Ser Phe Pro Glu Asp Thr Val Ile Thr Tyr Lys Cys Glu	
275 280 285	
gaa agc ttt gtg aaa att cct ggc gag aag gac tca gtg atc tgc ctt	1008
Glu Ser Phe Val Lys Ile Pro Gly Glu Lys Asp Ser Val Ile Cys Leu	
290 295 300	
aag ggc agt caa tgg tca gat att gaa gag ttc tgc aat cgt agc tgc	1056
Lys Gly Ser Gln Trp Ser Asp Ile Glu Glu Phe Cys Asn Arg Ser Cys	
305 310 315	
gag gtg cca aca agg cta aat tct gca tcc ctc aaa cag cct tat atc	1104
Glu Val Pro Thr Arg Leu Asn Ser Ala Ser Leu Lys Gln Pro Tyr Ile	
320 325 330	
act cag aat tac ttc cct gtc ggt act gtt gtg gaa tat gag tgc cgt	1152
Thr Gln Asn Tyr Phe Pro Val Gly Thr Val Val Glu Tyr Glu Cys Arg	
335 340 345 350	
cca ggt tac aga aga gaa cct tct cta tca cca aaa cta act tgc ctt	1200
Pro Gly Tyr Arg Arg Glu Pro Ser Leu Ser Pro Lys Leu Thr Cys Leu	
355 360 365	
cag aat tta aaa tgg tcc aca gca gtc gaa ttt tgt aaa aag aaa tca	1248
Gln Asn Leu Lys Trp Ser Thr Ala Val Glu Phe Cys Lys Lys Lys Ser	
370 375 380	
tgc cct aat ccg gga gaa ata cga aat ggt cag att gat gta cca ggt	1296
Cys Pro Asn Pro Gly Glu Ile Arg Asn Gly Gln Ile Asp Val Pro Gly	
385 390 395	
ggc ata tta ttt ggt gca acc atc tcc ttc tca tgt aac aca ggg tac	1344
Gly Ile Leu Phe Gly Ala Thr Ile Ser Phe Ser Cys Asn Thr Gly Tyr	
400 405 410	
aaa tta ttt ggc tcg act tct agt ttt tgt cta ata agc ggc tcg agt	1392
Lys Leu Phe Gly Ser Thr Ser Ser Phe Cys Leu Ile Ser Gly Ser Ser	
415 420 425 430	
gtc cag tgg agt gac ccg ttg cca gag tgc aga gaa att tat tgt cca	1440
Val Gln Trp Ser Asp Pro Leu Pro Glu Cys Arg Glu Ile Tyr Cys Pro	
435 440 445	
gca cca cca caa att gac aat gga ata att caa ggg gaa cgt gac cat	1488
Ala Pro Pro Gln Ile Asp Asn Gly Ile Ile Gln Gly Glu Arg Asp His	
450 455 460	
tat gga tat aga cag tct gta acg tat gca tgt aat aaa gga ttc acc	1536
Tyr Gly Tyr Arg Gln Ser Val Thr Tyr Ala Cys Asn Lys Gly Phe Thr	
465 470 475	
atg att gga gag cac tct att tat tgt act gtg aat aat gat gaa gga	1584
Met Ile Gly Glu His Ser Ile Tyr Cys Thr Val Asn Asn Asp Glu Gly	
480 485 490	
gag tgg agt ggc cca cca cct gaa tgc aga gga aaa tct cta act tcc	1632

-71-

Glu Trp Ser Gly Pro Pro Pro Glu Cys Arg Gly Lys Ser Leu Thr Ser
 495 500 505 510
 aag gtc cca cca aca gtt cag aaa cct acc aca gta aat gtt cca act 1680
 Lys Val Pro Pro Thr Val Gln Lys Pro Thr Thr Val Asn Val Pro Thr
 515 520 525
 aca gaa gtc tca cca act tct cag aaa acc acc aca aaa acc acc aca 1728
 Thr Glu Val Ser Pro Thr Ser Gln Lys Thr Thr Thr Lys Thr Thr Thr
 530 535 540
 cca aat gct caa gca aca cgg agt aca cct gtt tcc agg aca acc aag 1776
 Pro Asn Ala Gln Ala Thr Arg Ser Thr Pro Val Ser Arg Thr Thr Lys
 545 550 555
 cat ttt cat gaa aca acc cca aat aaa gga agt gga acc act tca ggt 1824
 His Phe His Glu Thr Thr Pro Asn Lys Gly Ser Gly Thr Thr Ser Gly
 560 565 570
 act acc cgt tga 1836
 Thr Thr Arg
 575

<210> 75
 <211> 611
 <212> PRT
 <213> Homo Sapiens

<400> 75

Met Glu Pro Pro Gly Arg Arg Glu Cys Pro Phe Pro Ser Trp Arg Phe
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 -15 -10 -5
 Asp Ala Cys Glu Glu Pro Pro Thr Phe Glu Ala Met Glu Leu Ile Gly
 -1 1 5 10
 Lys Pro Lys Pro Tyr Tyr Glu Ile Gly Glu Arg Val Asp Tyr Lys Cys
 15 20 25 30
 Lys Lys Gly Tyr Phe Tyr Ile Pro Pro Leu Ala Thr His Thr Ile Cys
 35 40 45
 Asp Arg Asn His Thr Trp Leu Pro Val Ser Asp Asp Ala Cys Tyr Arg
 50 55 60
 Glu Thr Cys Pro Tyr Ile Arg Asp Pro Leu Asn Gly Gln Ala Val Pro
 65 70 75

-72-

Ala Asn Gly Thr Tyr Glu Phe Gly Tyr Gln Met His Phe Ile Cys Asn
80 85 90

Glu Gly Tyr Tyr Leu Ile Gly Glu Glu Ile Leu Tyr Cys Glu Leu Lys
95 100 105 110

Gly Ser Val Ala Ile Trp Ser Gly Lys Pro Pro Ile Cys Glu Lys Val
115 120 125

Leu Cys Thr Pro Pro Pro Lys Ile Lys Asn Gly Lys His Thr Phe Ser
130 135 140

Glu Val Glu Val Phe Glu Tyr Leu Asp Ala Val Thr Tyr Ser Cys Asp
145 150 155

Pro Ala Pro Gly Pro Asp Pro Phe Ser Leu Ile Gly Glu Ser Thr Ile
160 165 170

Tyr Cys Gly Asp Asn Ser Val Trp Ser Arg Ala Ala Pro Glu Cys Lys
175 180 185 190

Val Val Lys Cys Arg Phe Pro Val Val Glu Asn Gly Lys Gln Ile Ser
195 200 205

Gly Phe Gly Lys Lys Phe Tyr Tyr Lys Ala Thr Val Met Phe Glu Cys
210 215 220

Asp Lys Gly Phe Tyr Leu Asp Gly Ser Asp Thr Ile Val Cys Asp Ser
225 230 235

Asn Ser Thr Trp Asp Pro Pro Val Pro Lys Cys Leu Lys Val Ser Thr
240 245 250

Asp Cys Gly Leu Pro Pro Asp Val Pro Asn Ala Gln Pro Ala Leu Glu
255 260 265 270

Gly Arg Thr Ser Phe Pro Glu Asp Thr Val Ile Thr Tyr Lys Cys Glu
275 280 285

Glu Ser Phe Val Lys Ile Pro Gly Glu Lys Asp Ser Val Ile Cys Leu
290 295 300

Lys Gly Ser Gln Trp Ser Asp Ile Glu Glu Phe Cys Asn Arg Ser Cys

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305		310		315
Glu Val Pro Thr Arg Leu Asn Ser Ala Ser Leu Lys Gln Pro Tyr Ile				
320		325		330
Thr Gln Asn Tyr Phe Pro Val Gly Thr Val Val Glu Tyr Glu Cys Arg				
335		340		345 350
Pro Gly Tyr Arg Arg Glu Pro Ser Leu Ser Pro Lys Leu Thr Cys Leu				
	355		360	365
Gln Asn Leu Lys Trp Ser Thr Ala Val Glu Phe Cys Lys Lys Lys Ser				
	370		375	380
Cys Pro Asn Pro Gly Glu Ile Arg Asn Gly Gln Ile Asp Val Pro Gly				
	385		390	395
Gly Ile Leu Phe Gly Ala Thr Ile Ser Phe Ser Cys Asn Thr Gly Tyr				
400		405		410
Lys Leu Phe Gly Ser Thr Ser Ser Phe Cys Leu Ile Ser Gly Ser Ser				
415		420		425 430
Val Gln Trp Ser Asp Pro Leu Pro Glu Cys Arg Glu Ile Tyr Cys Pro				
	435		440	445
Ala Pro Pro Gln Ile Asp Asn Gly Ile Ile Gln Gly Glu Arg Asp His				
	450		455	460
Tyr Gly Tyr Arg Gln Ser Val Thr Tyr Ala Cys Asn Lys Gly Phe Thr				
	465		470	475
Met Ile Gly Glu His Ser Ile Tyr Cys Thr Val Asn Asn Asp Glu Gly				
480		485		490
Glu Trp Ser Gly Pro Pro Pro Glu Cys Arg Gly Lys Ser Leu Thr Ser				
495		500		505 510
Lys Val Pro Pro Thr Val Gln Lys Pro Thr Thr Val Asn Val Pro Thr				
	515		520	525
Thr Glu Val Ser Pro Thr Ser Gln Lys Thr Thr Thr Lys Thr Thr Thr				
	530		535	540

-75-

<213> Homo sapiens

<400> 79

gtatcccttg agttacgtat tgagctcggt

30

CLAIMS

What is claimed is:

1. A method of producing a recombinant polypeptide comprising:
 - (a) culturing cells which have been transformed or transfected with a vector or segment thereof comprising multiple copies of a transcription unit separated by at least one selective marker gene, wherein the transcription unit encodes a polypeptide, under selective conditions; and
 - (b) expressing said polypeptide from the multiple copies of the transcription unit.
2. A method of producing a recombinant polypeptide from multiple copies of a transcription unit comprising the following steps:
 - (a) transfecting or transforming cells with a vector or segment thereof comprising multiple copies of a transcription unit separated by at least one selective marker gene wherein the transcription unit encodes a polypeptide;
 - (b) culturing cells from step (a) under selective conditions of at least one of the selective marker gene(s) thereby selecting for cells transfected or transformed with the vector or segment thereof;
 - (c) optionally repeating steps (a) and (b) on the cells to sequentially transfect or transform the cells with an additional vector or segment thereof from step (a) wherein each additional vector or segment thereof comprises a different selective marker gene; and
 - (d) expressing the polypeptide from the multiple copies of the transcription unit.
3. The method of Claim 1 or 2 wherein the cells have been transformed or transfected sequentially with an additional vector or segment thereof, and wherein each additional vector or segment thereof comprises a different selective marker gene.
4. The method of Claim 1 or 2 wherein the number of multiple copies of the transcription unit is at least two and not more than eight.

5. The method of Claim 1 or 2 wherein the number of multiple copies of the transcription unit is two.

6. The method of Claim 1 or 2 wherein the expression vector is linearized such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.

7. The method of Claim 1 or 2 wherein the expression vector is linearized with a restriction enzyme, such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.

8. The method of Claim 1 or 2 wherein each transcription unit is under the control of its own promoter and 3' untranslated region.

9. The method of Claim 1 or 2 wherein each transcription unit is under the control of its own promoter and 3' untranslated region, and wherein the promoter is an SV40, HSV, bovine growth hormone, thymidine kinase, MPSV, mouse beta globin, human EF1, MSV-LTR, RSV, MMTV-LTR, CMV, MLV, Chinese hamster elongation factor, or mouse Abelson LTR promoter.

10. The method of Claim 1 or 2 wherein each transcription unit is under the control of its own promoter and 3' untranslated region, and wherein the expression vector further comprises multiple enhancers.

11. The method of Claim 1 or 2 and wherein the transcription unit encodes two different subunits of a multimeric protein.

12. The method of Claim 1 or 2 wherein the transcription unit encodes immunoglobulin light and heavy chain polypeptides.

13. The method of Claim 1 or 2 wherein the transcription unit encodes at least the variable regions of immunoglobulin light and heavy chain polypeptides.

14. The method of Claim 1 or 2 wherein the transcription unit encodes a BPI protein product.

15. The method of Claim 1 or 2 wherein the transcription unit encodes a BPI protein product that is a BPI fragment, BPI analog, BPI variant, or BPI-derived peptide.

16. The method of Claim 1 or 2 wherein the multiple copies of the transcription unit are in a direct repeat orientation.

17. The method of Claim 1 or 2 wherein the multiple copies of the transcription unit are in an inverted repeat orientation.

18. The method of Claim 1 or 2 wherein the transcription unit is a bi-cistronic or poly-cistronic unit.

19. The method of Claim 1 or 2 wherein the transcription unit comprises an internal ribosome entry site.

20. The method of Claim 1 or 2 wherein the multiple copies of the transcription unit are between 25% and 100% identical.

21. The method of Claim 1 or 2 wherein the multiple copies of the transcription unit are 25% identical.

22. The method of Claim 1 or 2 wherein the multiple copies of the transcription unit are at least 70%, at least 80%, or at least 90% identical.

23. The method of Claim 1 or 2 wherein the multiple copies of the transcription unit are 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical.

24. The method of Claim 1 or 2 wherein the multiple copies of the transcription unit are 100% identical.

25. The method of Claim 1 or 2 wherein the selective marker gene is a *gpt*, *res*, *neo*, *his*, DHFR, GS, adenosine deaminase (ADA), thymidine kinase (TK), adenine phosphoribosyl transferase (APRT), zeocin resistance, hygromycin resistance or puromycin resistance gene.

26. The method of Claim 1 or 2, wherein the selective marker gene is an amplifiable selective marker gene.

27. The method of Claim 1 or 2 wherein the expression vector or segment thereof comprising at least two copies of a transcription unit separated by at least one selective marker gene is integrated into the cell chromosome.

28. The method of Claim 1 or 2 wherein the cell is transformed or transfected by at least one and not more than six expression vectors or segments thereof.

29. The method of Claim 1 or 2 wherein the cells are eukaryotic cells.

30. The method of Claim 1 or 2 wherein the cells are plant, insect, mammalian or yeast cells.

31. The method of Claim 1 or 2 wherein the cells are Chinese hamster ovary (CHO) cells or CHO-K1 cells.

32. The method of Claim 1 or 2 wherein the cells are from a DHFR⁺ CHO-K1 cell line, a DHFR⁻ DUKX-B11 (DXB11) cell line or DG-44 cell line.

33. The method of Claim 1 or 2 wherein the cells are in an attached state.

34. The method of Claim 1 or 2 wherein the cells are in a suspension state.

35. The method of Claim 1 or 2 wherein the polypeptide is accumulated in an intracellular compartment.

36. The method of Claim 1 or 2 wherein the polypeptide is secreted from the cells into the culture supernatant.

37. The method of Claim 1 or 2 wherein the polypeptide is isolated.
38. A vector or segment thereof comprising multiple copies of a transcription unit separated by at least one selective marker gene wherein the transcription unit encodes a polypeptide.
39. The vector or segment thereof of Claim 38 wherein the number of multiple copies of the transcription unit is at least two and no more than eight.
40. The vector or segment thereof of Claim 38 wherein the number of multiple copies of the transcription unit is two.
41. The vector or segment thereof of Claim 38 wherein the vector is linearized such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.
42. The vector or segment thereof of Claim 38 wherein the vector is linearized with a restriction enzyme, such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.
43. The vector or segment thereof of Claim 38 wherein each transcription unit is under the control of its own promoter and 3' untranslated region.
44. The vector or segment thereof of Claim 38 wherein each transcription unit is under the control of its own promoter and 3' untranslated region, and wherein the promoter is an SV40, HSV, bovine growth hormone, thymidine kinase, MPSV, mouse beta globin, human EF1, MSV-LTR, RSV, MMTV-LTR, CMV, MLV, Chinese hamster elongation factor, or mouse Abelson LTR promoter.
45. The vector or segment thereof any one of Claims 38 to 44 wherein the vector further comprises multiple enhancers.
46. The vector or segment thereof any one of Claims 38 to 44 wherein the transcription unit encodes two different subunits of a multimeric protein.

47. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit encode immunoglobulin light and heavy chain polypeptides.

48. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit encode at least the variable regions of immunoglobulin light and heavy chain polypeptides.

49. The vector or segment thereof any one of Claims 38 to 44 wherein the transcription unit encodes a BPI protein product.

50. The vector or segment thereof any one of Claims 38 to 44 wherein the transcription unit encodes a BPI fragment, BPI analog, BPI variant, or BPI-derived peptide.

51. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit are in a direct repeat orientation.

52. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit are in an inverted repeat orientation.

53. The vector or segment thereof any one of Claims 38 to 44 wherein the transcription unit is a bi-cistronic or poly-cistronic unit.

54. The vector or segment thereof any one of Claims 38 to 44 wherein the transcription unit comprises an internal ribosome entry site.

55. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit are between 25% and 100% identical.

56. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit are 25% identical.

57. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit are at least 70%, at least 80%, or at least 90% identical.

58. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit are 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical.

59. The vector or segment thereof any one of Claims 38 to 44 wherein the multiple copies of the transcription unit are 100% identical.

60. The vector or segment thereof any one of Claims 38 to 44 wherein the selective marker gene is a *gpt*, *res*, *neo*, *his*, DHFR, GS, adenosine deaminase (ADA), thymidine kinase (TK), adenine phosphoribosyl transferase (APRT), zeocin resistance, hygromycin resistance or puromycin resistance gene.

61. The vector or segment thereof any one of Claims 38 to 44 wherein the transcription unit encodes rBPI₂₁ and is under the control of an hCMV promoter and mouse light chain 3' untranslated region, and wherein the vector further comprises 0, 1 or 2 copies of a human heavy chain enhancer and either a *gpt* or *neo* gene.

62. A cloning vector or segment thereof according to Claim 38.

63. An expression vector or segment thereof according to Claim 38.

64. A host cell comprising an expression vector or segment thereof according to Claim 63.

65. The host cell according to Claim 64 comprising an additional expression vector or segment thereof according to Claim 63, said additional expression vector or segment thereof comprising a different selective marker gene.

66. The host cell according to Claim 64 wherein the host cell is a eukaryotic cell.

67. The host cell according to Claim 64, wherein the host cell is a plant, insect, mammalian or yeast cell.

68. The host cell according to Claim 64 wherein the host cell is a Chinese hamster ovary (CHO) cell or CHO-K1 cell.

69. The host cell according to Claim 64 wherein the expression vector or segment thereof is integrated into the host cell chromosome.

70. A stable cell line comprising an expression vector or segment thereof according to Claim 63.

71. The stable cell line according to Claim 70 wherein the cells are transformed or transfected by at least one and not more than six expression vectors or segments thereof.

72. A DHFR⁺ CHO-K1 cell line, DHFR⁻ DUKX-B11 (DXB11) cell line or DG-44 cell line according to Claim 70 or 71.

73. A method of producing a recombinant polypeptide comprising:

(a) culturing cells which have been transformed or transfected with a vector or segment thereof comprising multiple copies of a transcription unit each separated by a selective marker gene, wherein the transcription unit encodes a polypeptide, under selective conditions; and

(b) expressing said polypeptide from the multiple copies of the transcription unit.

74. The method of Claim 73 or 74 wherein the cells have been transformed or transfected sequentially with an additional vector or segment thereof, and wherein each additional vector or segment thereof comprises a different selective marker gene.

75. The method of Claim 73 or 74 wherein the number of multiple copies of the transcription unit is at least two and not more than eight.

76. The method of Claim 73 or 74 wherein the number of multiple copies of the transcription unit is two.

77. The method of Claim 73 or 74 wherein the expression vector is linearized such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.

78. The method of Claim 73 or 74 wherein the expression vector is linearized with a restriction enzyme, such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.

79. The method of Claim 73 or 74 wherein each transcription unit is under the control of its own promoter and 3' untranslated region.

80. The method of Claim 73 or 74 wherein each transcription unit is under the control of its own promoter and 3' untranslated region, and wherein the promoter is an SV40, HSV, bovine growth hormone, thymidine kinase, MPSV, mouse beta globin, human EF1, MSV-LTR, RSV, MMTV-LTR, CMV, MLV, Chinese hamster elongation factor, or mouse Abelson LTR promoter.

81. The method of Claim 73 or 74 wherein each transcription unit is under the control of its own promoter and 3' untranslated region, and wherein the expression vector further comprises multiple enhancers.

82. The method of Claim 73 or 74 and wherein the transcription unit encodes two different subunits of a multimeric protein.

83. The method of Claim 73 or 74 wherein the transcription unit encodes immunoglobulin light and heavy chain polypeptides.

84. The method of Claim 73 or 74 wherein the transcription unit encodes at least the variable regions of immunoglobulin light and heavy chain polypeptides.

85. The method of Claim 73 or 74 wherein the transcription unit encodes a BPI protein product.

86. The method of Claim 73 or 74 wherein the transcription unit encodes a BPI protein product that is a BPI fragment, BPI analog, BPI variant, or BPI-derived peptide.

87. The method of Claim 73 or 74 wherein the multiple copies of the transcription unit are in a direct repeat orientation.

88. The method of Claim 73 or 74 wherein the multiple copies of the transcription unit are in an inverted repeat orientation.

89. The method of Claim 73 or 74 wherein the transcription unit is a bi-cistronic or poly-cistronic unit.

90. The method of Claim 73 or 74 wherein the transcription unit comprises an internal ribosome entry site.

91. The method of Claim 73 or 74 wherein the multiple copies of the transcription unit are between 25% and 100% identical.

92. The method of Claim 73 or 74 wherein the multiple copies of the transcription unit are 25% identical.

93. The method of Claim 73 or 74 wherein the multiple copies of the transcription unit are at least 70%, at least 80%, or at least 90% identical.

94. The method of Claim 73 or 74 wherein the multiple copies of the transcription unit are 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical.

95. The method of Claim 73 or 74 wherein the multiple copies of the transcription unit are 100% identical.

96. The method of Claim 73 or 74 wherein the selective marker gene is a *gpt*, *res*, *neo*, *his*, DHFR, GS, adenosine deaminase (ADA), thymidine kinase (TK), adenine phosphoribosyl transferase (APRT), zeocin resistance, hygromycin resistance or puromycin resistance gene.

97. The method of Claim 73 or 74, wherein the selective marker gene is an amplifiable selective marker gene.

98. The method of Claim 73 or 74 wherein the expression vector or segment thereof comprising at least two copies of a transcription unit separated by at least one selective marker gene is integrated into the cell chromosome.

99. The method of Claim 73 or 74 wherein the cell is transformed or transfected by at least one and not more than six expression vectors or segments thereof.

100. The method of Claim 73 or 74 wherein the cells are eukaryotic cells.

101. The method of Claim 73 or 74 wherein the cells are plant, insect, mammalian or yeast cells.

102. The method of Claim 73 or 74 wherein the cells are Chinese hamster ovary (CHO) cells or CHO-K1 cells.

103. The method of Claim 73 or 74 wherein the cells are from a DHFR⁺ CHO-K1 cell line, a DHFR⁻ DUKX-B11 (DXB11) cell line or DG-44 cell line.

104. The method of Claim 73 or 74 wherein the cells are in an attached state.

105. The method of Claim 73 or 74 wherein the cells are in a suspension state.

106. The method of Claim 73 or 74 wherein the polypeptide is accumulated in an intracellular compartment.

107. The method of Claim 73 or 74 wherein the polypeptide is secreted from the cells into the culture supernatant.

108. The method of Claim 73 or 74 wherein the polypeptide is isolated.

109. A vector or segment thereof comprising multiple copies of a transcription unit each separated by a selective marker gene wherein the transcription unit encodes a polypeptide.

110. The vector or segment thereof of Claim 109 wherein the number of multiple copies of the transcription unit is at least two and no more than eight.

111. The vector or segment thereof of Claim 109 wherein the number of multiple copies of the transcription unit is two.

112. The vector or segment thereof of Claim 109 wherein the vector is linearized such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.

113. The vector or segment thereof of Claim 109 wherein the vector is linearized with a restriction enzyme, such that the selective marker gene is positioned between at least two transcription unit copies, as [(transcription unit)_x - selective marker gene - (transcription unit)_x], and wherein x is 1-4.

114. The vector or segment thereof of Claim 109 wherein each transcription unit is under the control of its own promoter and 3' untranslated region.

115. The vector or segment thereof of Claim 109 wherein each transcription unit is under the control of its own promoter and 3' untranslated region, and wherein the promoter is an SV40, HSV, bovine growth hormone, thymidine kinase, MPSV, mouse beta globin, human EF1, MSV-LTR, RSV, MMTV-LTR, CMV, MLV, Chinese hamster elongation factor, or mouse Abelson LTR promoter.

116. The vector or segment thereof any one of Claims 109 to 115 wherein the vector further comprises multiple enhancers.

117. The vector or segment thereof any one of Claims 109 to 115 wherein the transcription unit encodes two different subunits of a multimeric protein.

118. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit encode immunoglobulin light and heavy chain polypeptides.

119. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit encode at least the variable regions of immunoglobulin light and heavy chain polypeptides.

120. The vector or segment thereof any one of Claims 109 to 115 wherein the transcription unit encodes a BPI protein product.

121. The vector or segment thereof any one of Claims 109 to 115 wherein the transcription unit encodes a BPI fragment, BPI analog, BPI variant, or BPI-derived peptide.

122. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit are in a direct repeat orientation.

123. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit are in an inverted repeat orientation.

124. The vector or segment thereof any one of Claims 109 to 115 wherein the transcription unit is a bi-cistronic or poly-cistronic unit.

125. The vector or segment thereof any one of Claims 109 to 115 wherein the transcription unit comprises an internal ribosome entry site.

126. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit are between 25% and 100% identical.

127. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit are 25% identical.

128. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit are at least 70%, at least 80%, or at least 90% identical.

129. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit are 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical.

130. The vector or segment thereof any one of Claims 109 to 115 wherein the multiple copies of the transcription unit are 100% identical.

131. The vector or segment thereof any one of Claims 109 to 115 wherein the selective marker gene is a *gpt*, *res*, *neo*, *his*, DHFR, GS, adenosine deaminase (ADA), thymidine kinase (TK), adenine phosphoribosyl transferase (APRT), zeocin resistance, hygromycin resistance or puromycin resistance gene.

132. The vector or segment thereof any one of Claims 109 to 115 wherein the transcription unit encodes rBPI₂₁ and is under the control of an hCMV promoter and mouse light chain 3' untranslated region, and wherein the vector further comprises 0, 73 or 74 or 74 copies of a human heavy chain enhancer and either a *gpt* or *neo* gene.

133. A cloning vector or segment thereof according to Claim 109.

134. An expression vector or segment thereof according to Claim 109.

135. A host cell comprising an expression vector or segment thereof according to Claim 134.

136. The host cell according to Claim 135 comprising an additional expression vector or segment thereof according to Claim 134, said additional expression vector or segment thereof comprising a different selective marker gene.

137. The host cell according to Claim 135 wherein the host cell is a eukaryotic cell.

138. The host cell according to Claim 135, wherein the host cell is a plant, insect, mammalian or yeast cell.

139. The host cell according to Claim 135 wherein the host cell is a Chinese hamster ovary (CHO) cell or CHO-K1 cell.

140. The host cell according to Claim 135 wherein the expression vector or segment thereof is integrated into the host cell chromosome.

141. A stable cell line comprising an expression vector or segment thereof according to Claim 134.

142. The stable cell line according to Claim 141 wherein the cells are transformed or transfected by at least one and not more than six expression vectors or segments thereof.

143. A DHFR⁺ CHO-K1 cell line, DHFR⁻ DUKX-B11 (DXB11) cell line or DG-44 cell line according to Claim 141 or 142.

Figure 1.

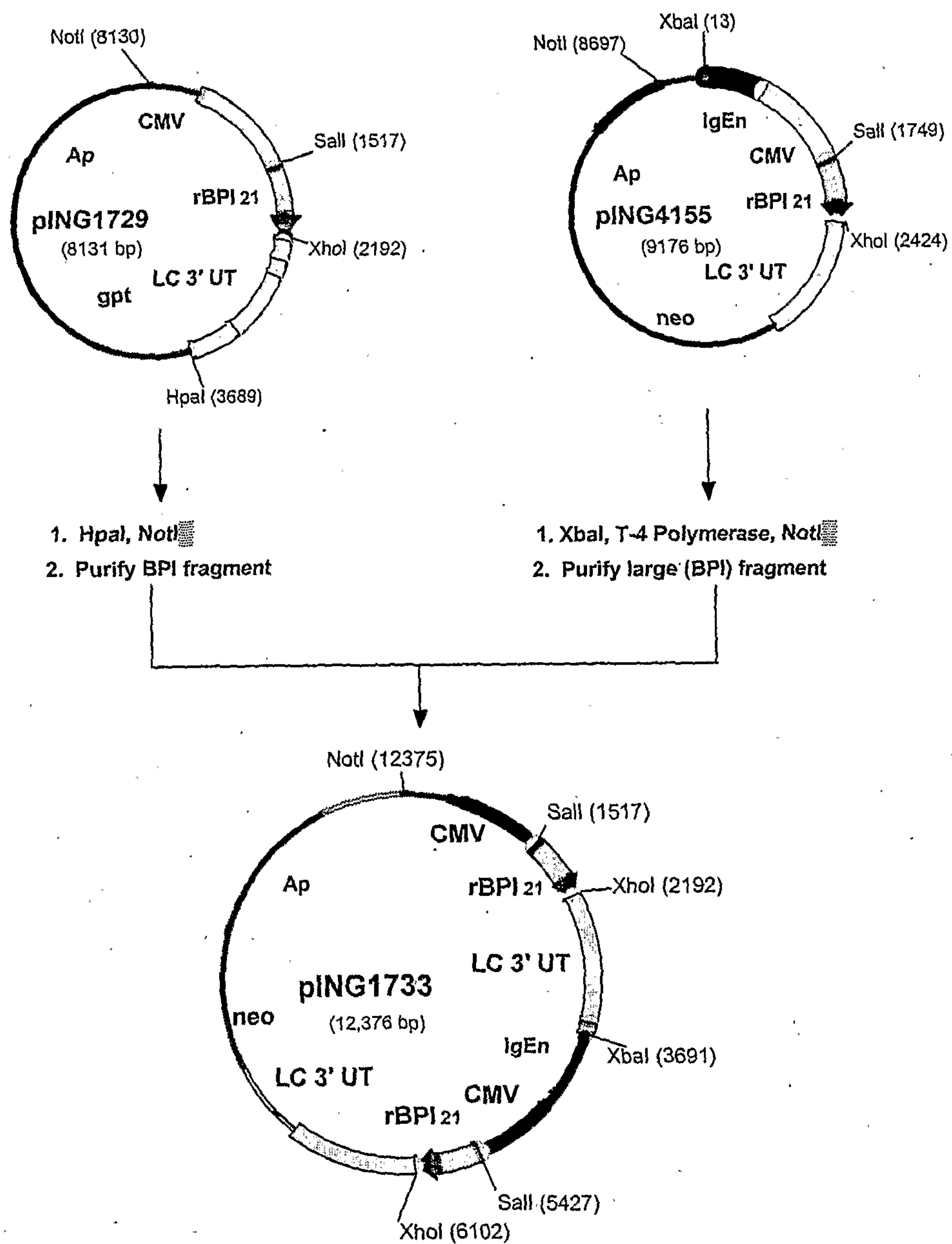


Figure 2.

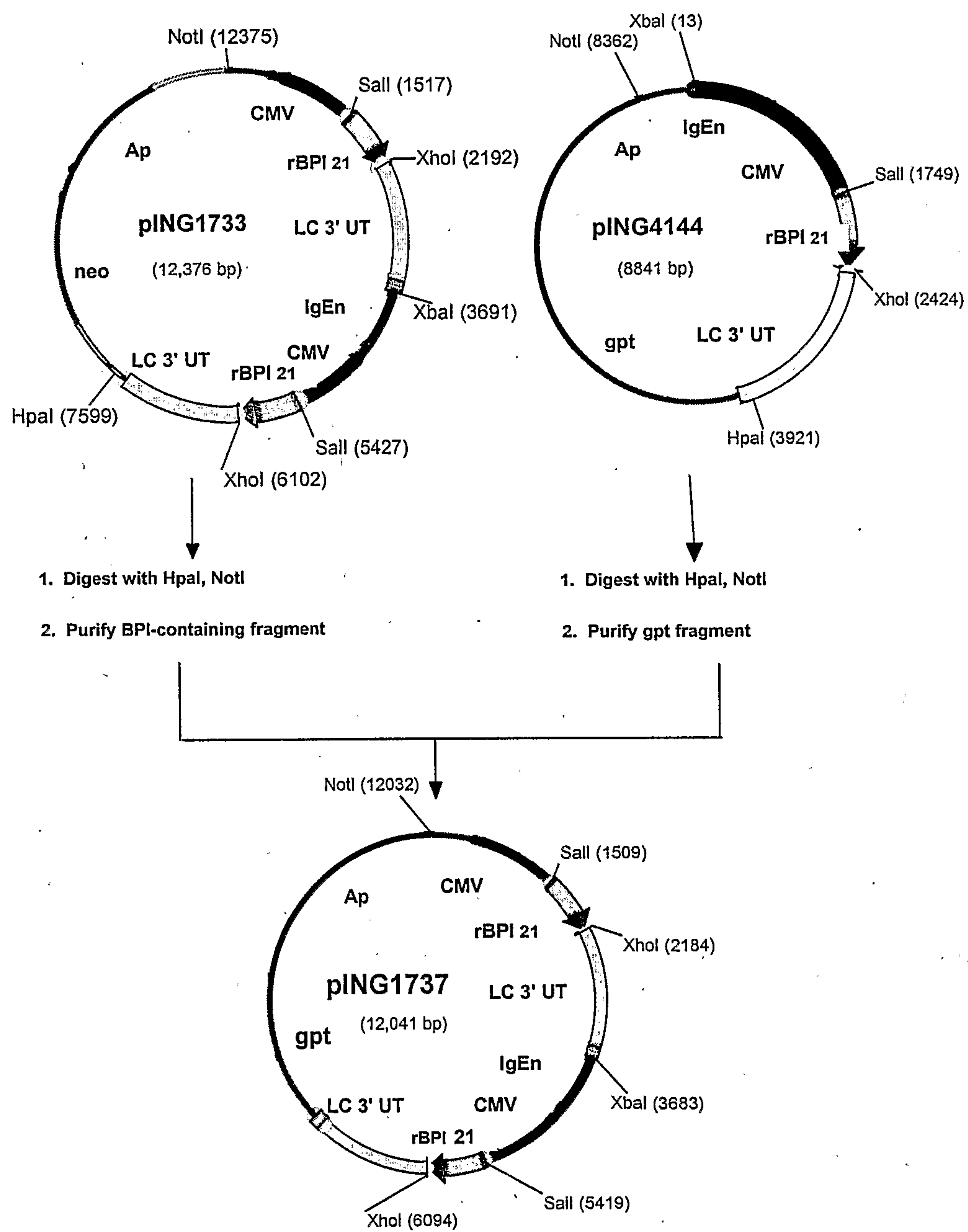
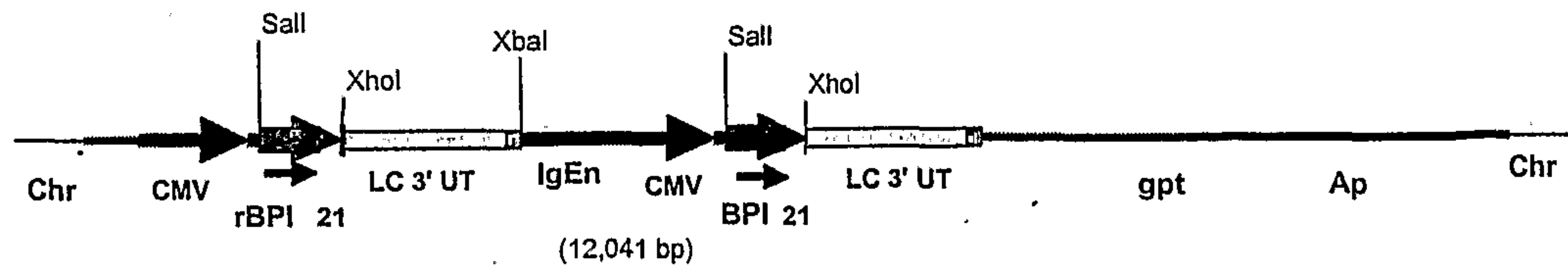


Figure 3.

A.



B.

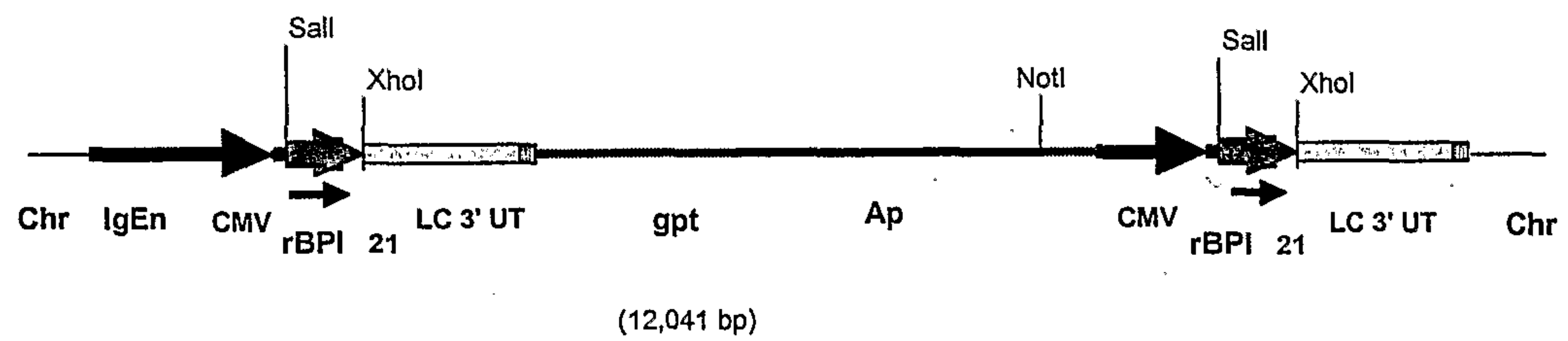


Figure 4.

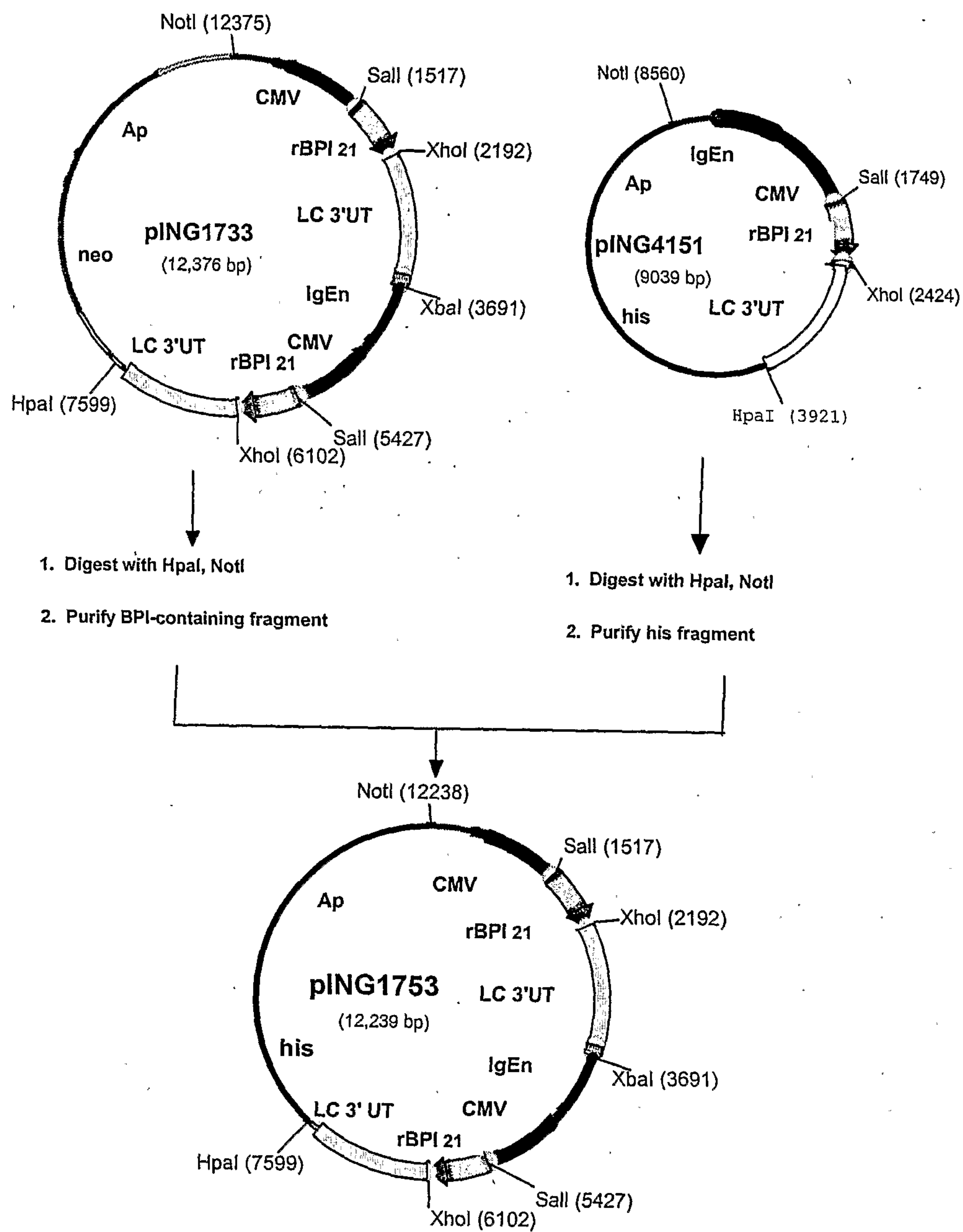


Figure 5.

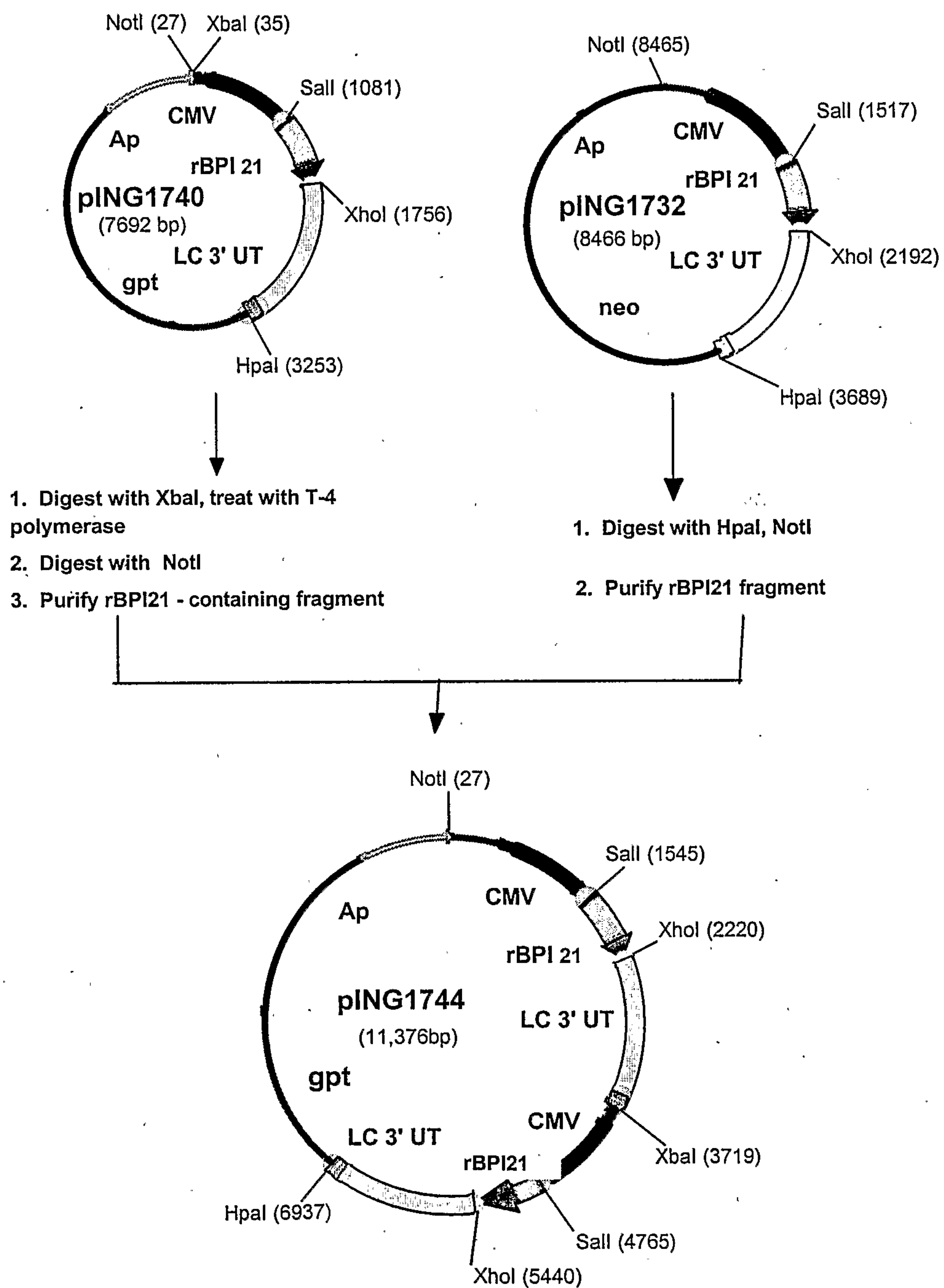


Figure 6.

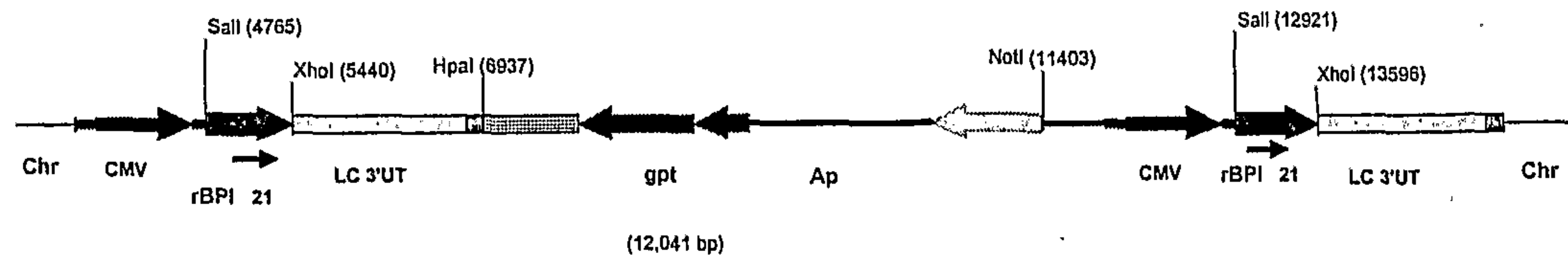


Figure 7.

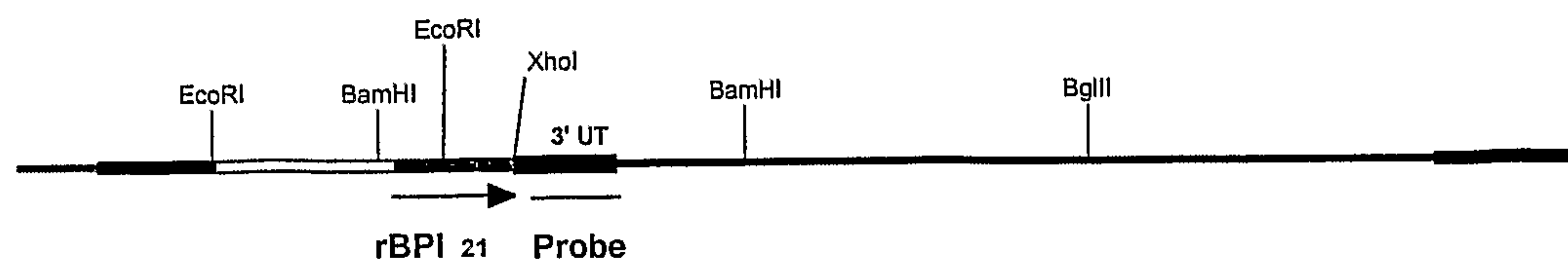


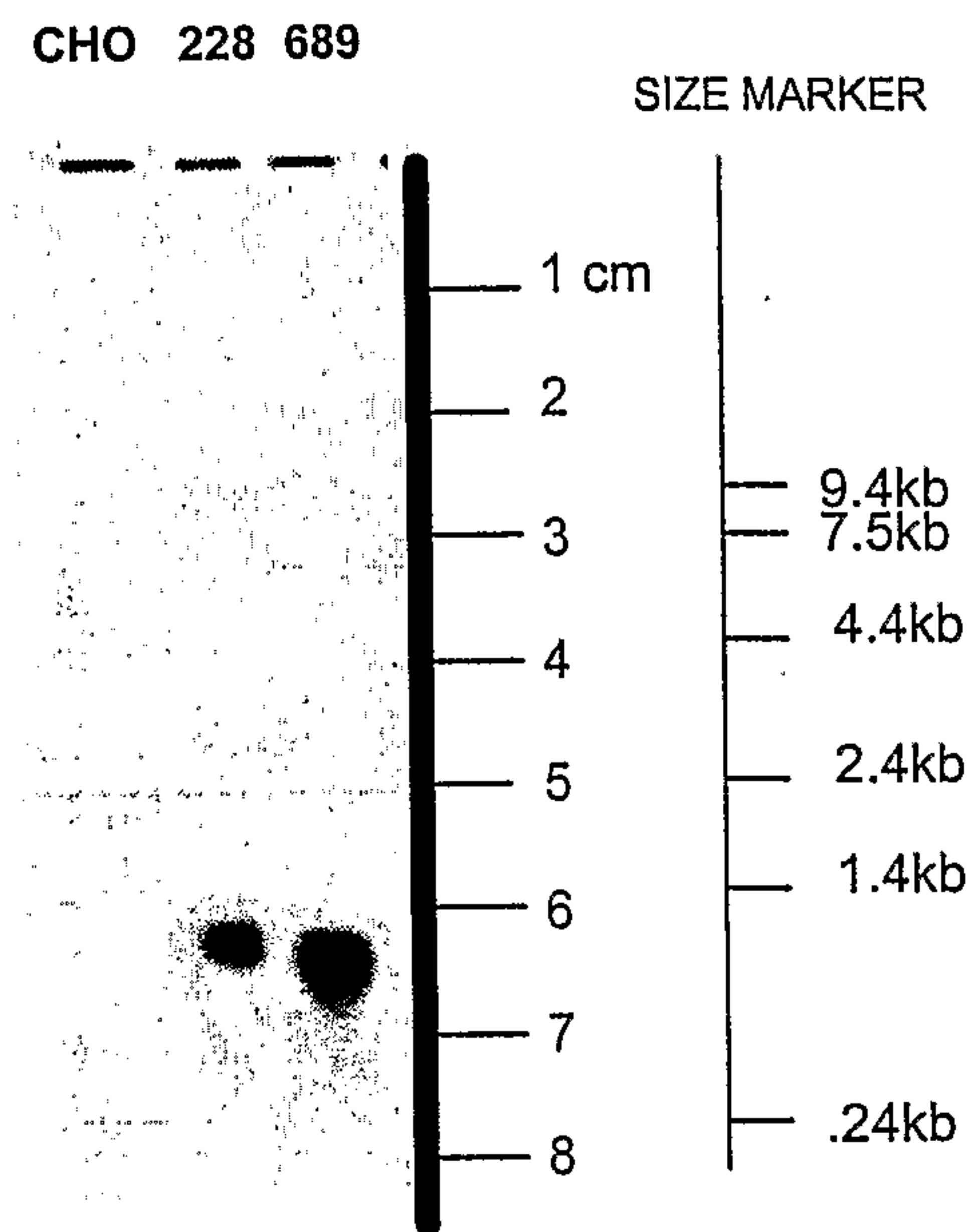
Figure 8.

Figure 9.

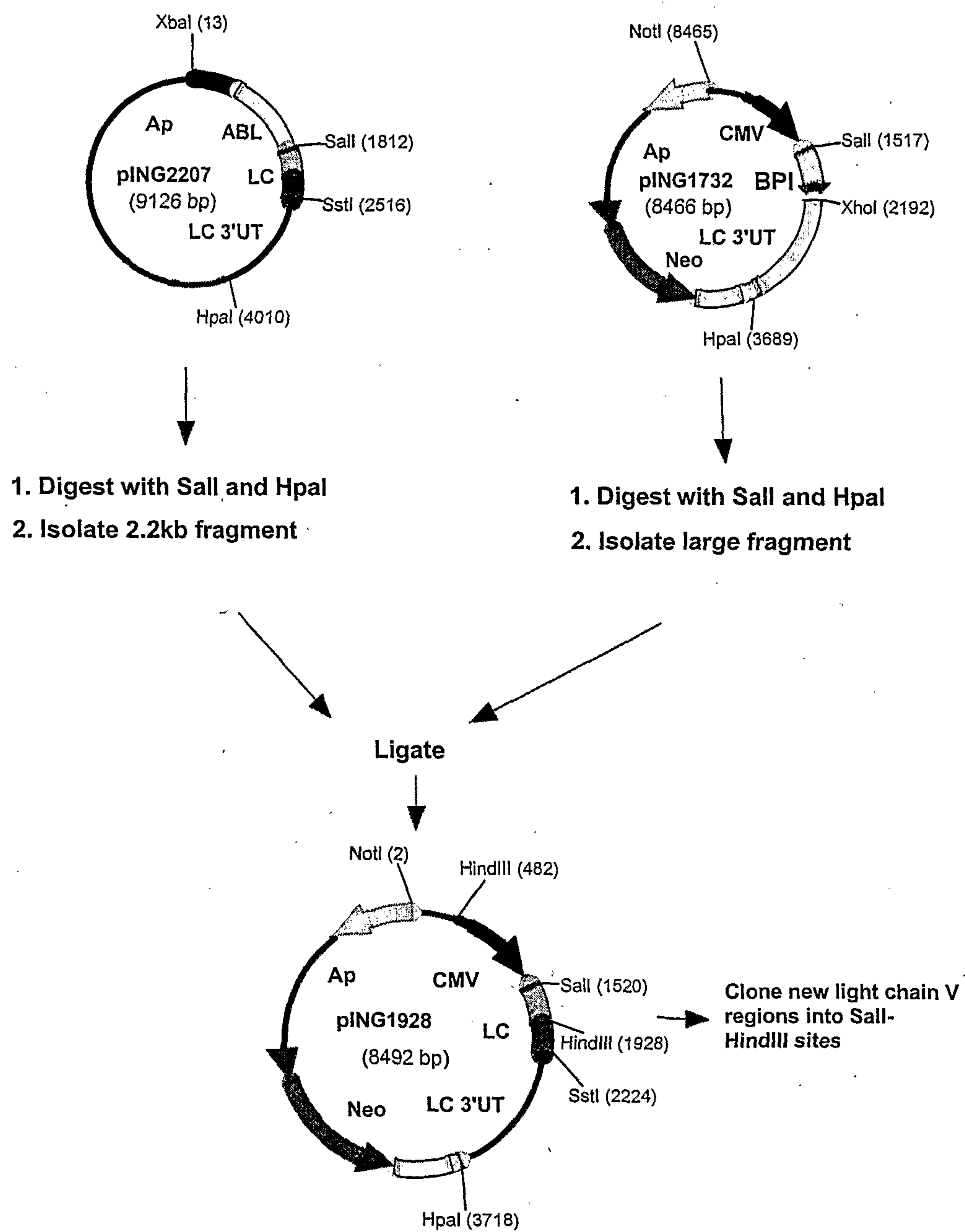


Figure 10.

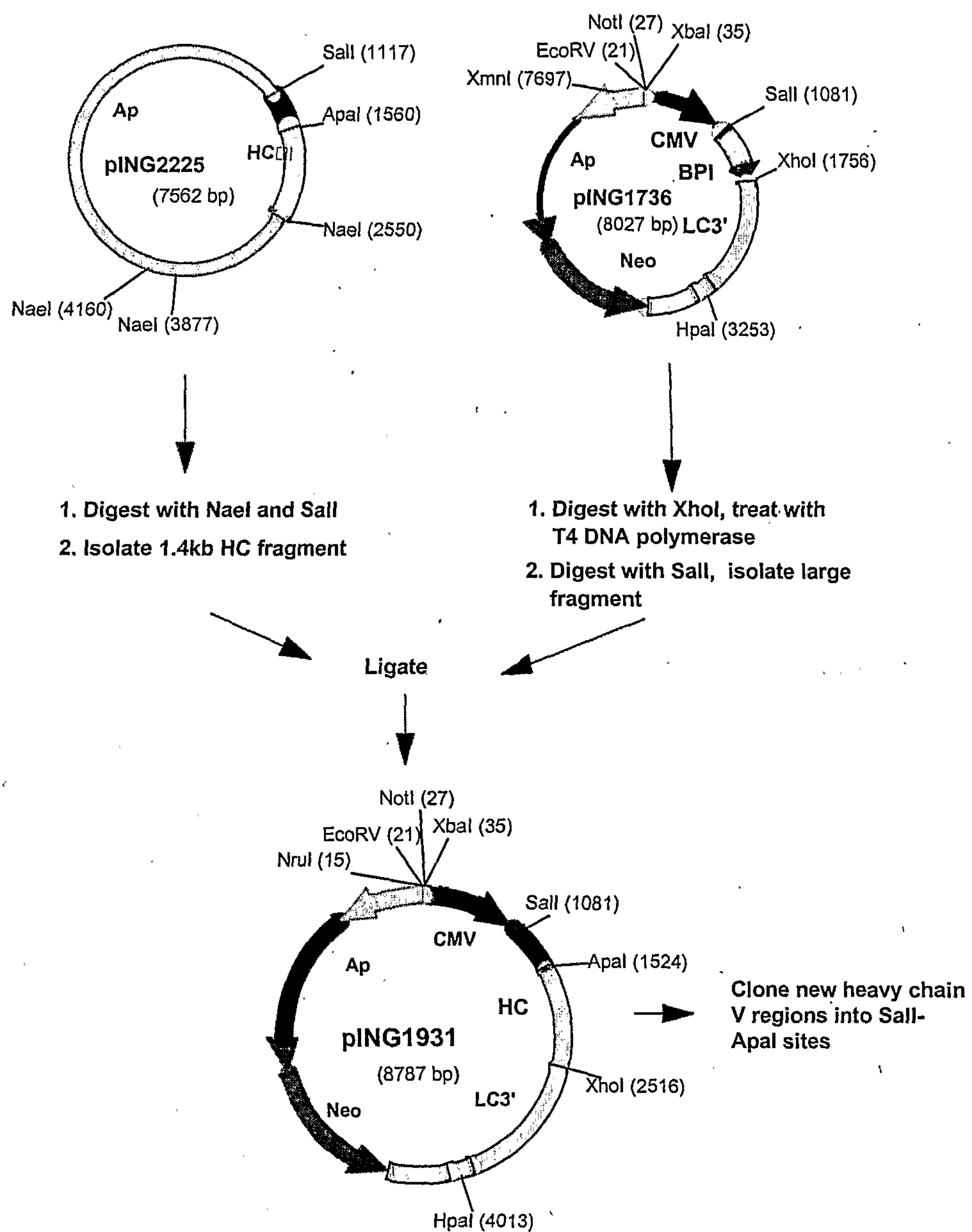


Figure 11.

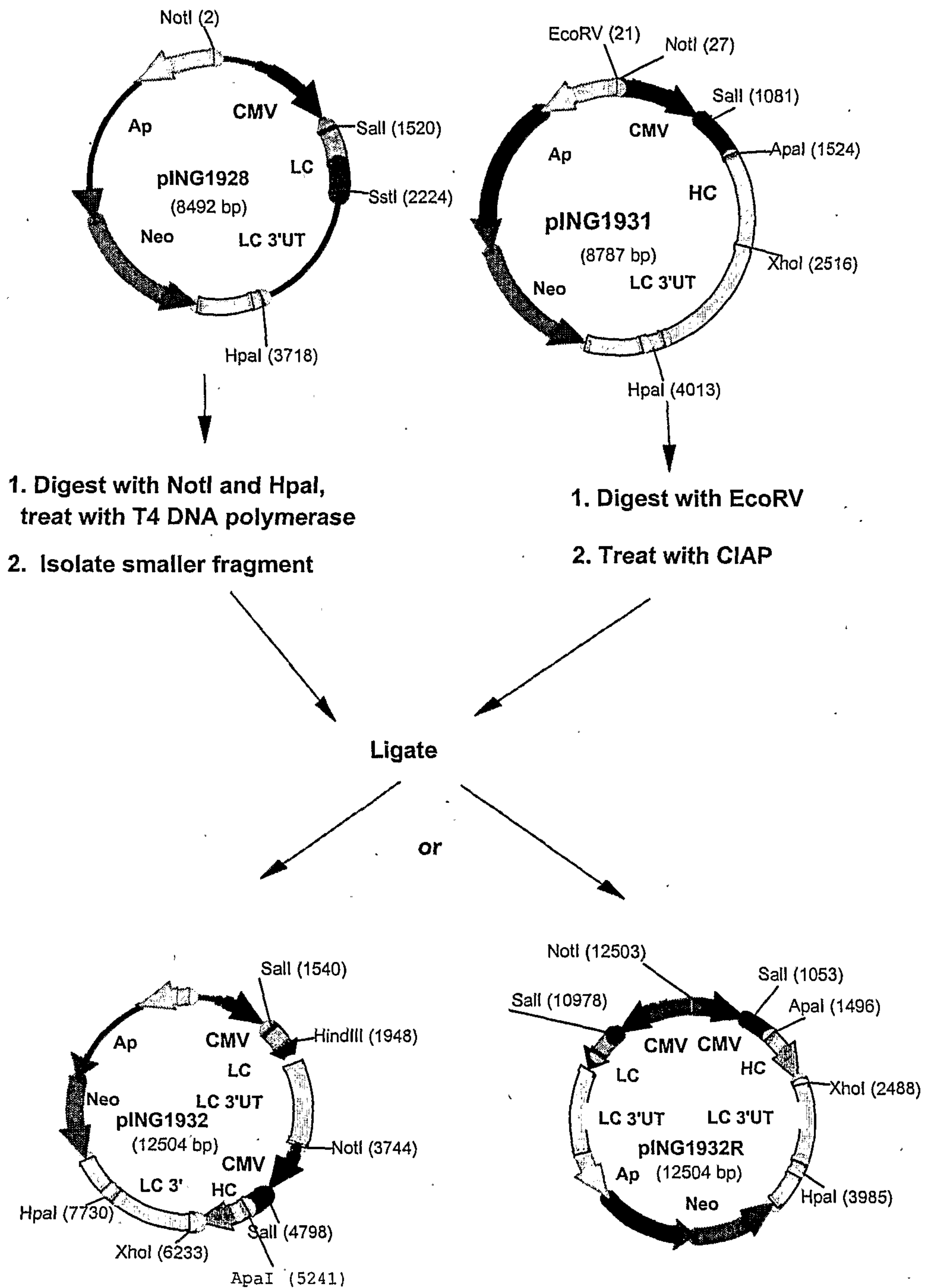


Figure 12.

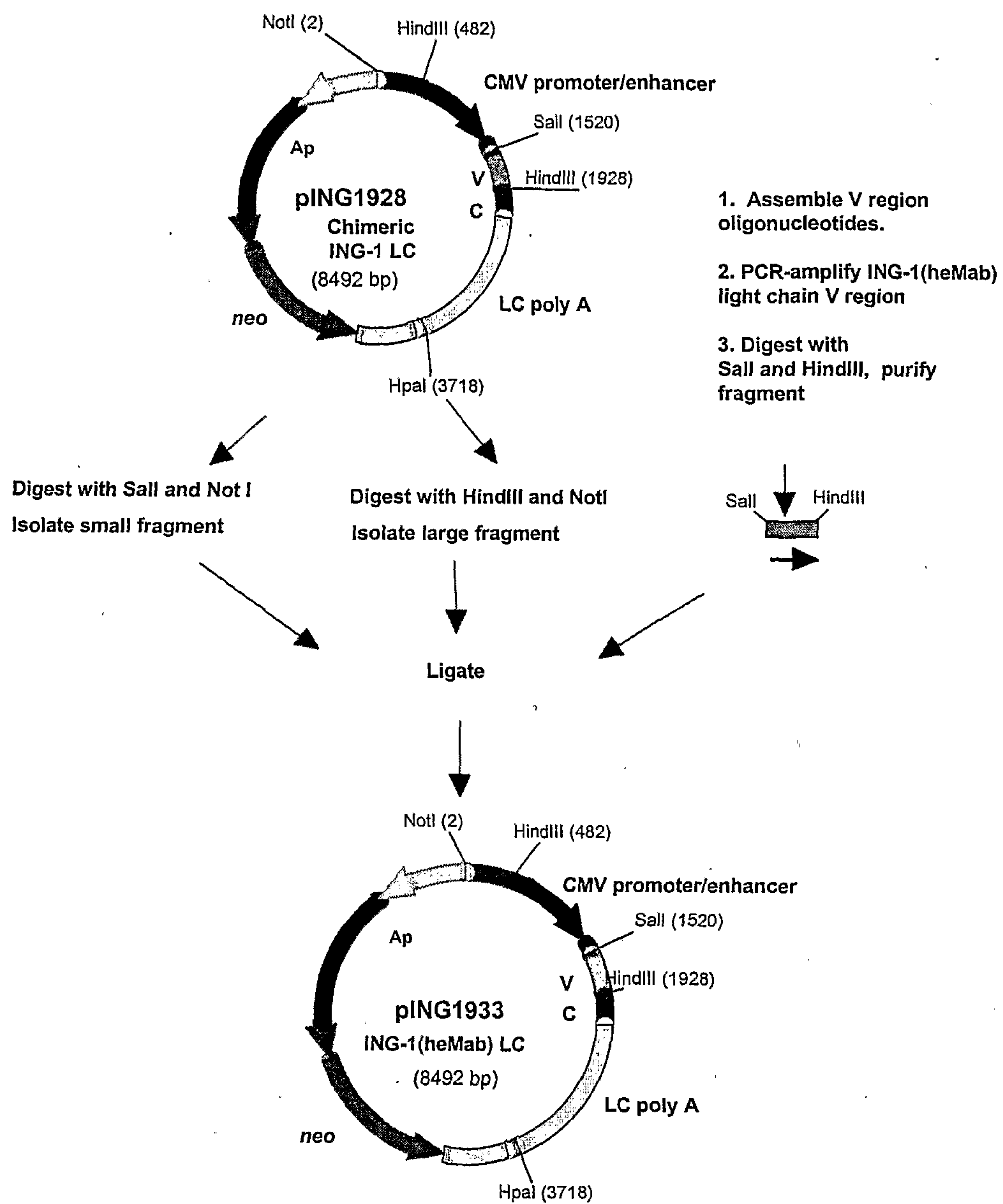


Figure 13.

Variable Region	No. of Changes	Amino Acids 1-59
Risk Line		LHLHLHMLLMMLHLMLLLHLHLHLMHHHHHHHHHHHHHHHMLLMMLMHMHH HHHHHHL
Mouse		DIVMTQAAFSNPVTLGTSGSISCRSSKSLHNSNGITYLYWYLQKPGQSPQLLIYQ MSNL
Human		DIVMTQSPLSLPVTGPGEPAISCRSSQSLLHSLN----- WYLQKPGQSPQLLIYLVSNR
Low Risk	4	DIVMTQ <u>S</u> <u>A</u> <u>L</u> SNPVTLG <u>E</u> SGSISCRSSKSLHNSNGITYLYWYLQKPGQSPQLLIYQ MS <u>N</u> <u>R</u>
Low plus Moderate Risk	8	DIVMTQ <u>S</u> <u>P</u> <u>L</u> <u>S</u> <u>L</u> PVTP <u>G</u> <u>E</u> <u>P</u> GSISCRSSKSLHNSNGITYLYWYLQKPGQSPQLLIYQ MS <u>N</u> <u>R</u>
Variable Region	No. of Changes	Amino Acids 60-112
Risk Line		MMLHMLMHLHLHLHLLHLHLHLLHLLHLLHHHHHHHHHHHHHHHHHLLHHLHLLL
Mouse		ASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELPRTFGGGTKLEMK
Human		ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQAXQXPXTFGQGGTKVEIK
Low Risk	2	ASGVPDRFSSSGSGTDFTL <u>K</u> ISRVEAEDVGVYYCAQNLELPRTFG <u>Q</u> GTKLEMK
Low plus Moderate Risk	2	ASGVPDRFSSSGSGTDFTL <u>K</u> ISRVEAEDVGVYYCAQNLELPRTFG <u>Q</u> GTKLEMK

Figure 14.

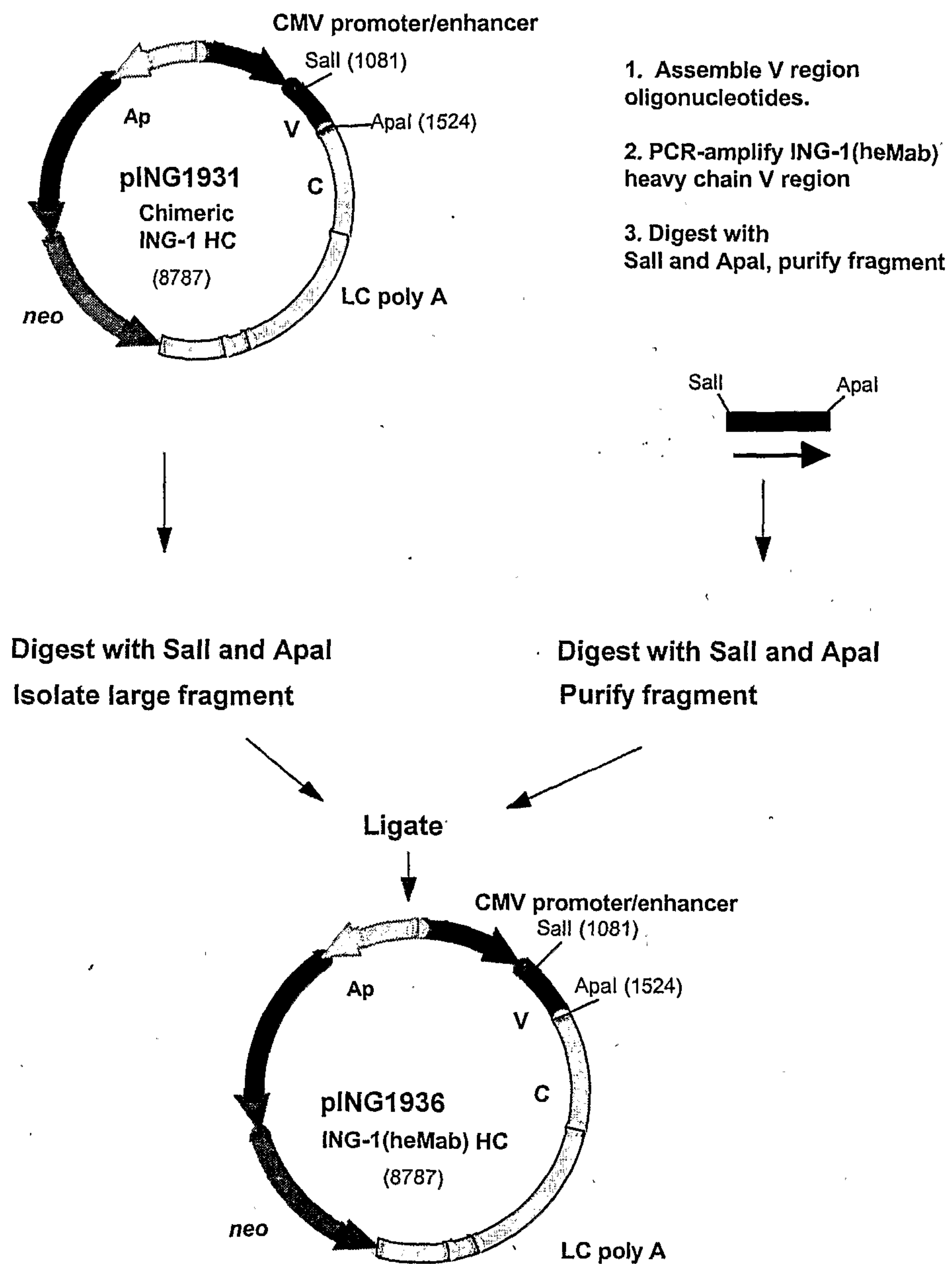


Figure 15.

Variable Region	No. of Changes	Amino Acids 1-59
Risk Line		MHLHLHLHMLLMLMLLLHLHLHLHMHHHHHHHHHHHHHMLLMLMHMHHHHH HHHHHHHH
Mouse		QIQLVQSGPELKKPGETVKISCKASGYTFTKYGMNWVKQAPGKGLKWMGWIN TYTEPT
Human		QVQLVQSGAEVKKPGXSVKVSCASGYTFXSYXIXWVRQAPGQGLEWMGXIX PXGXT-X
Low Risk	3	QIQLVQSGPE <u>V</u> KKPGE <u>S</u> VKISCKASGYTFTKYGMNWVKQAPG <u>Q</u> GLKWMGWIN TYTEPT
Low plus Moderate Risk	6	QIQLVQSGA <u>E</u> <u>V</u> KKPGE <u>S</u> VKISCKASGYTFTKYGMNWV <u>R</u> QAPG <u>Q</u> GLE <u>W</u> MGWIN TYTEPT
Variable Region	No. of Changes	Amino Acids 60-116
Risk Line		MHMMHMLMHLHLHMLLMLHLHLHLLHLLHLMHHHHHHHHHHHHHHLHHL HLHLL
Mouse		YGDDFKGRFAFSLETSASTANLQINNPKSEDATYFCARFGSAVDYWGQGTSV TVSS
Human		YAQKFQGRVTITXDXSTSTAYMELSSLRSEDATVYYCARXXXXXXXXXWGQGTLV TVSS
Low Risk	10	YGDDFKGRF <u>I</u> <u>F</u> <u>I</u> <u>L</u> <u>D</u> <u>T</u> <u>S</u> <u>I</u> <u>S</u> <u>T</u> <u>A</u> <u>L</u> <u>E</u> <u>I</u> <u>S</u> <u>S</u> <u>L</u> <u>R</u> <u>S</u> <u>E</u> <u>D</u> <u>A</u> <u>T</u> <u>Y</u> <u>F</u> <u>C</u> <u>A</u> <u>R</u> <u>F</u> <u>G</u> <u>S</u> <u>A</u> <u>V</u> <u>D</u> <u>Y</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>L</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u>
Low plus Moderate Risk	14	YG <u>Q</u> <u>K</u> <u>F</u> <u>Q</u> GRF <u>I</u> <u>F</u> <u>I</u> <u>L</u> <u>D</u> <u>T</u> <u>S</u> <u>I</u> <u>S</u> <u>T</u> <u>A</u> <u>L</u> <u>E</u> <u>I</u> <u>S</u> <u>S</u> <u>L</u> <u>R</u> <u>S</u> <u>E</u> <u>D</u> <u>A</u> <u>V</u> <u>Y</u> <u>F</u> <u>C</u> <u>A</u> <u>R</u> <u>F</u> <u>G</u> <u>S</u> <u>A</u> <u>V</u> <u>D</u> <u>Y</u> <u>W</u> <u>G</u> <u>Q</u> <u>G</u> <u>T</u> <u>L</u> <u>V</u> <u>T</u> <u>V</u> <u>S</u> <u>S</u>

Figure 16.

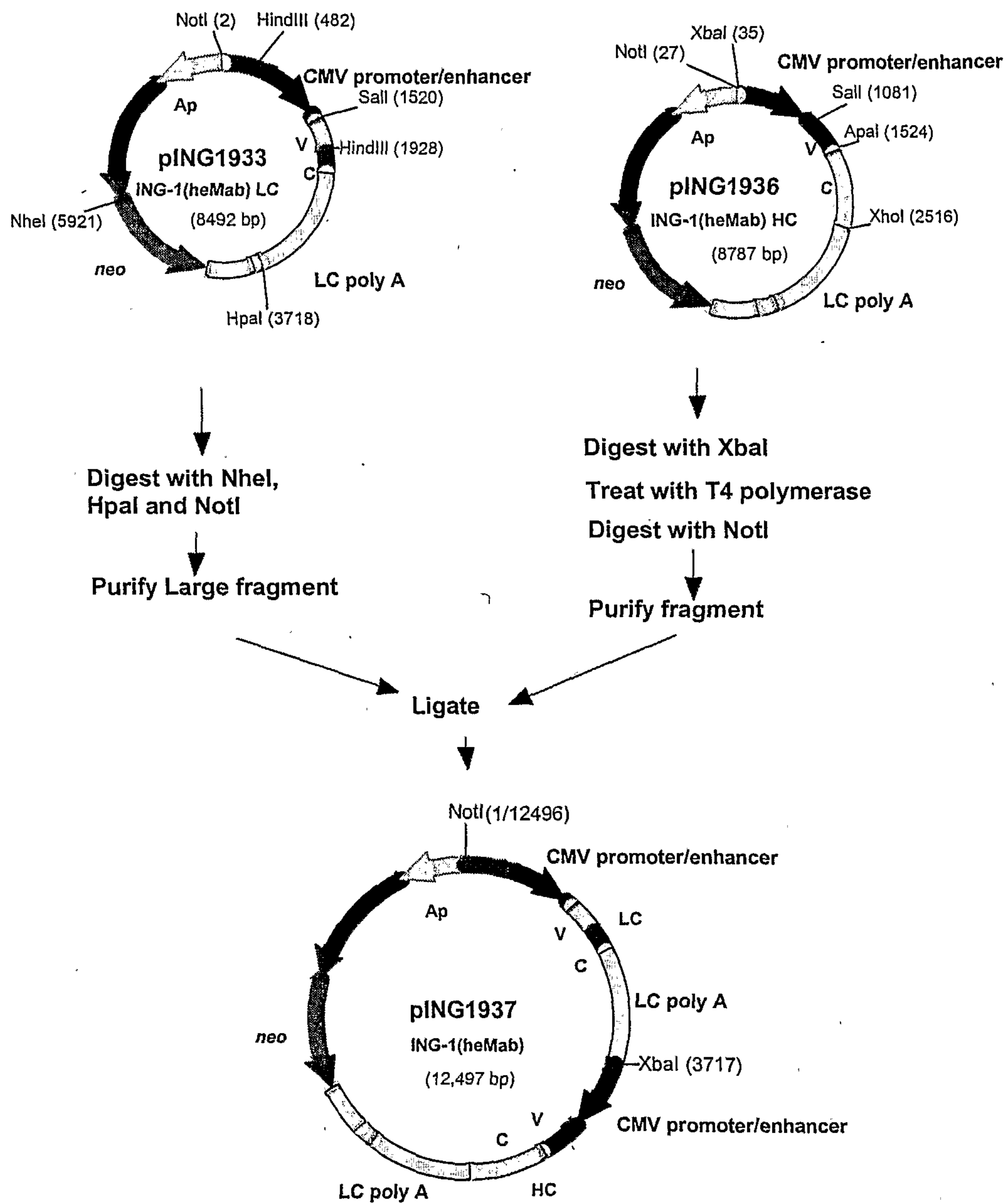


Figure 17.

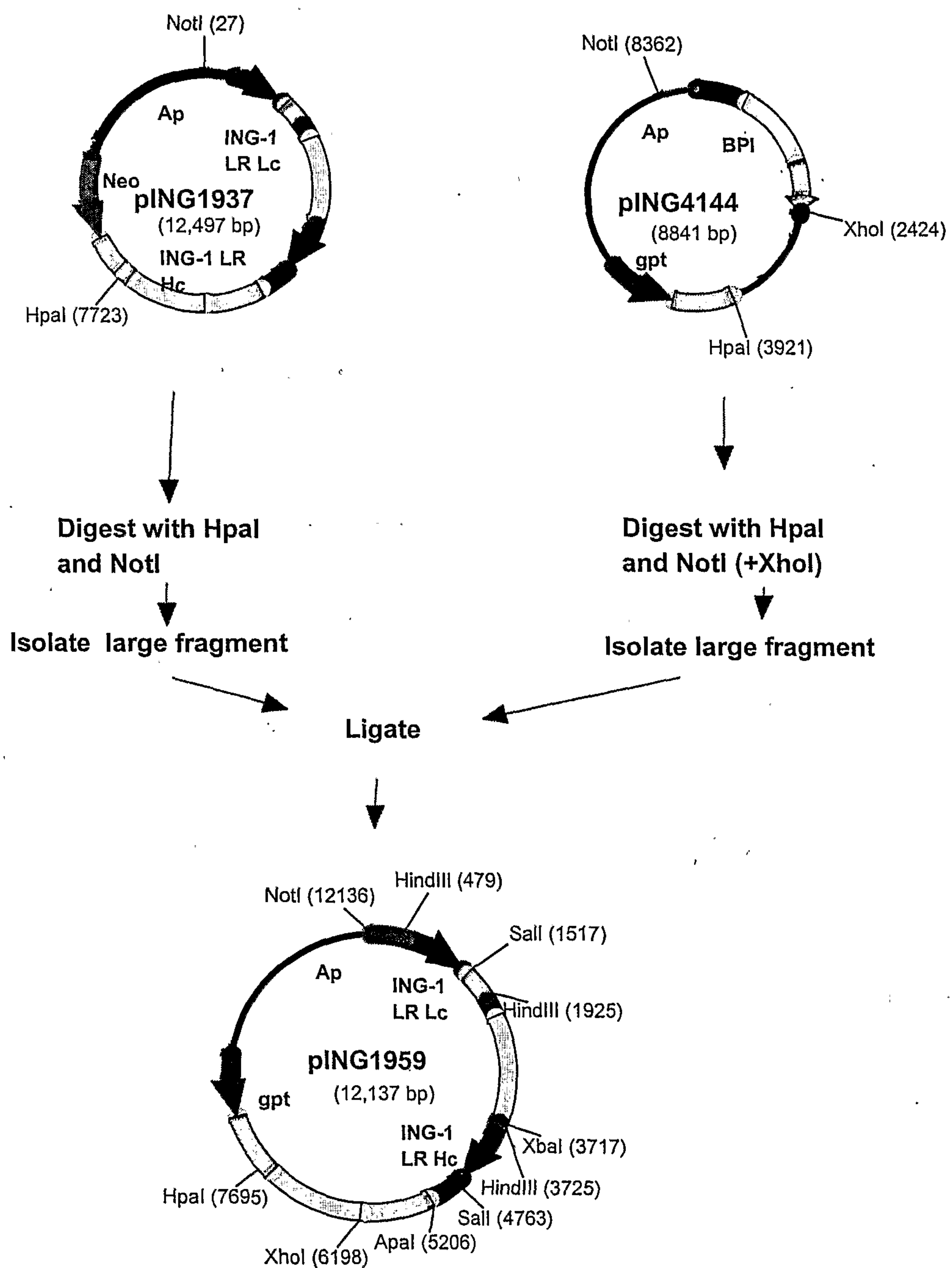


Figure 18.

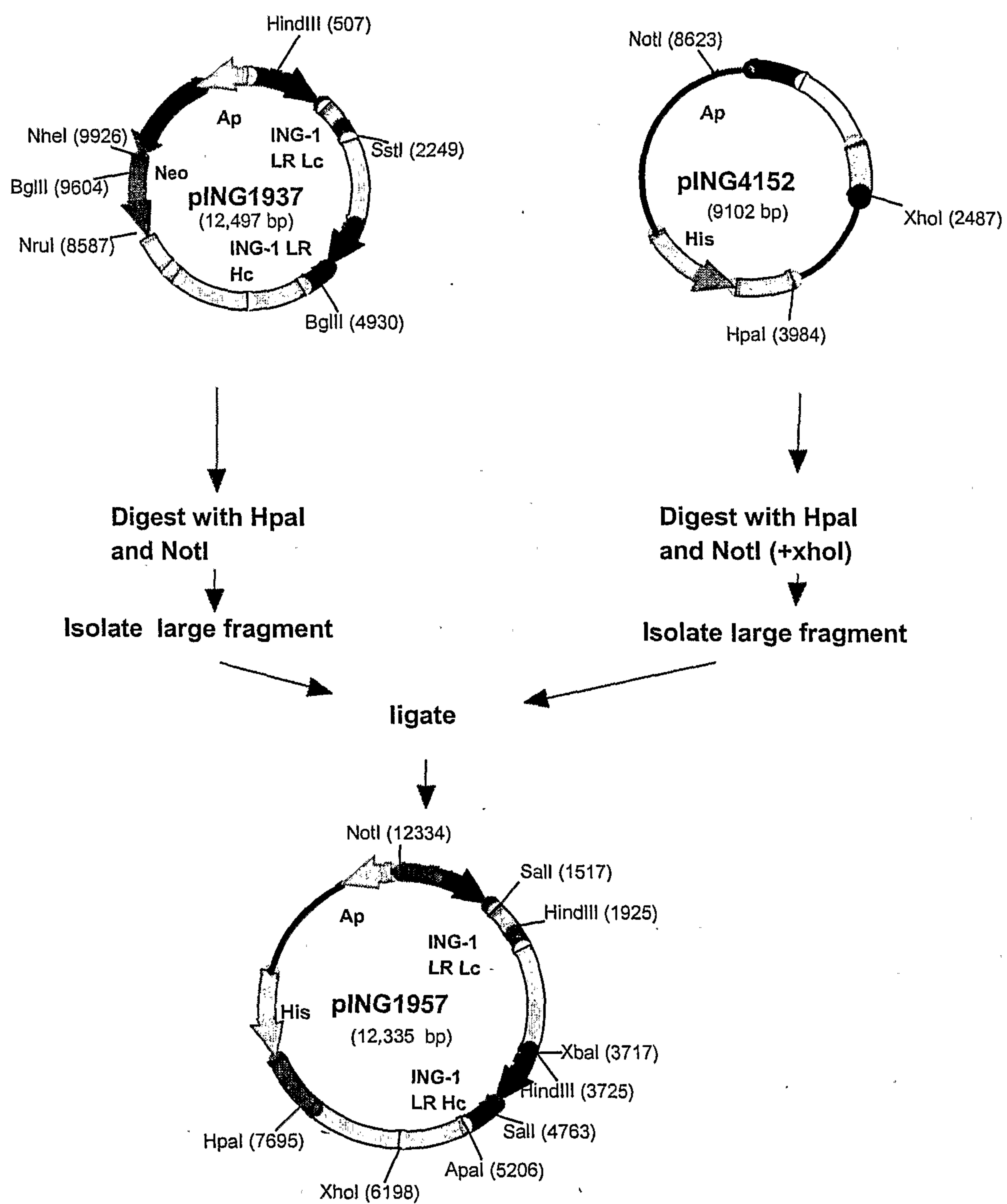


Figure 19.

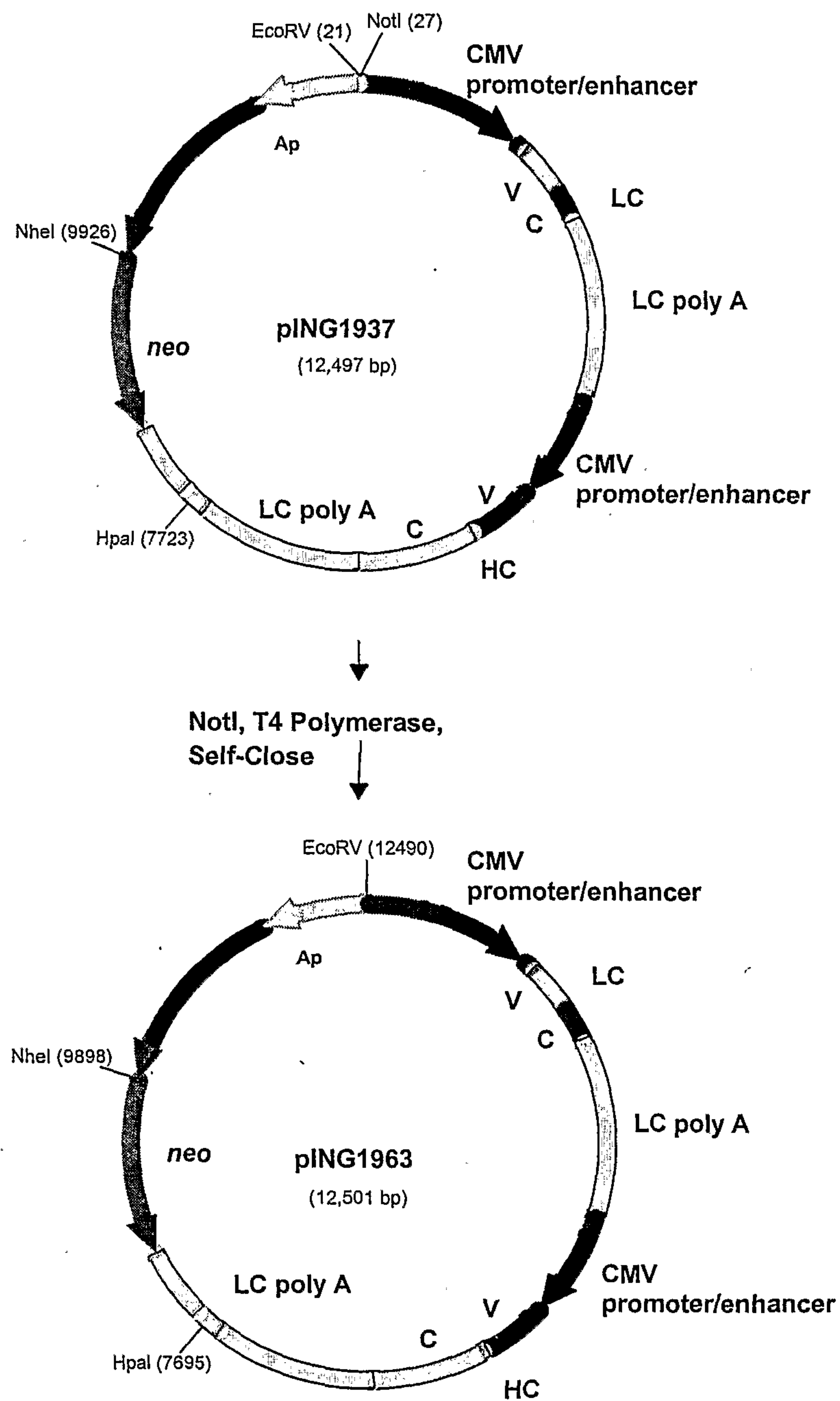


Figure 20.

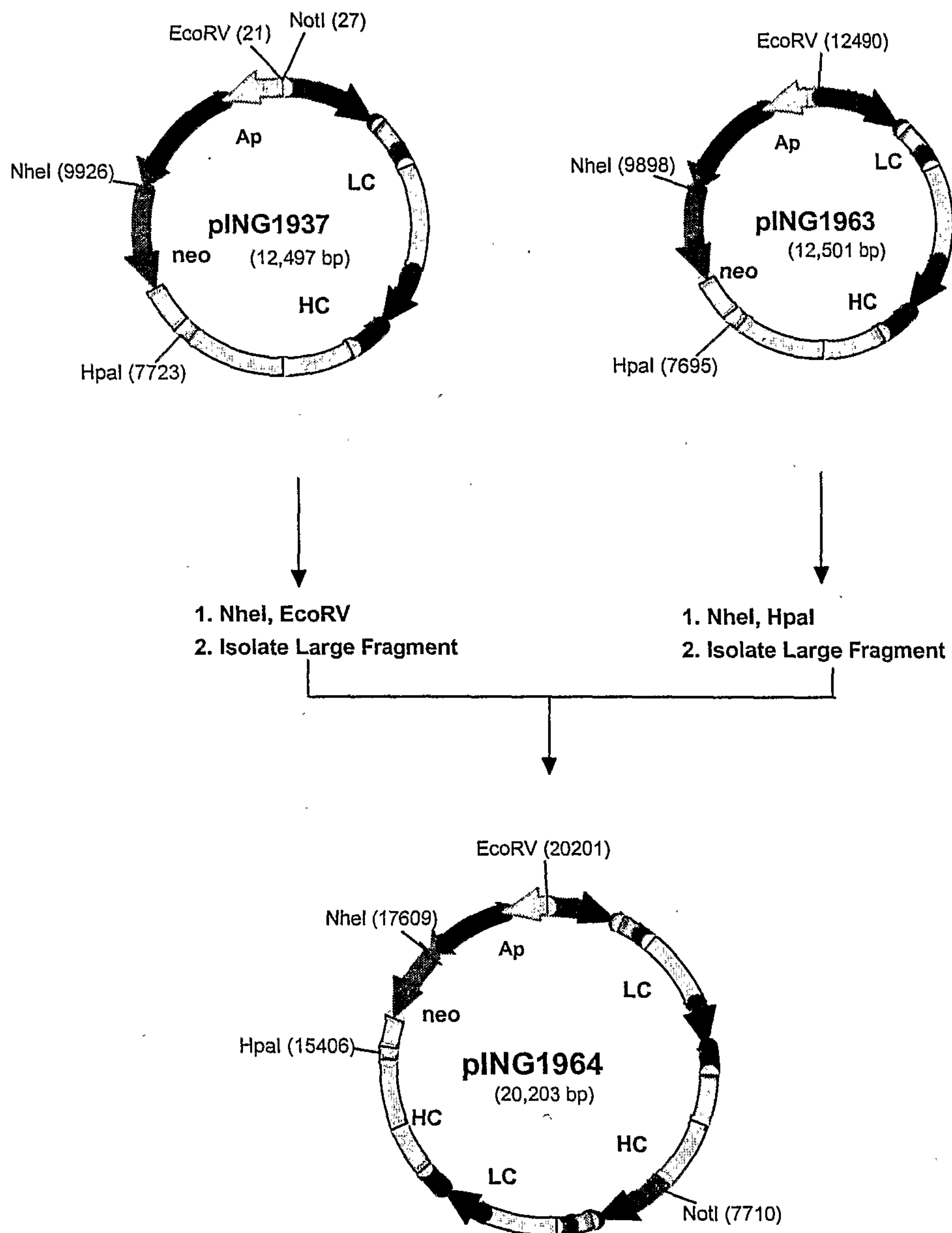


Figure 21.

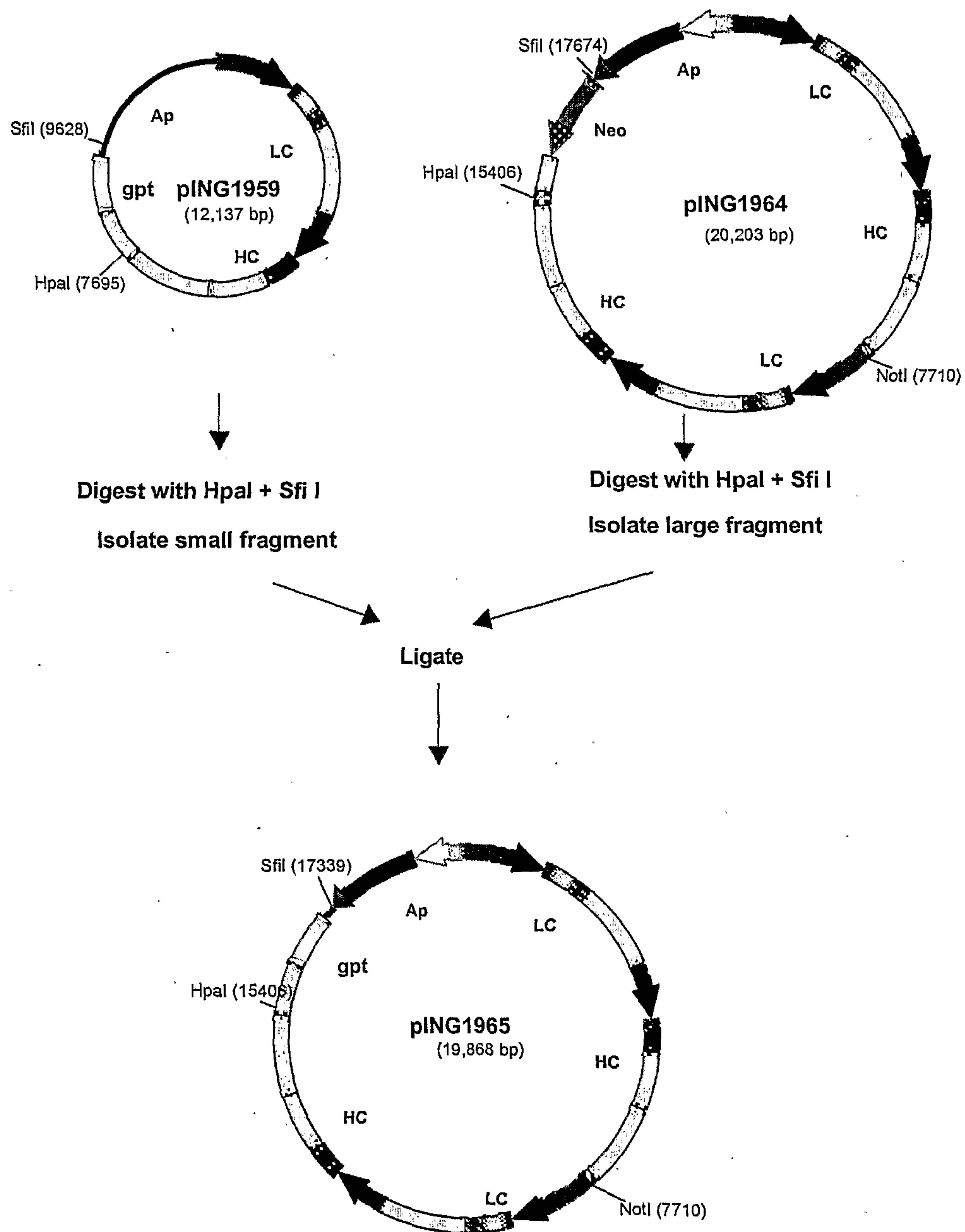


Figure 22.

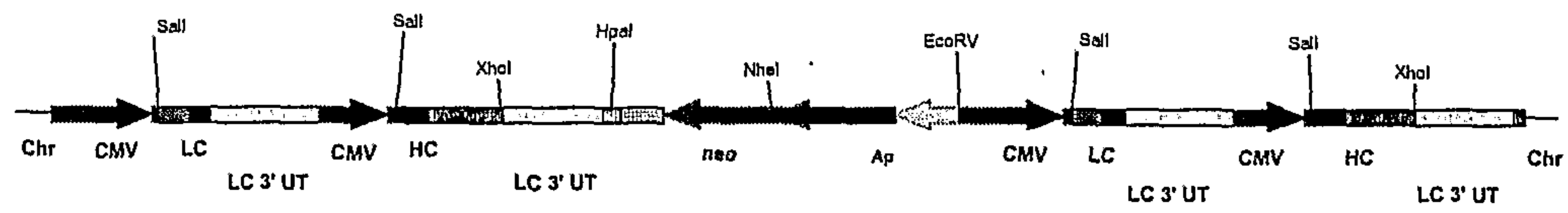


Figure 23.

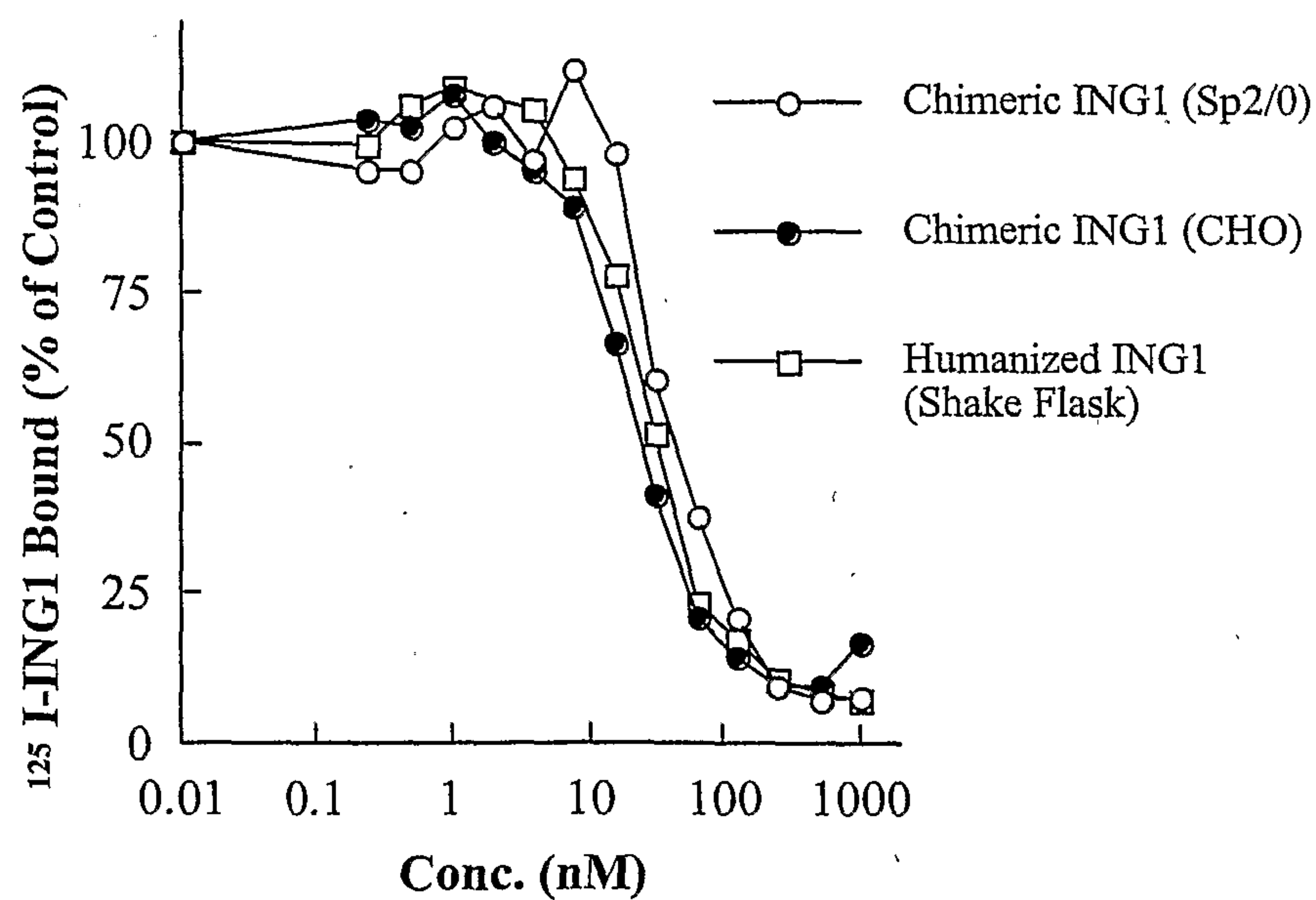
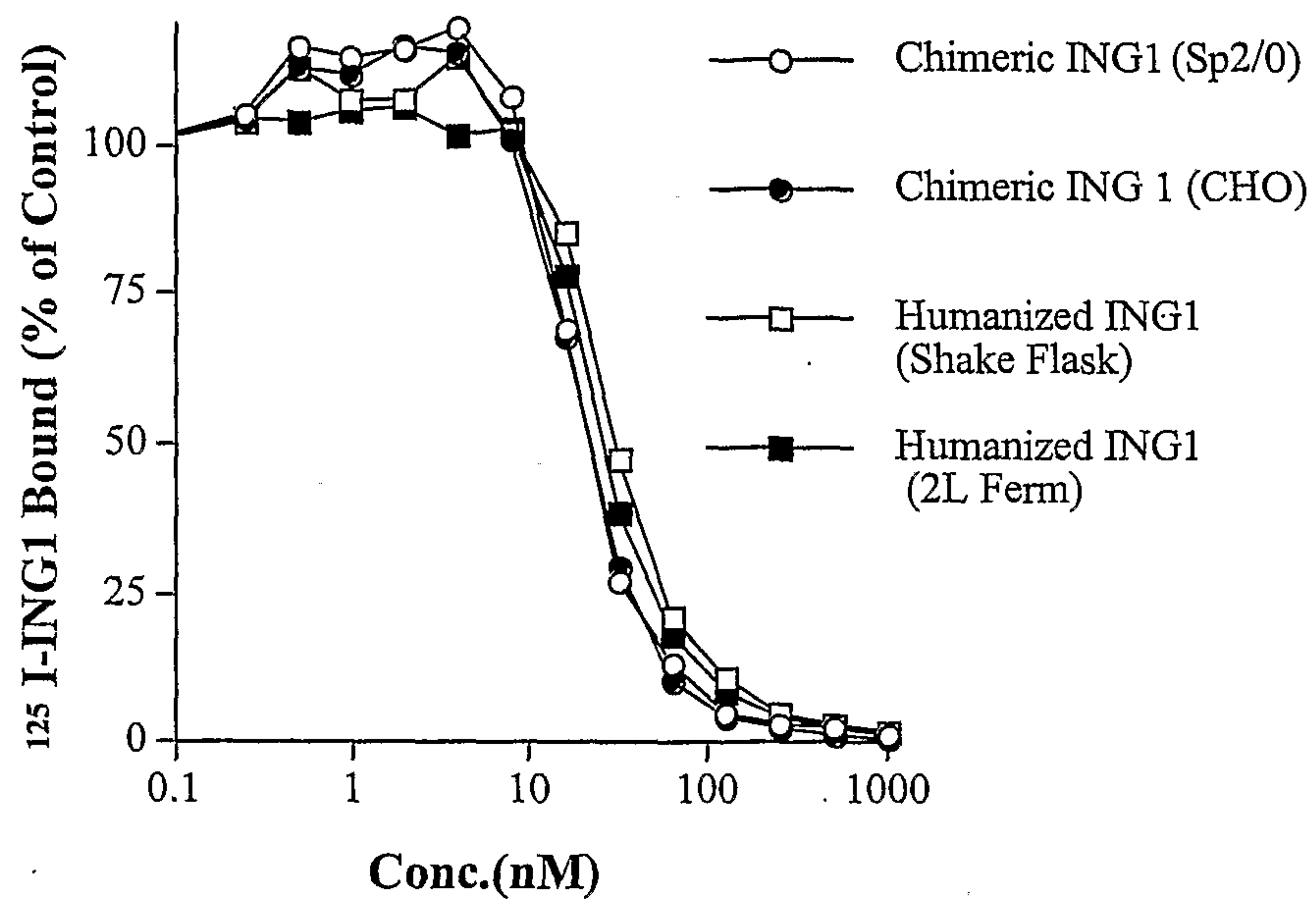


Figure 24.

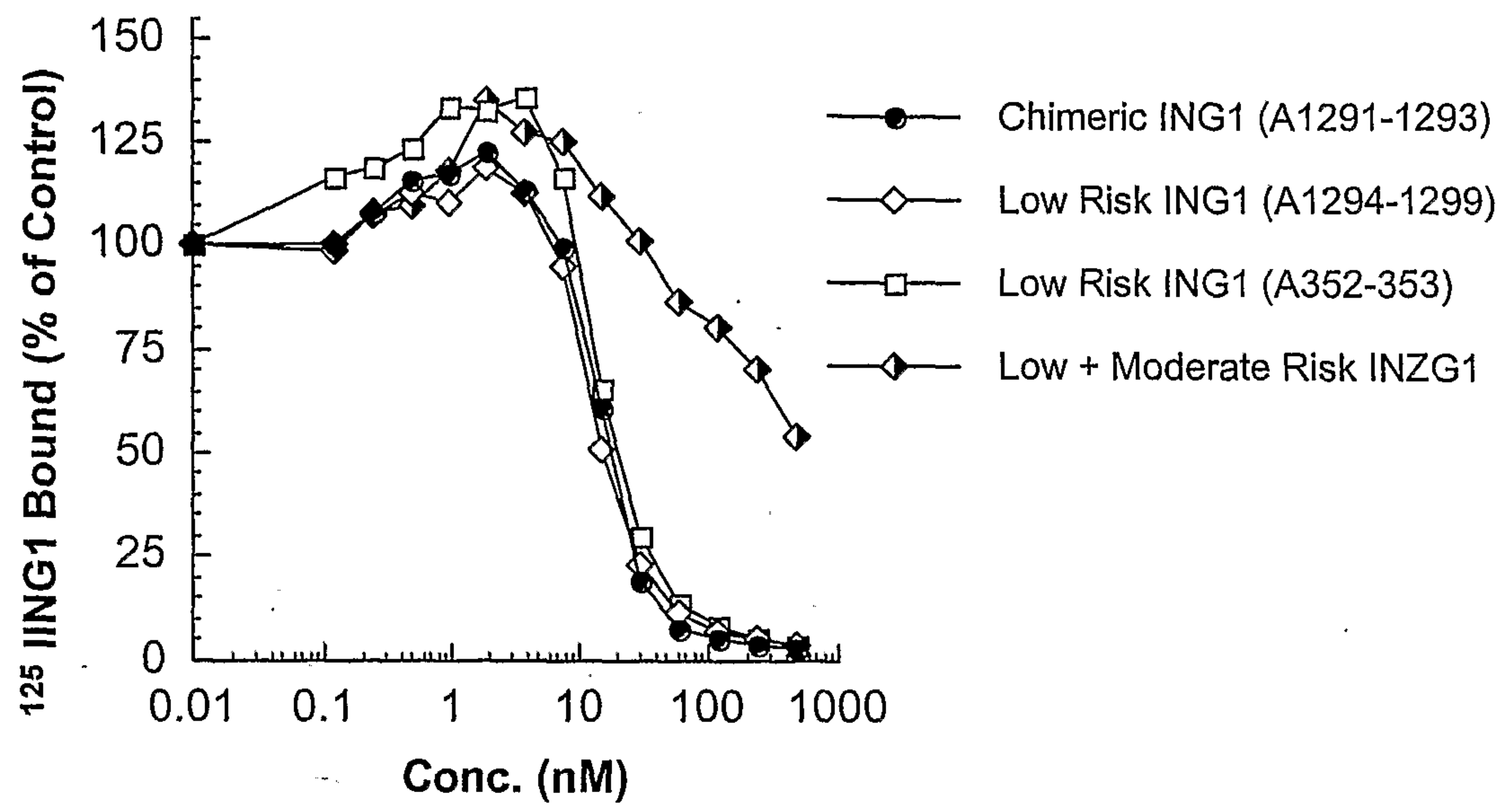


Figure 25.

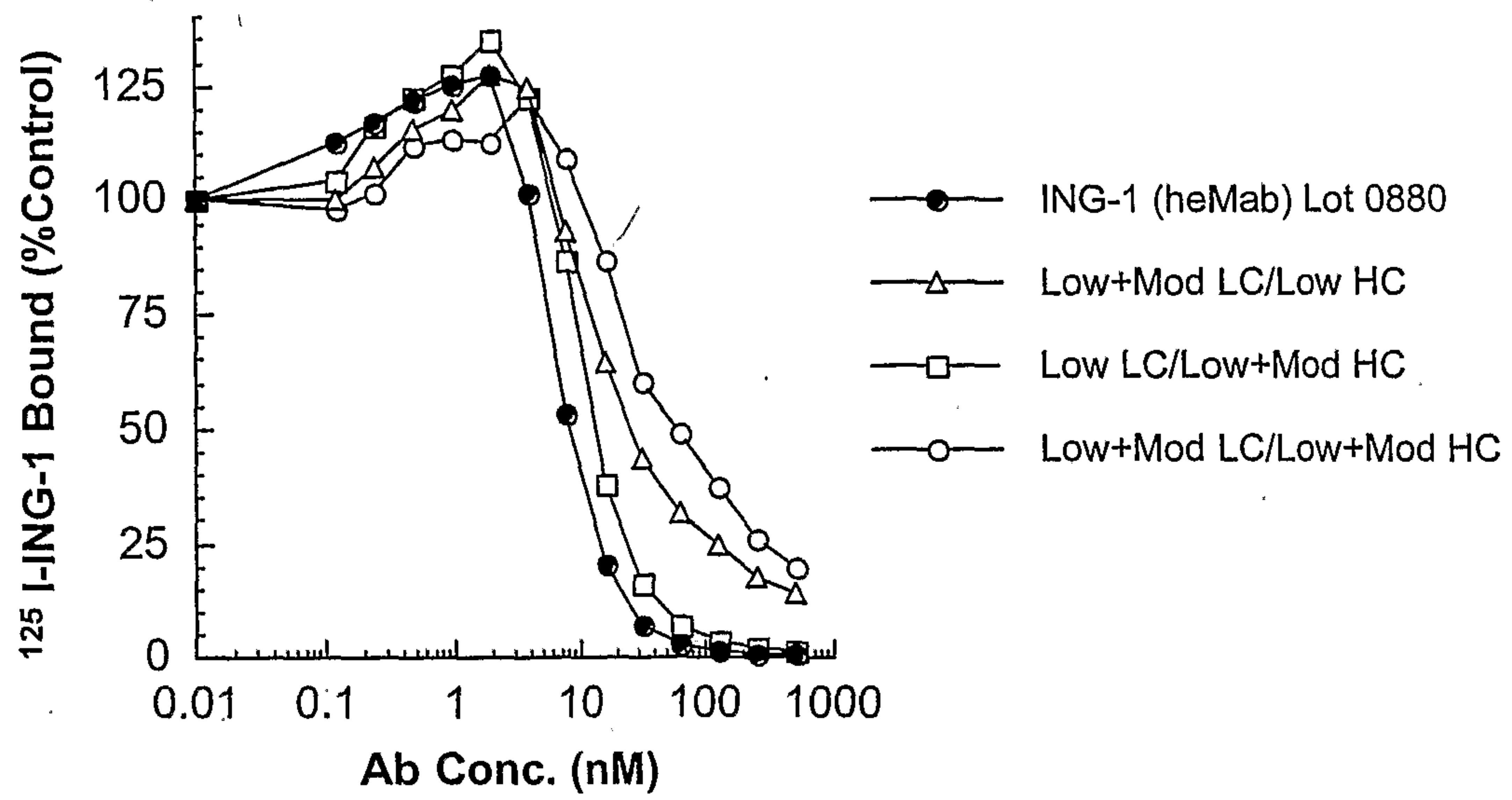


Figure 26A.

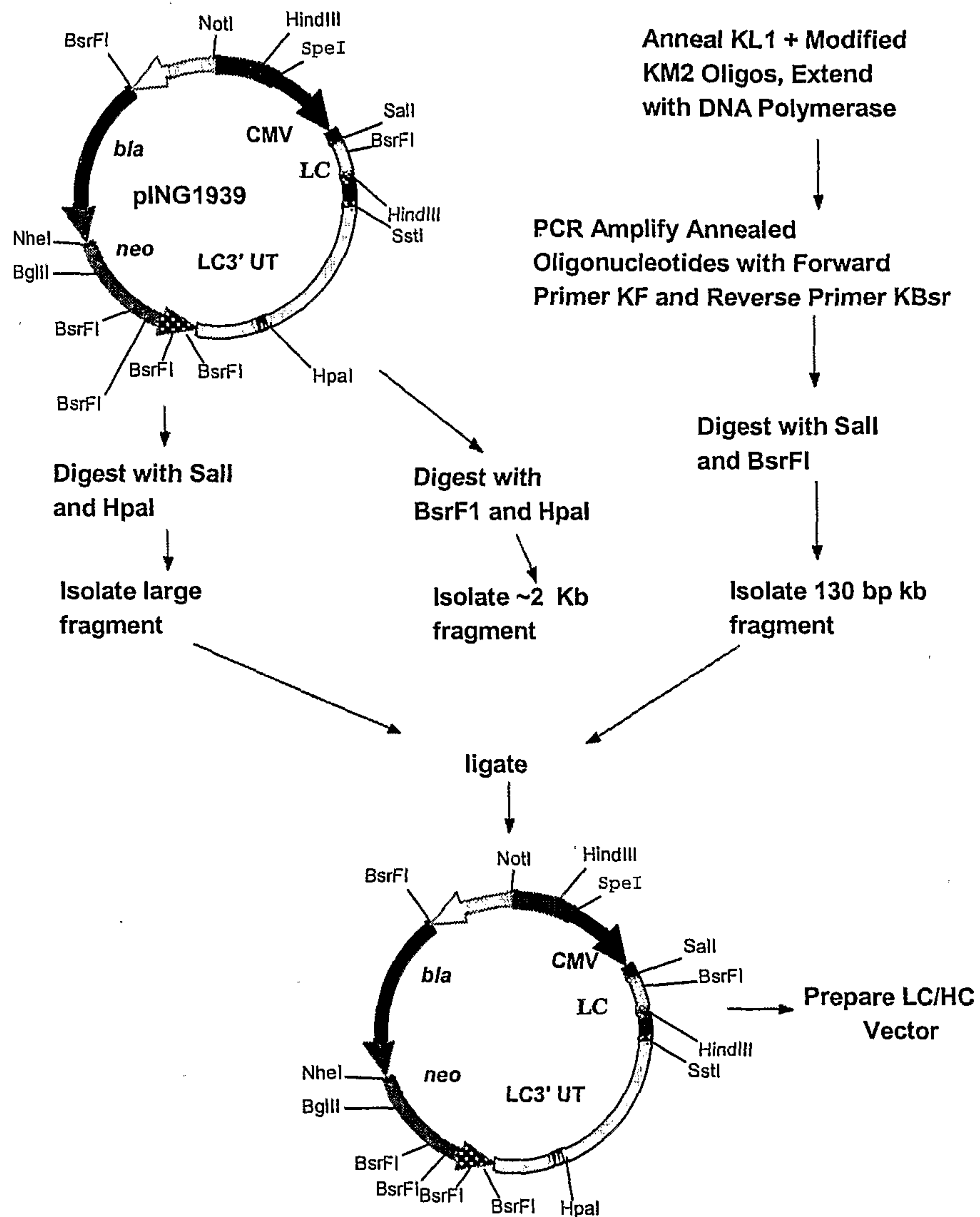


Figure 26B.

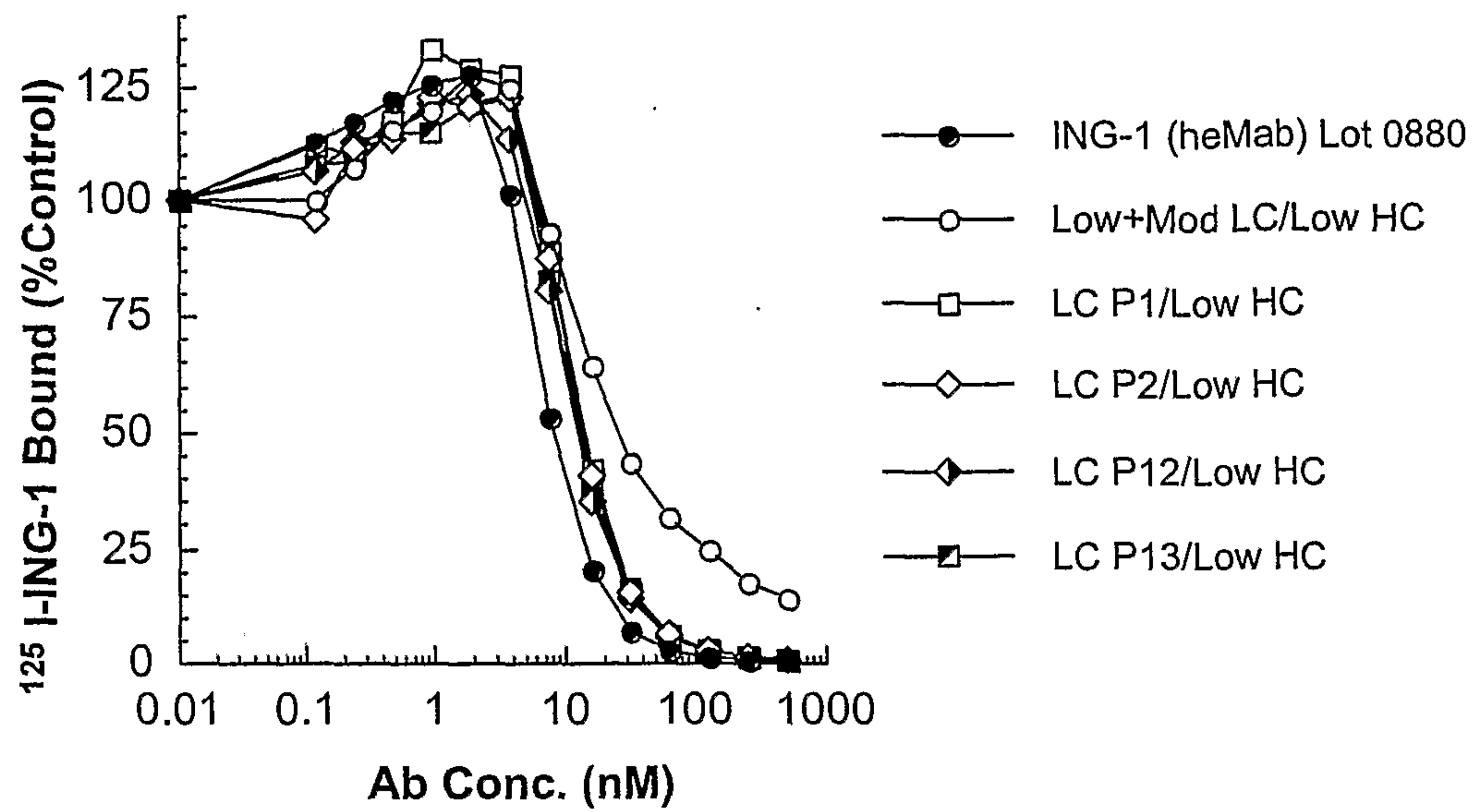


Figure 27.

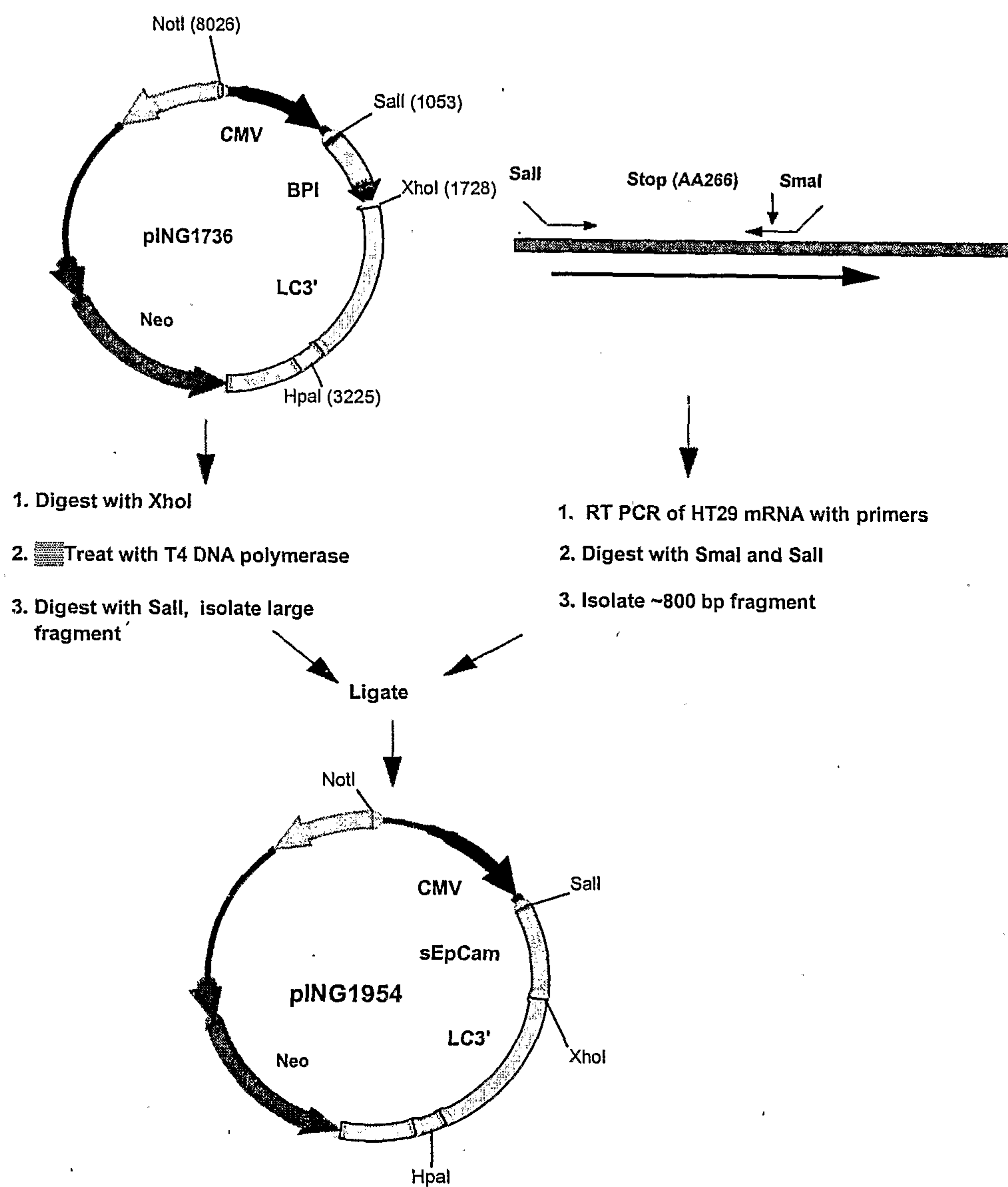


Figure 28.

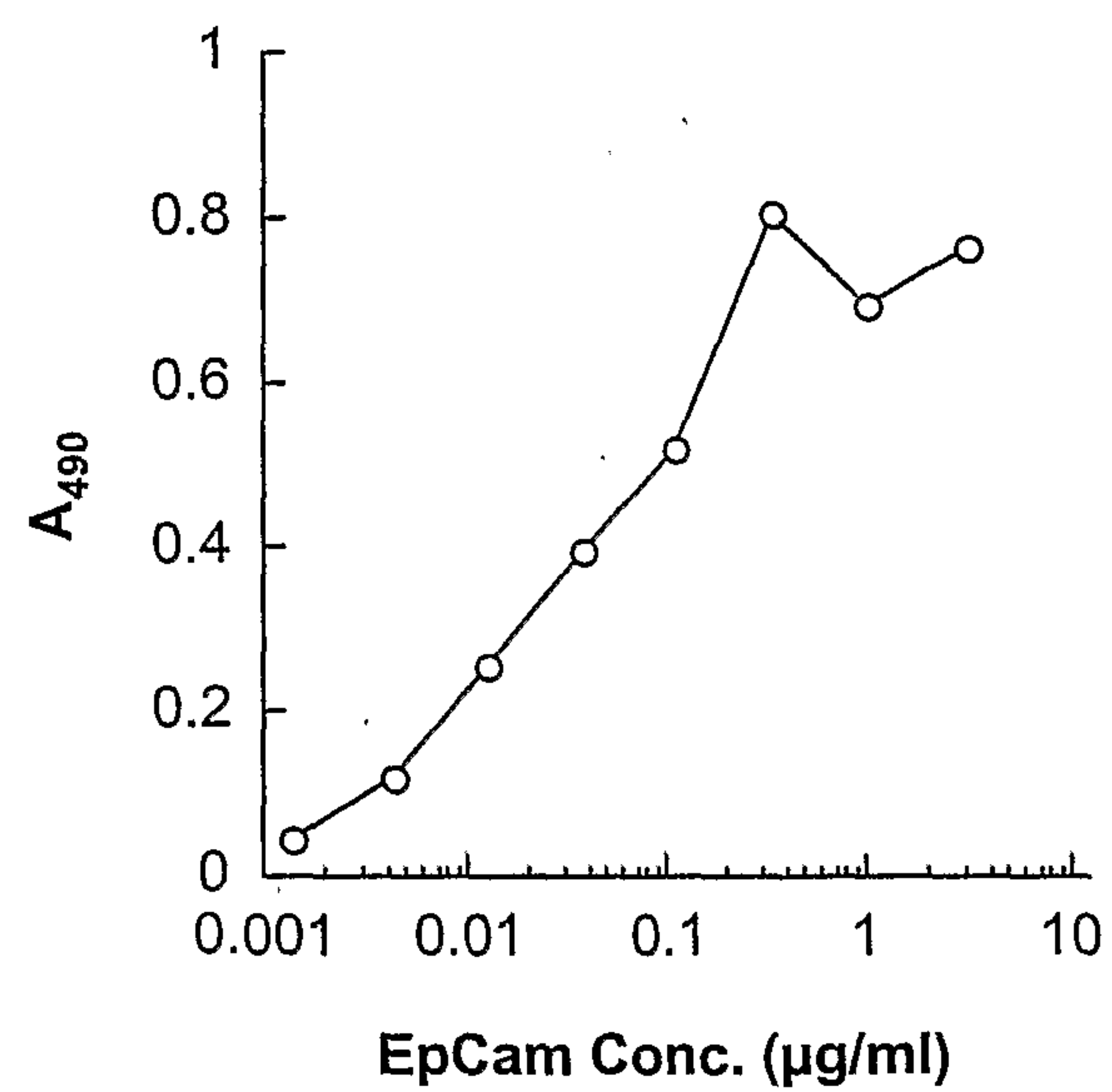
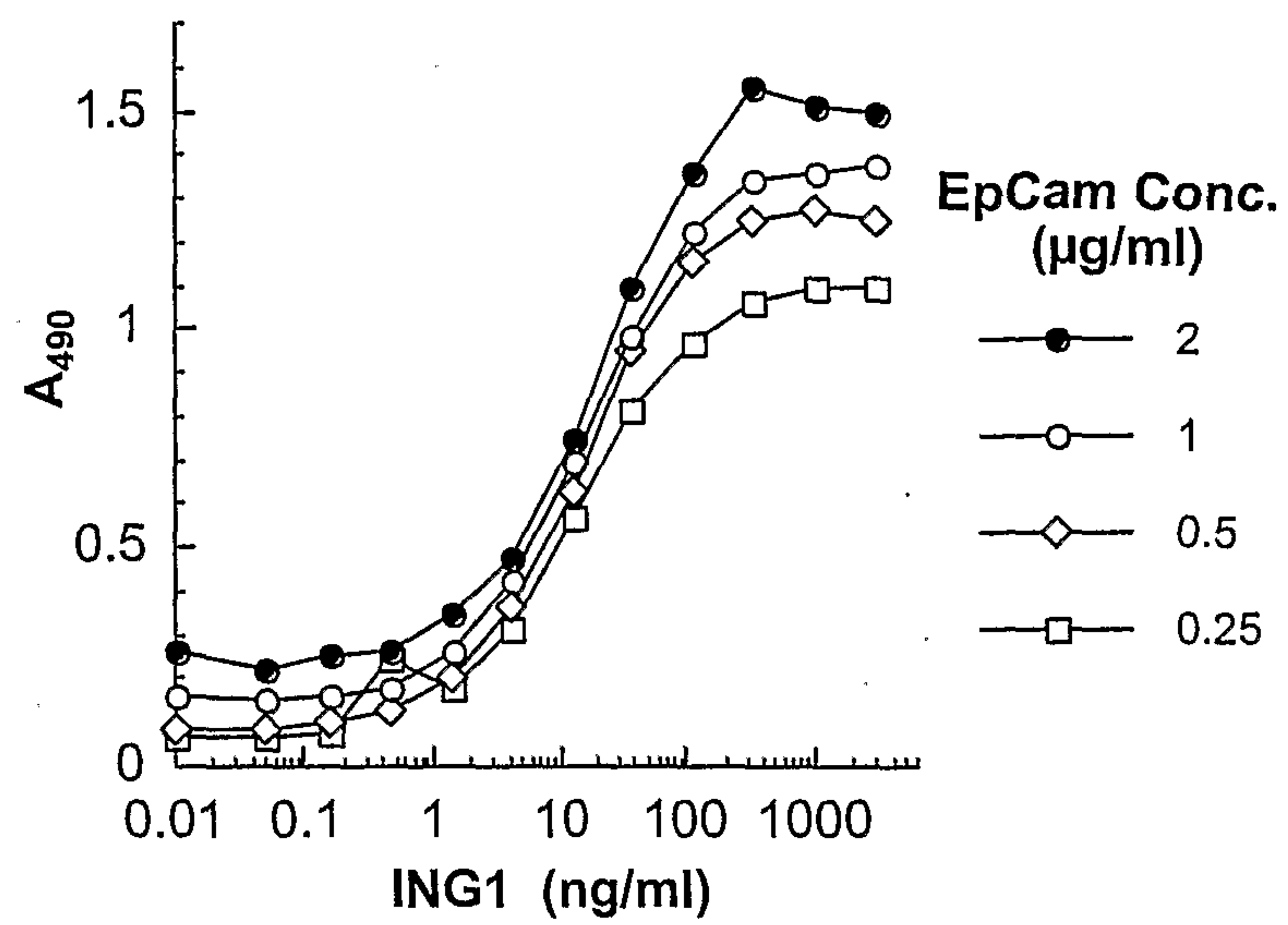


Figure 29.

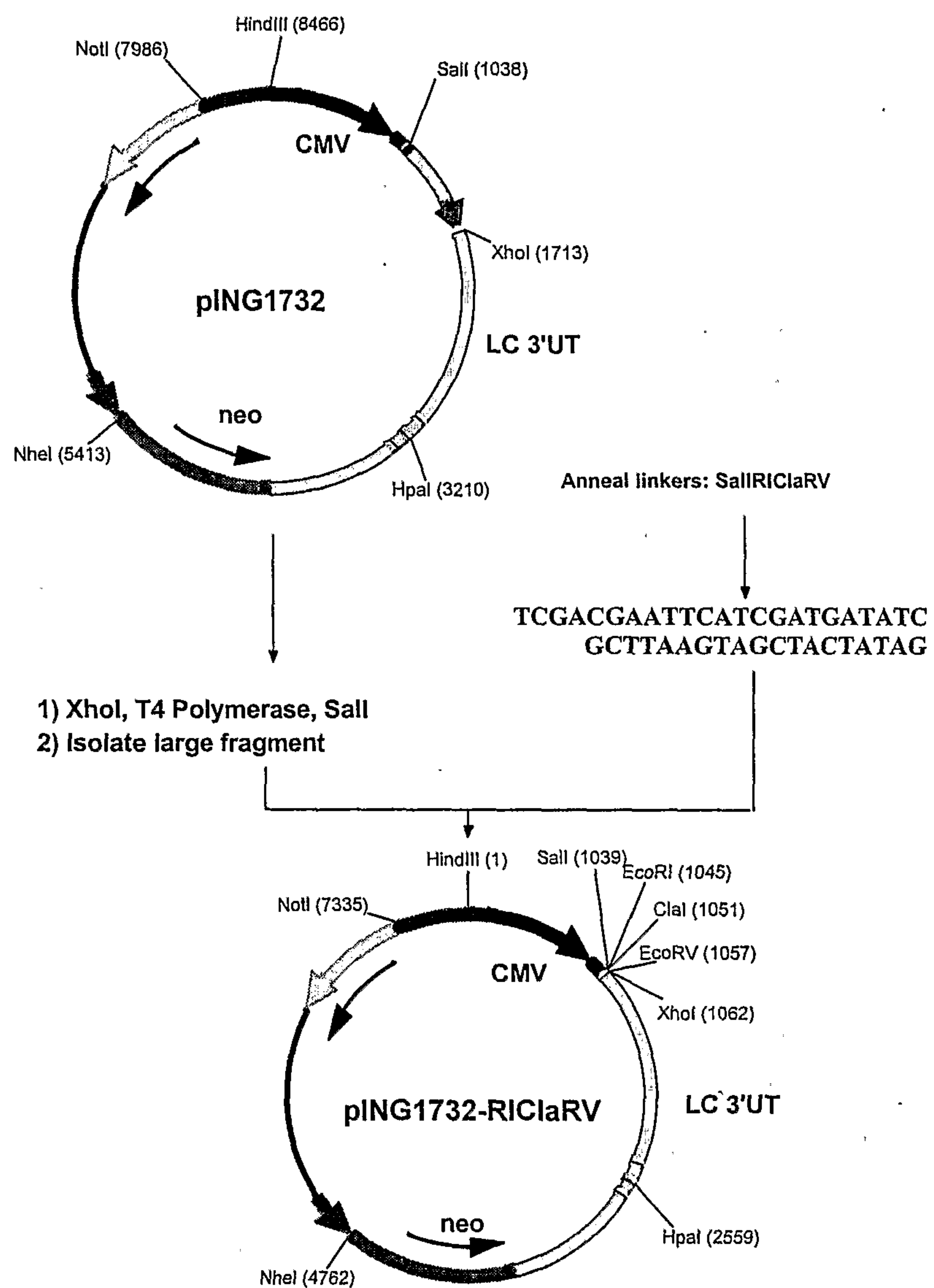


Figure 30.

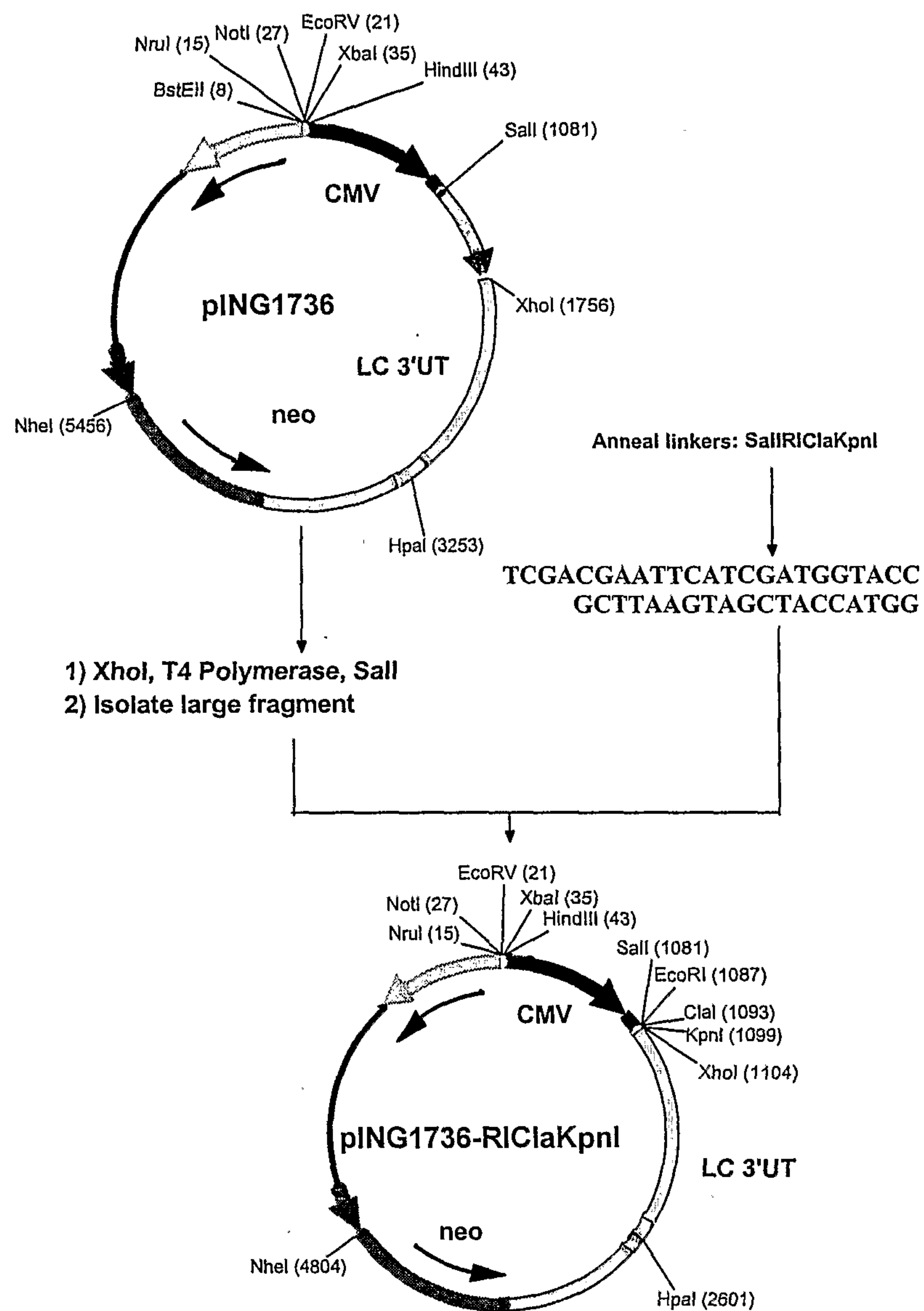


Figure 31.

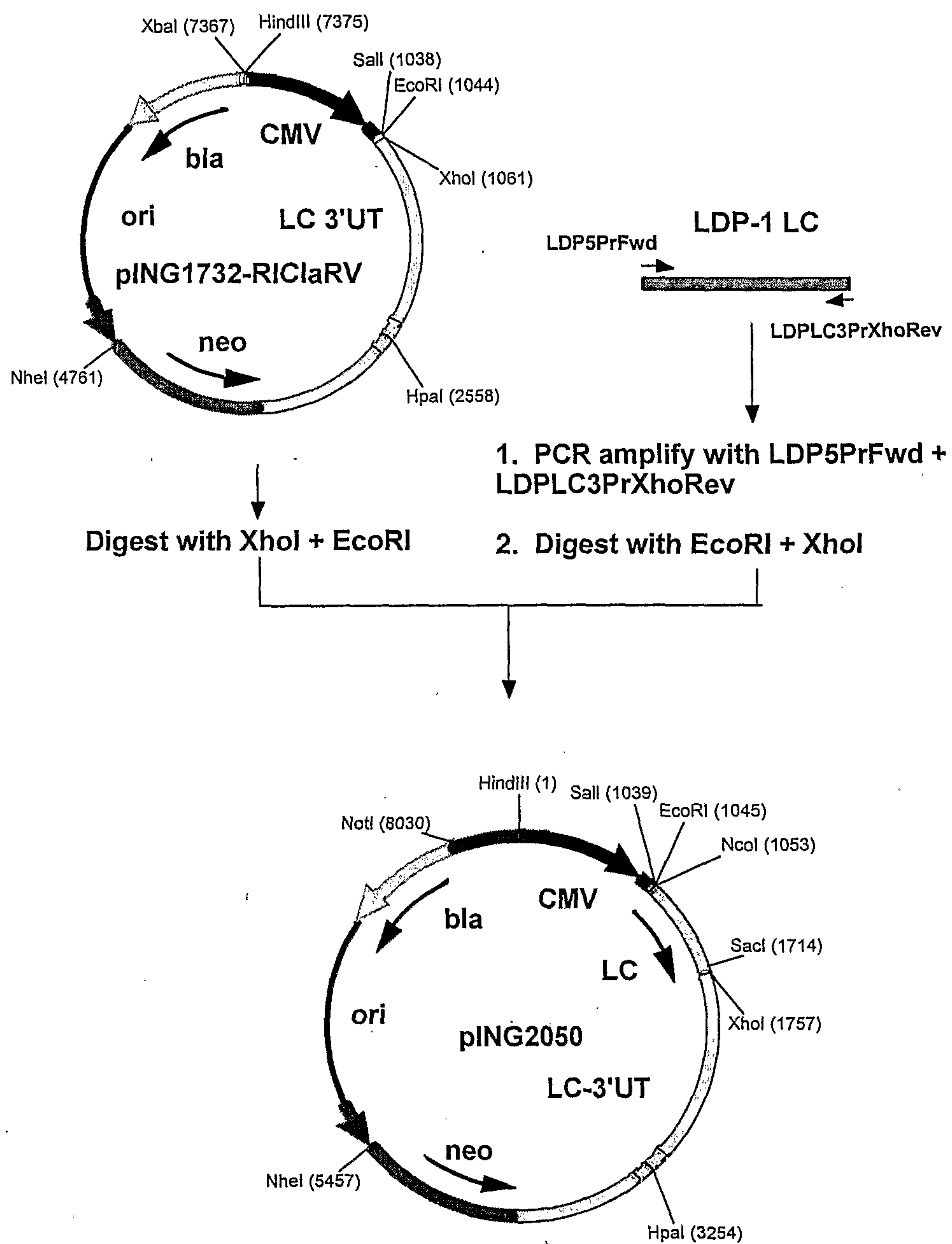


Figure 32.

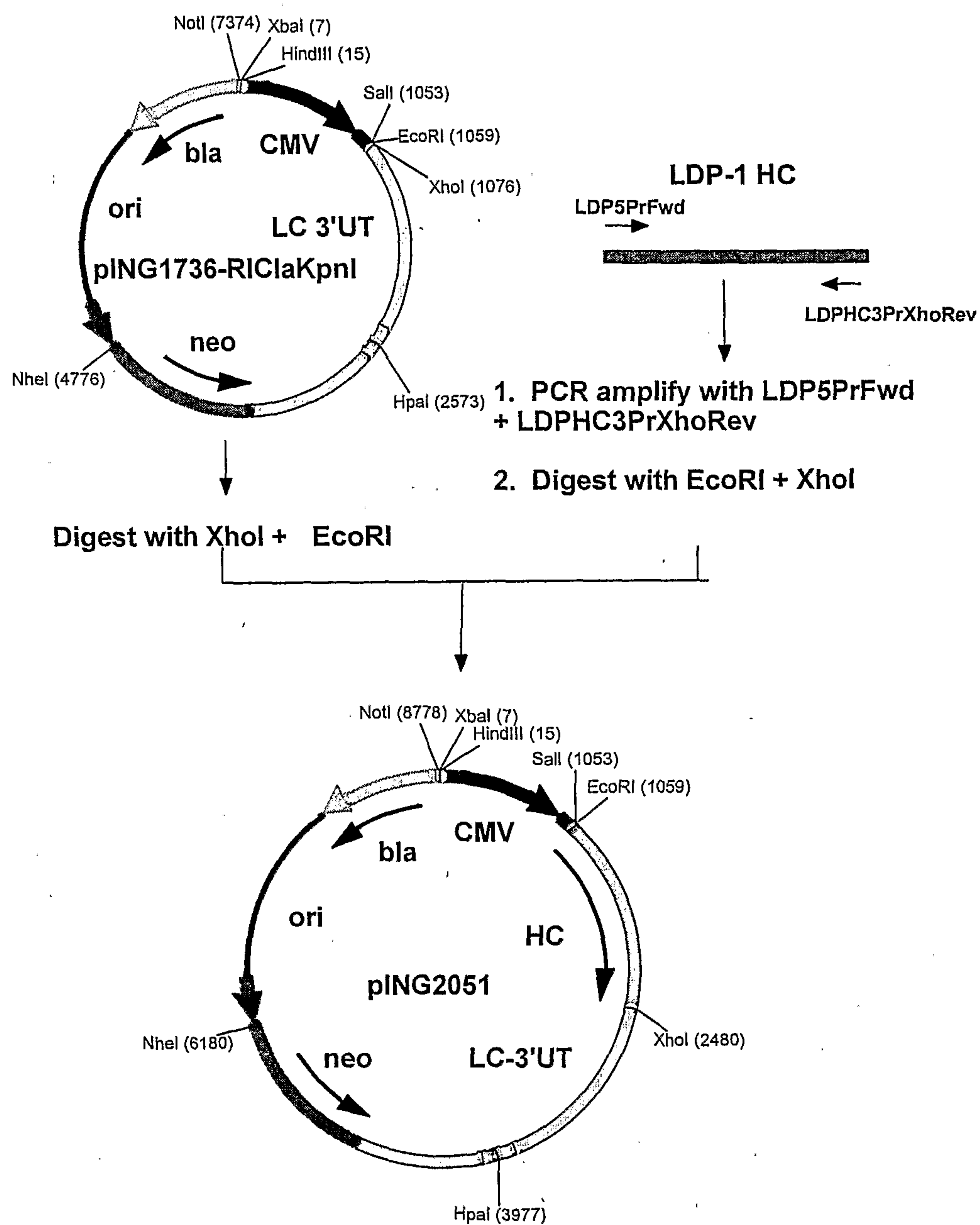


Figure 33.

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1  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT
1▶ M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G

49  GTC CAC TCC GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC
17▶ V  H  S  D  I  Q  M  T  Q  S  P  S  S  L  S  A
      +1

97  AGC GTG GGT GAC AGA GTG ACC ATC ACC TGT AAA GCA AGT AAG AGC ATT
33▶ S  V  G  D  R  V  T  I  T  C  K  A  S  K  S  I

145 AGC AAT TAT TTA GCC TGG TAC CAG CAG AAG CCA GGT AAG GCT CCA AAG
49▶ S  N  Y  L  A  W  Y  Q  Q  K  P  G  K  A  P  K

193 CTG CTG ATC TAC TAT GGG TCA ACT TTG CGA TCT GGT GTG CCA AGC AGA
65▶ L  L  I  Y  Y  G  S  T  L  R  S  G  V  P  S  R

241 TTC AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC
81▶ F  S  G  S  G  S  G  T  D  F  T  F  T  I  S  S

289 CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAA CAG TAT TAT GAA
97▶ L  Q  P  E  D  I  A  T  Y  Y  C  Q  Q  Y  Y  E

337 AGA CCG CTC ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA CGA ACT
113▶ R  P  L  T  F  G  Q  G  T  K  V  E  I  K  R  T

385 GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG
129▶ V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L

433 AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC
145▶ K  S  G  T  A  S  V  V  C  L  L  N  N  F  Y  P

481 AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT
161▶ R  E  A  K  V  Q  W  K  V  D  N  A  L  Q  S  G

529 AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC
177▶ N  S  Q  E  S  V  T  E  Q  D  S  K  D  S  T  Y

577 AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC
193▶ S  L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H

625 AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC
209▶ K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V

673 ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG
225▶ T  K  S  F  N  R  G  E  C

```

Figure 34.

```

1  ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC TCC CAG
1▶ M  G  W  S  C  I  I  L  F  L  V  A  T  A  T  G  V  H  S  Q
61  GTC CAA CTG CAG GAG AGC GGT CCA GGT CTT GTG AGA CCT AGC CAG ACC CTG AGC CTG ACC
21▶ V  Q  L  Q  E  S  G  P  G  L  V  R  P  S  Q  T  L  S  L  T
121 TGC ACC GTG TCT GGC TTC ACC TTC ACC GAT TAC CTT CTG CAC TGG GTG AGA CAG CCA CCT
41▶ C  T  V  S  G  F  T  F  T  D  Y  L  L  H  W  V  R  Q  P  P
181 GGA CGA GGT CTT GAG TGG ATT GGA TGG ATT GAT CCT GAG GAT GGT GAA ACA AAG TAT GGT
61▶ G  R  G  L  E  W  I  G  W  I  D  P  E  D  G  E  T  K  Y  G
241 CAG AAG TTT CAA AGC AGA GTG ACA ATG CTG GTA GAC ACC AGC AAG AAC CAG TTC AGC CTG
81▶ Q  K  F  Q  S  R  V  T  M  L  V  D  T  S  K  N  Q  F  S  L
301 AGA CTC AGC AGC GTG ACA GCC GCC GAC ACC GCG GTC TAT TAT TGT GCA AGA GGC GAA TAT
101▶ R  L  S  S  V  T  A  A  D  T  A  V  Y  Y  C  A  R  G  E  Y
361 AGA TAC AAC TCG TGG TTT GAT TAC TGG GGT CAA GGC TCA CTA GTC ACA GTC TCC TCA GCC
121▶ R  Y  N  S  W  F  D  Y  W  G  Q  G  S  L  V  T  V  S  S  A
421 TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC
141▶ S  T  K  G  P  S  V  F  P  L  A  P  S  S  K  S  T  S  G  G
481 ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG
161▶ T  A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V  S  W
541 AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA
181▶ N  S  G  A  L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G
601 CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC
201▶ L  Y  S  L  S  S  V  V  T  V  P  S  S  S  L  G  T  Q  T  Y
661 ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA
221▶ I  C  N  V  N  H  K  P  S  N  T  K  V  D  K  K  V  E  P  K
721 TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC GCG GGG GCA CCG
241▶ S  C  D  K  T  H  T  C  P  P  C  P  A  P  E  L  A  G  A  P
781 TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG
261▶ S  V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E
841 GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC
281▶ V  T  C  V  V  V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y
901 GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC
301▶ V  D  G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y  N  S
961 ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG
321▶ T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W  L  N  G  K  E
1021 TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA
341▶ Y  K  C  K  V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K
1081 GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG
361▶ A  K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  D  E  L
1141 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC
381▶ T  K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A
1201 GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG
401▶ V  E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  V  L
1261 GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG
421▶ D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q
1321 CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG
441▶ Q  G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q
1381 AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
461▶ K  S  L  S  L  S  P  G  K  .

```

Figure 35.

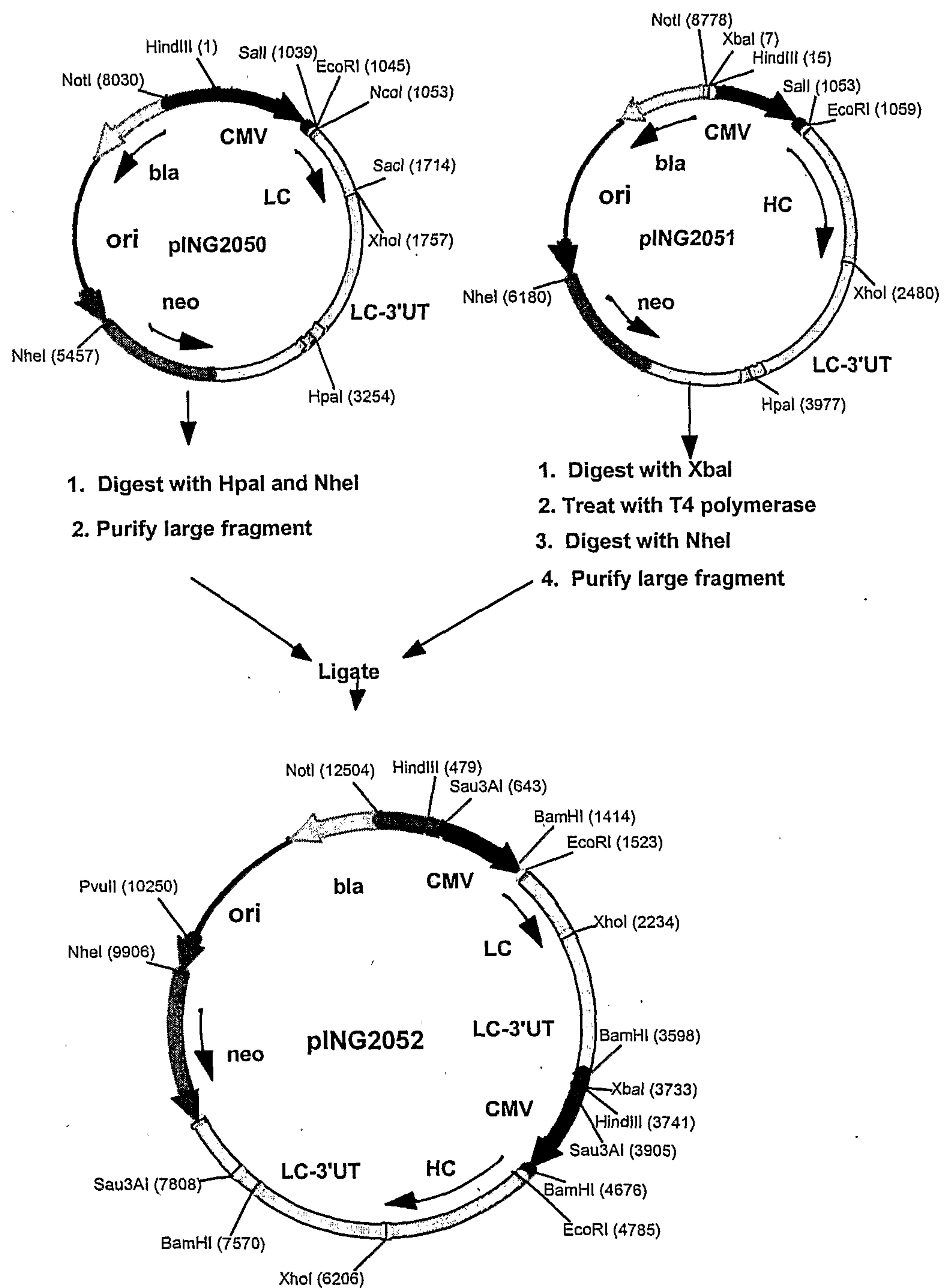


Figure 36.

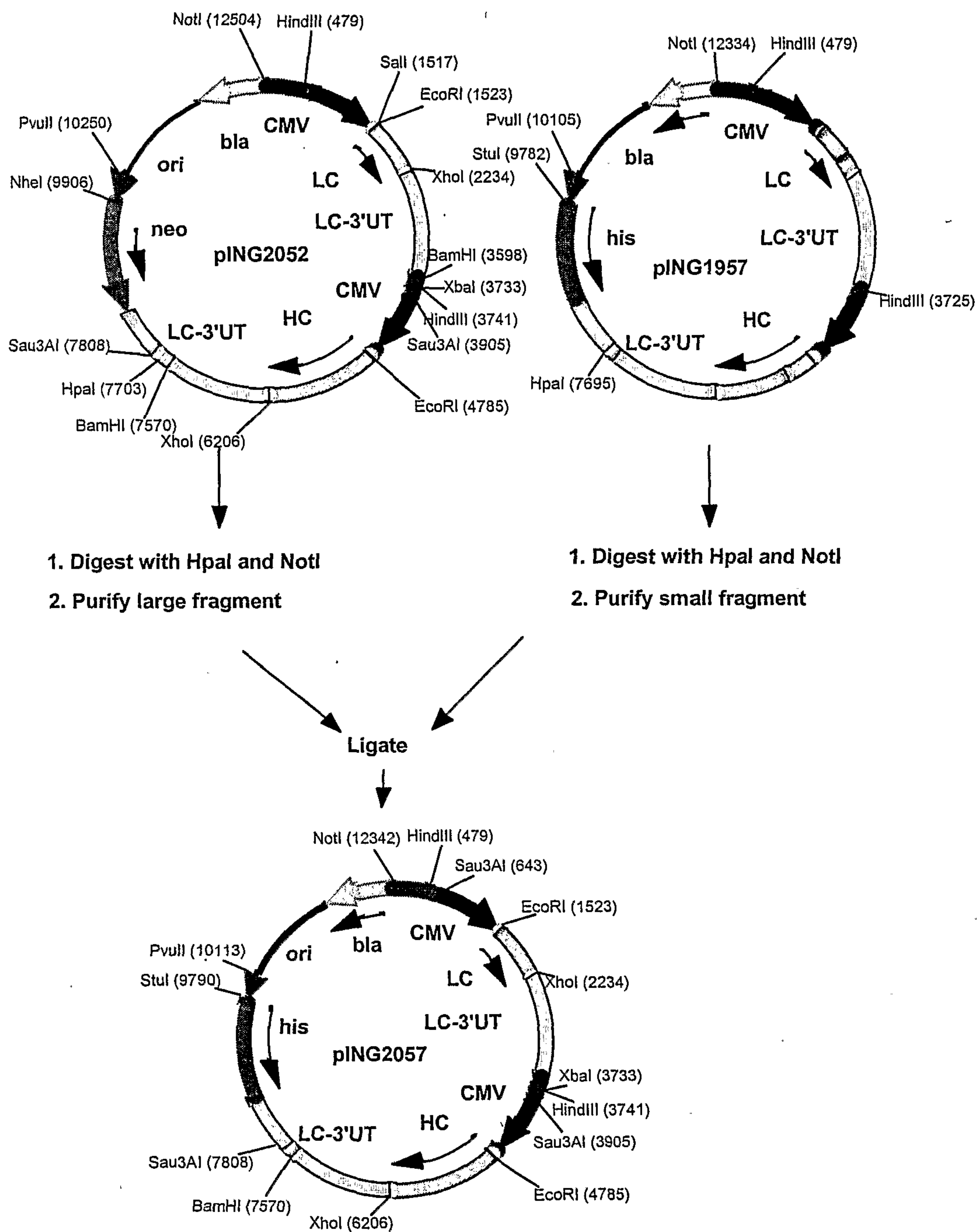


Figure 37.

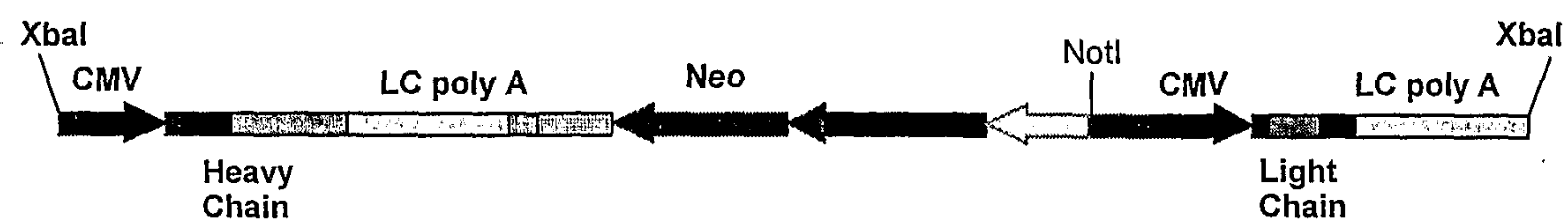


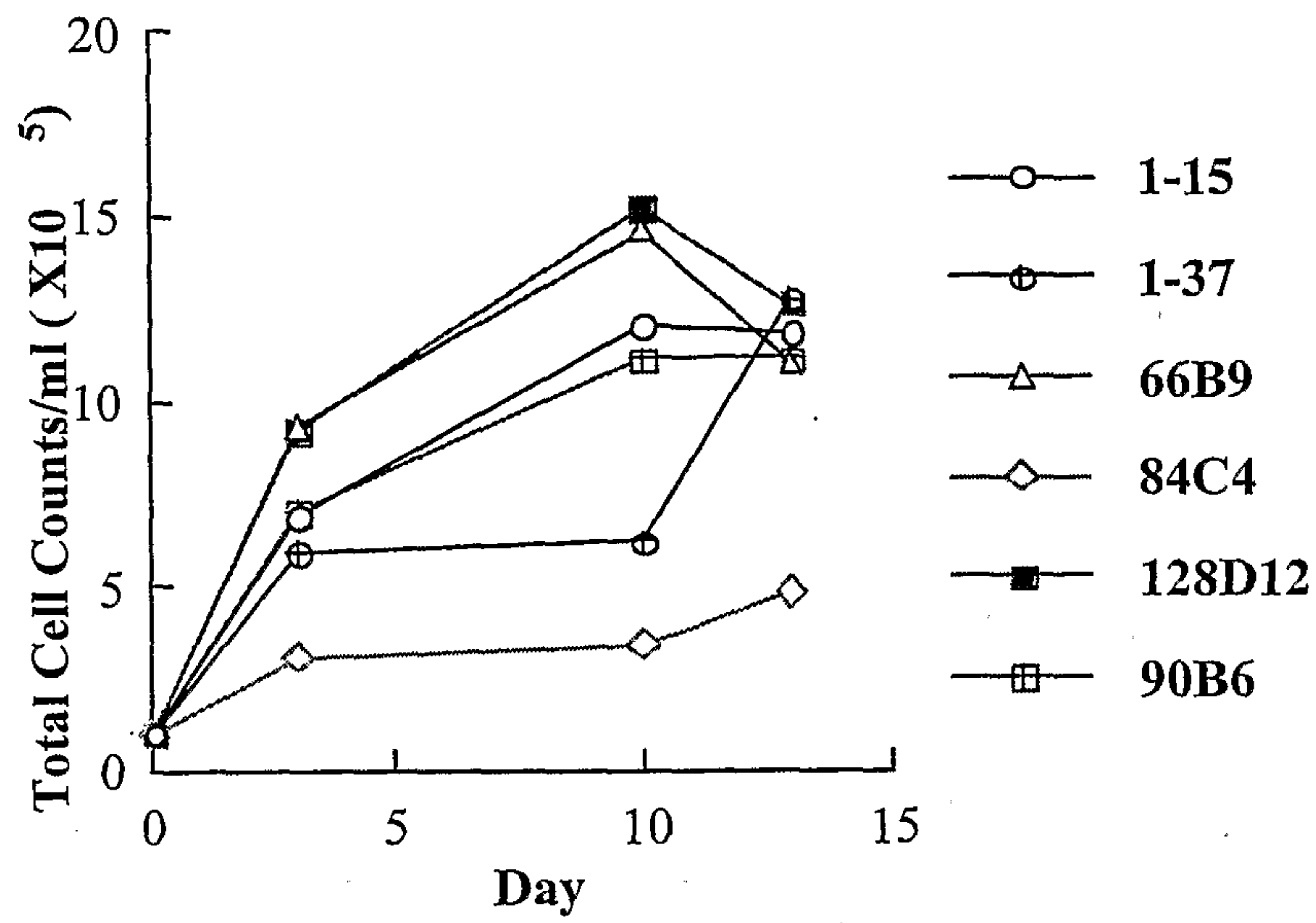
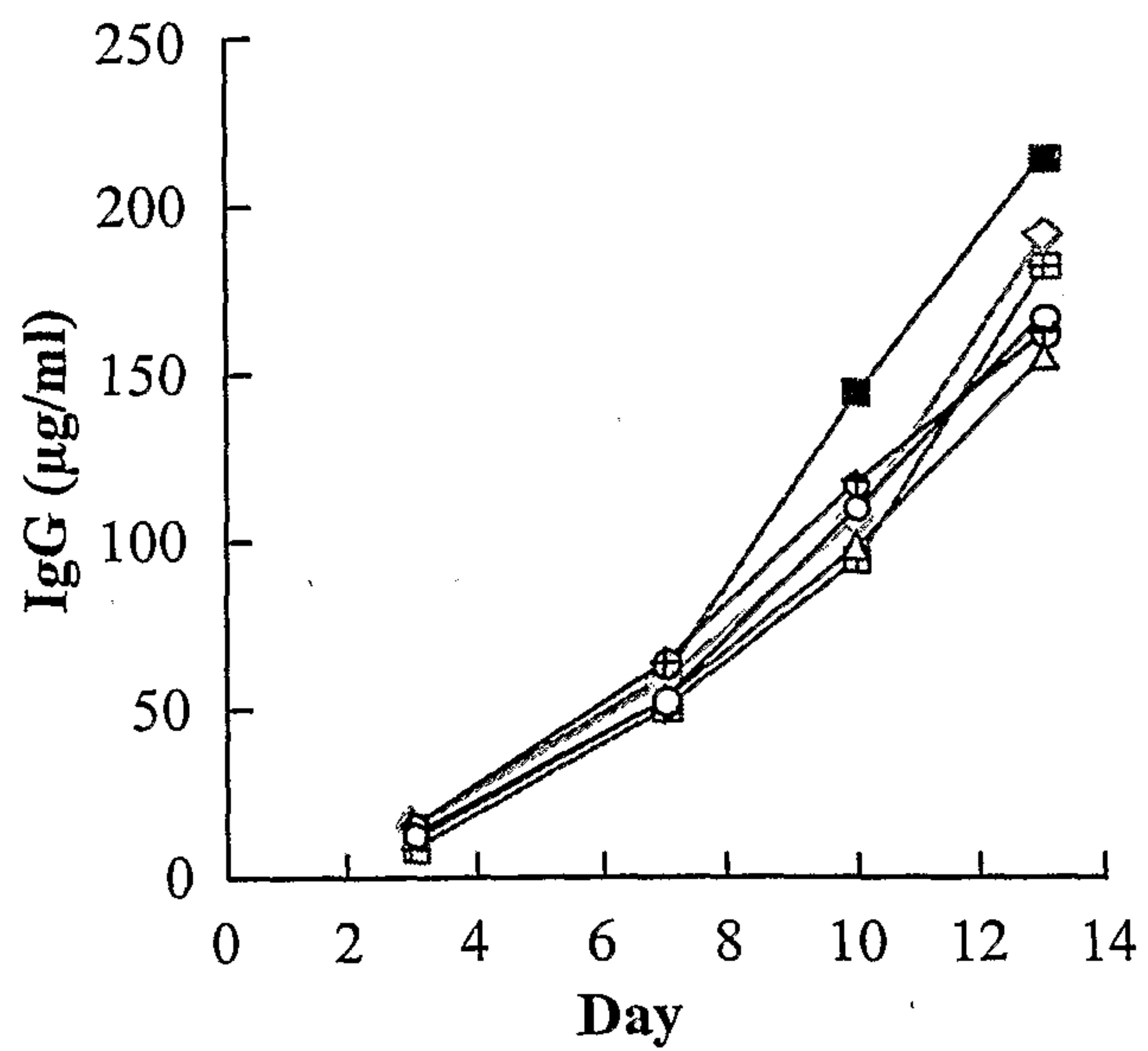
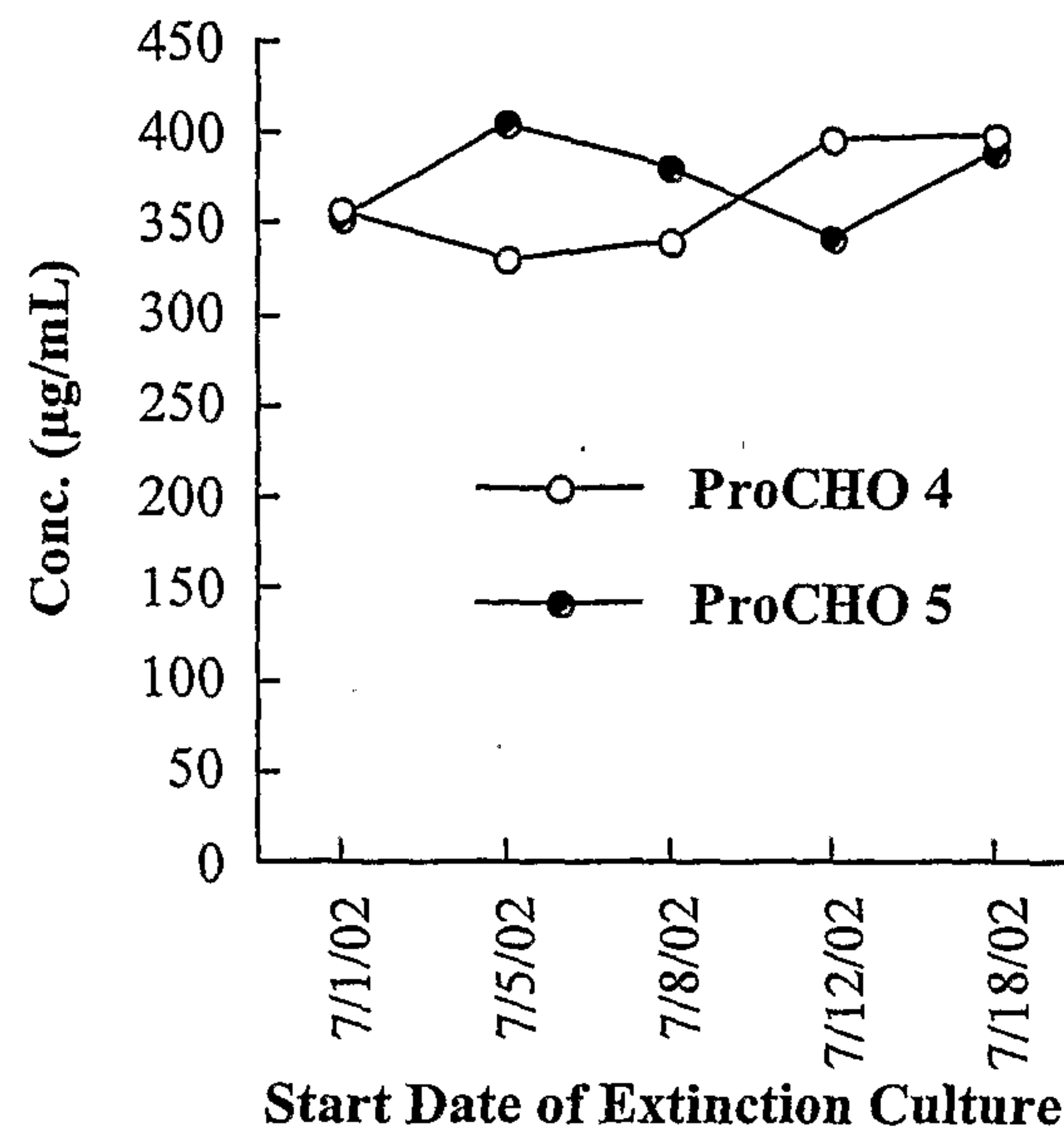
Figure 38.**A. Growth****B. Productivity**

Figure 39.

A. Clone 264E2



B. Clone 254G12

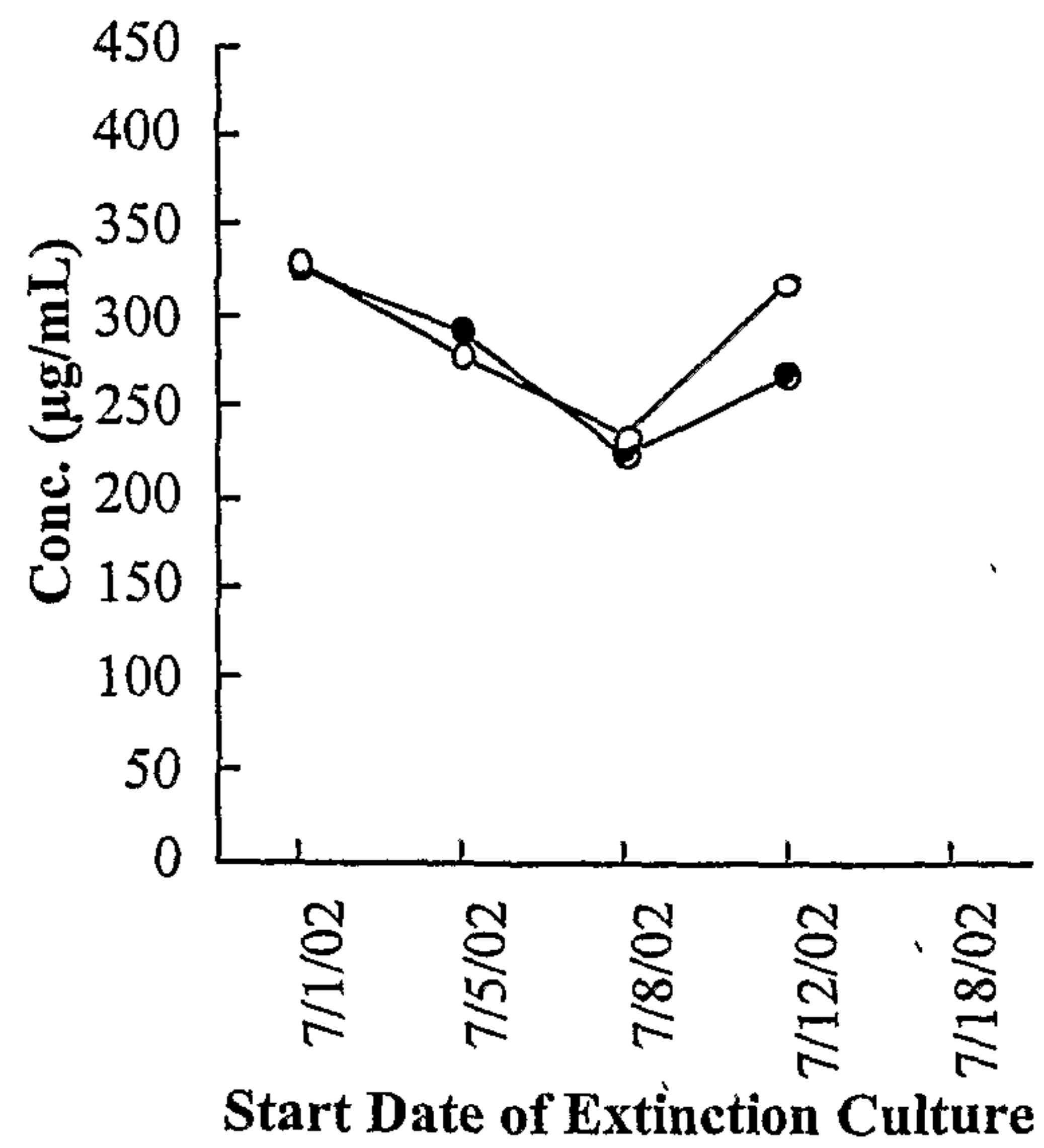


Figure 40.

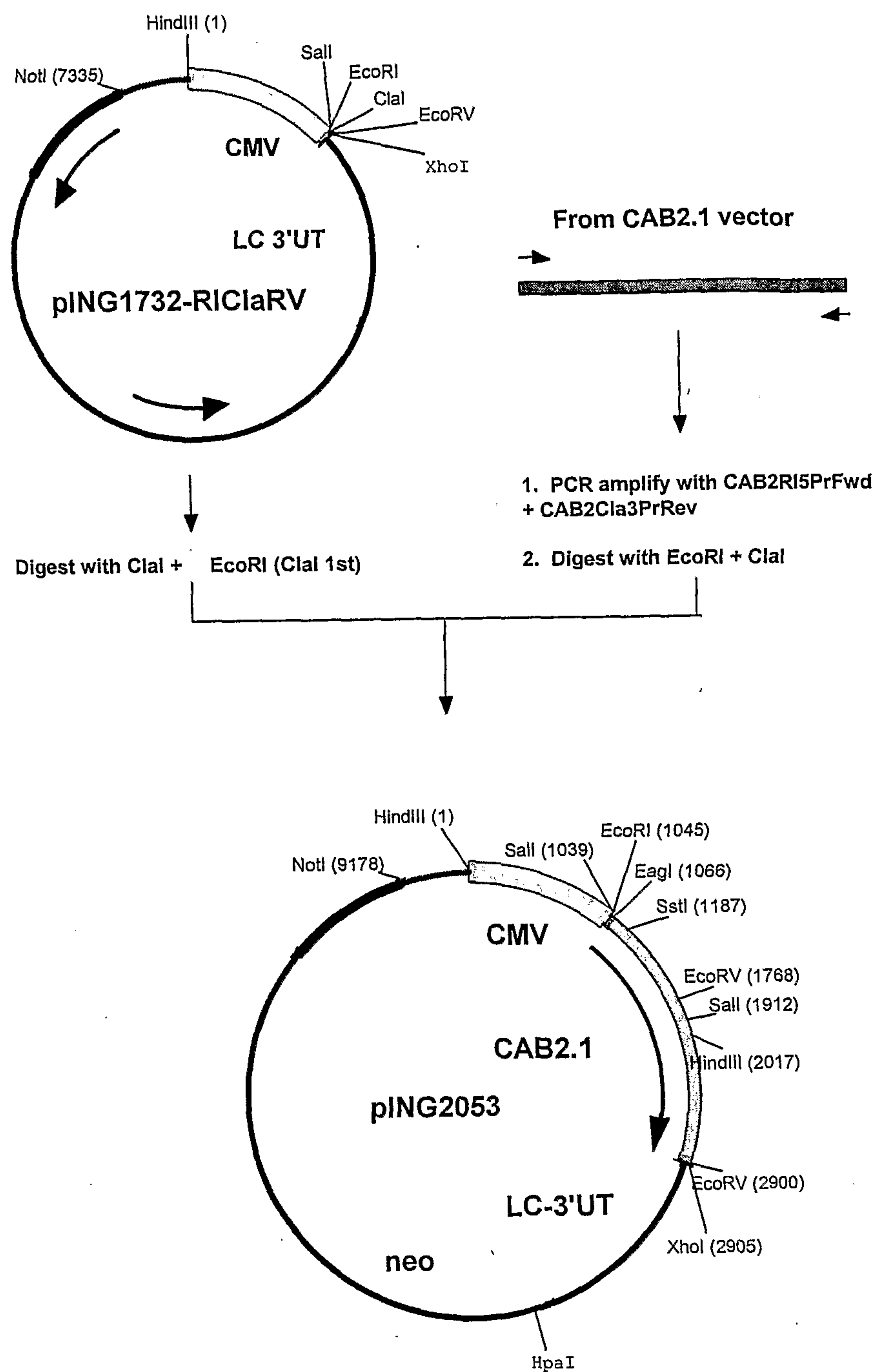


Figure 41.

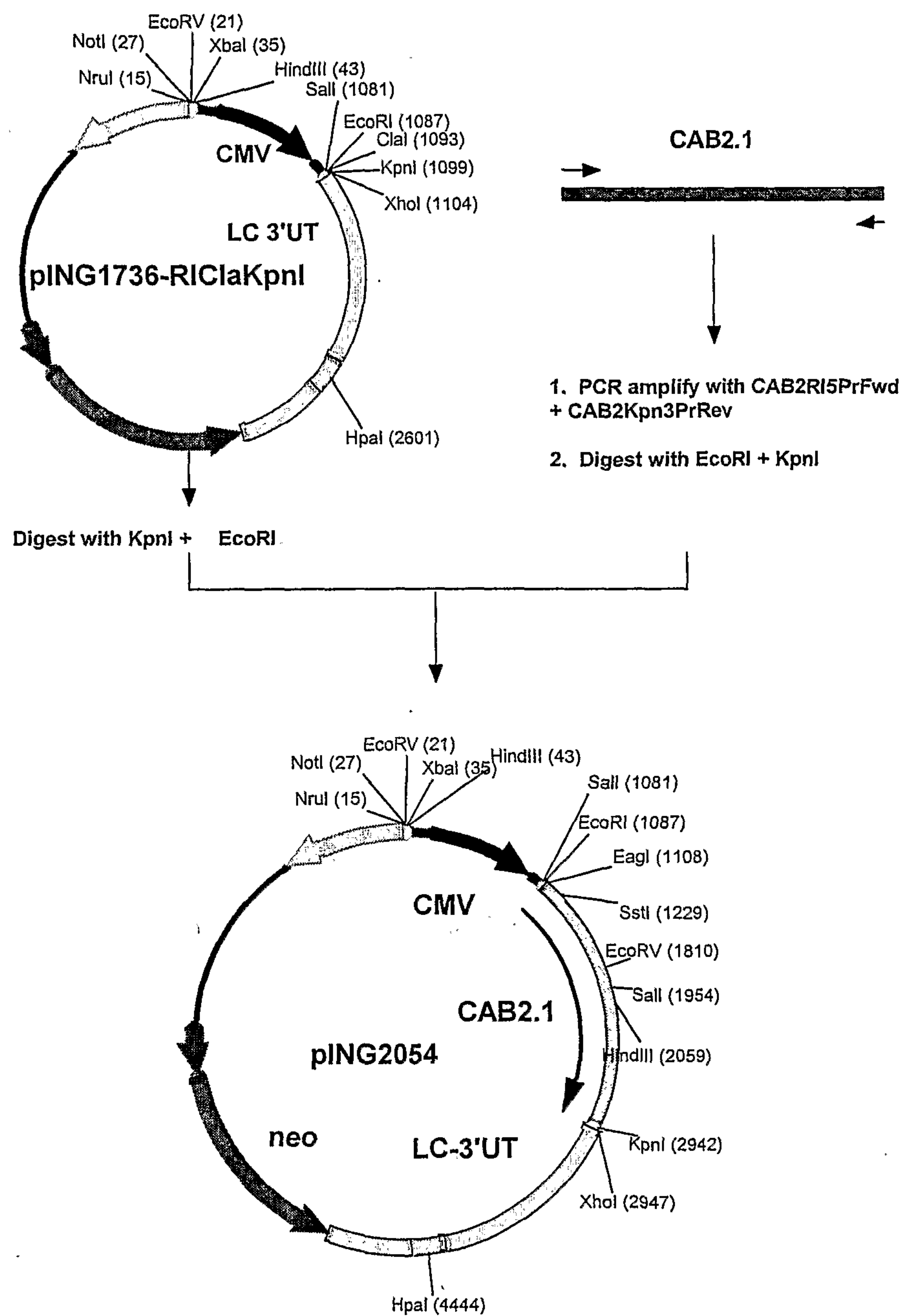


Figure 42.

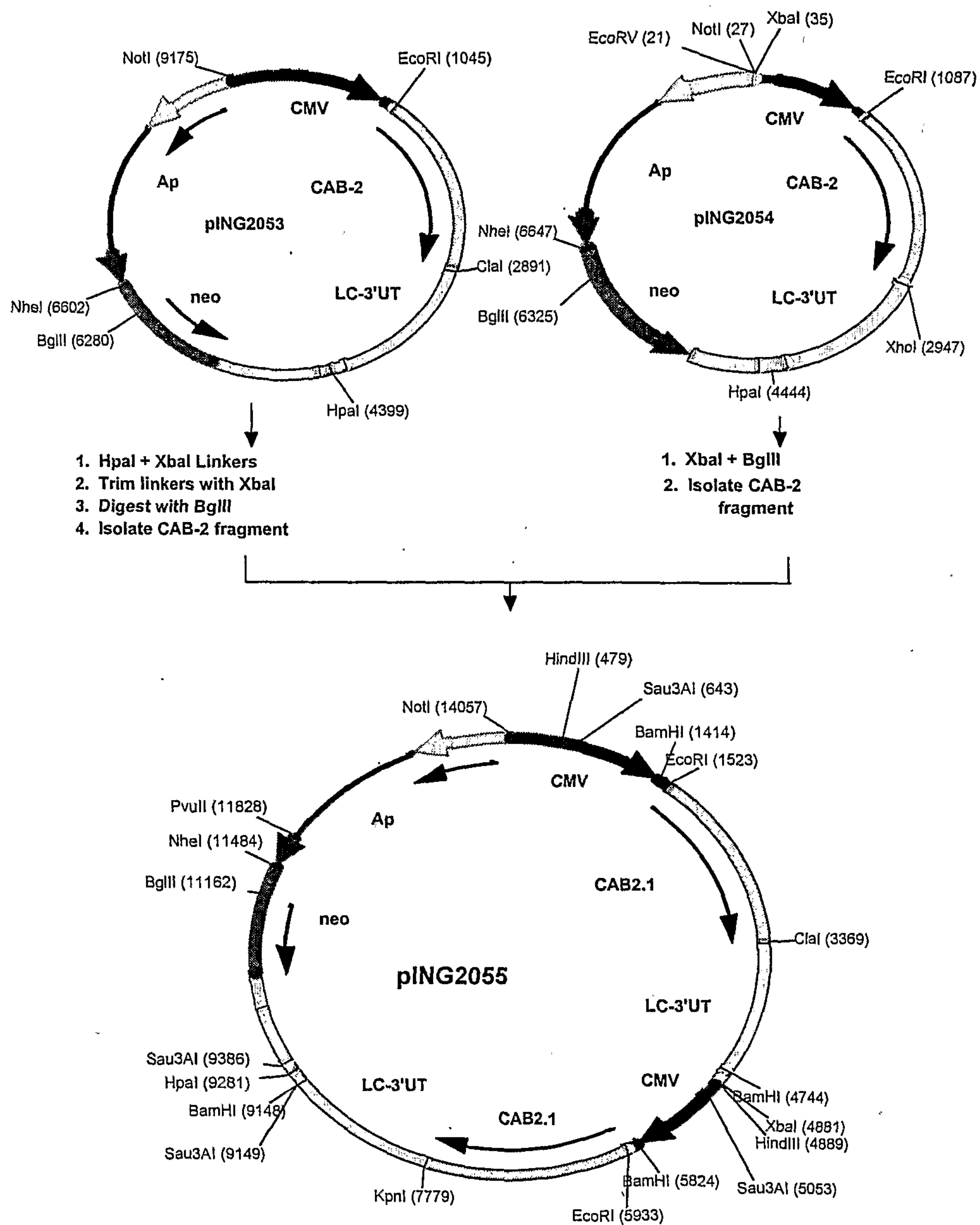


Figure 43.

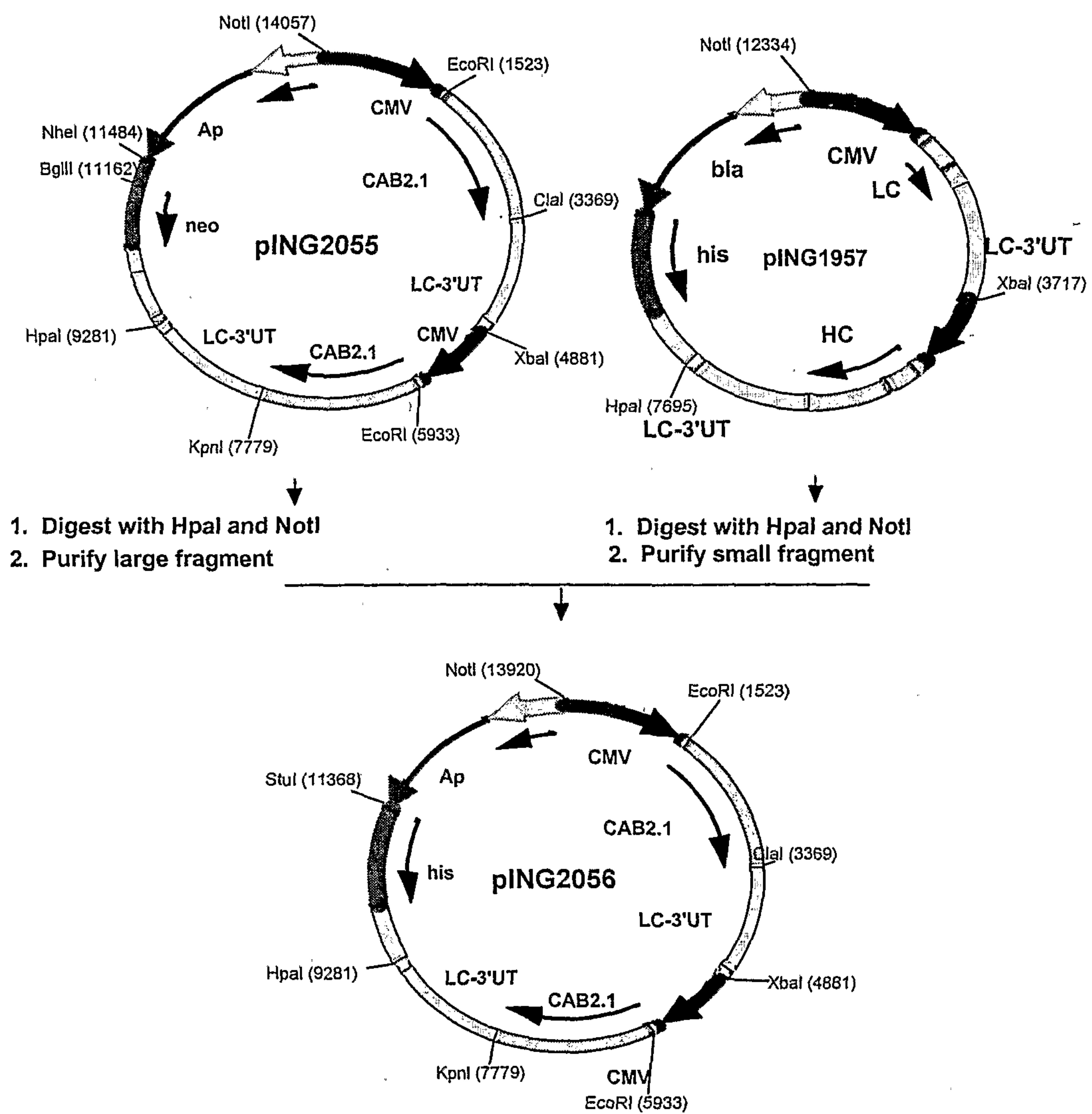
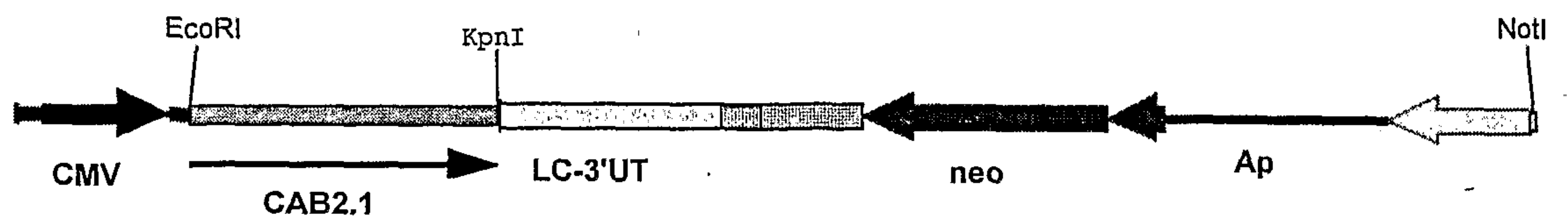


Figure 44.

A. pING2054: "single gene" vector.



B. pING2055: "two-gene" vector.

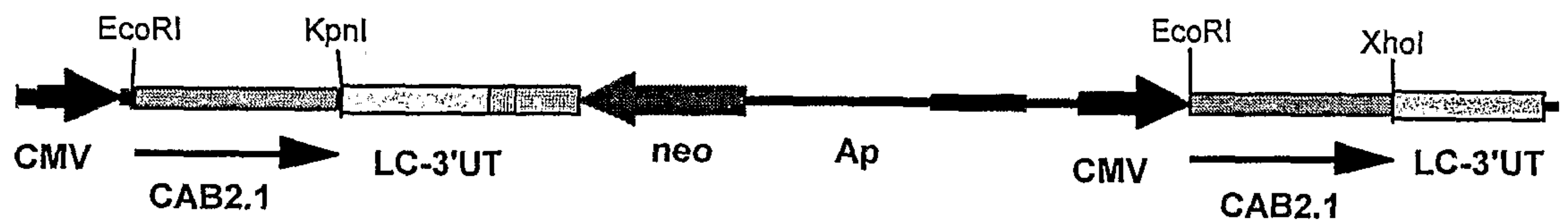
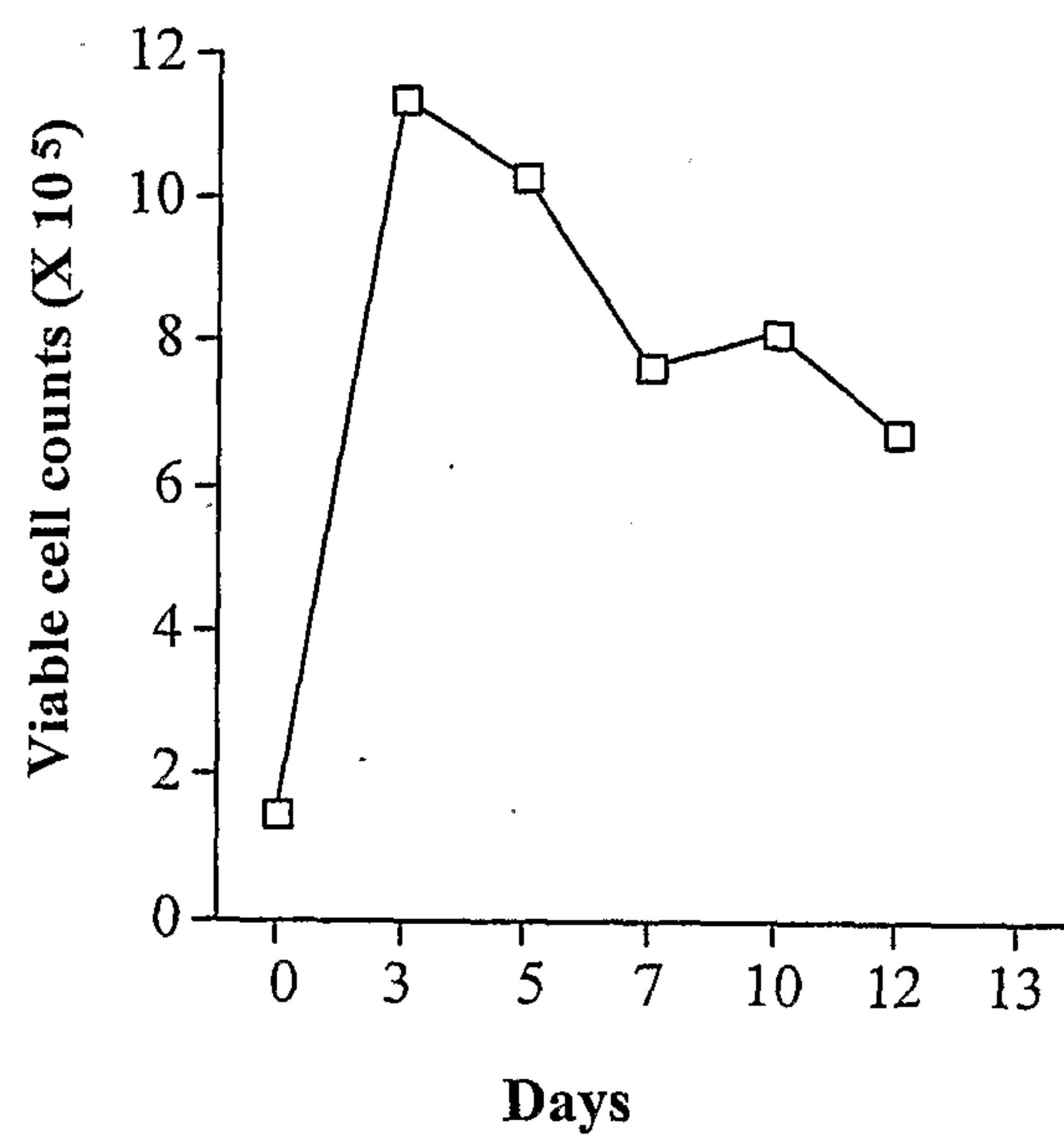


Figure 45.**A. Growth.****B. Productivity**