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(54) Title: ANTIBODY PRODUCING NON-HUMAN MAMMALS

(57) **Abrégé/Abstract:**

The invention provides transgenic, non-human animals comprising a nucleic acid encoding an immunoglobulin light chain, whereby the immunoglobulin light chain is human, human-like, or humanized. The nucleic acid is provided with a means that renders it resistant to DNA rearrangements and/or somatic hypermutations. In one embodiment, the nucleic acid comprises an expression cassette for the expression of a desired molecule in cells during a certain stage of development in cells developing into mature B cells. The invention further provides methods for producing an immunoglobulin from the transgenic, non-human animal.



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(54) Title: ANTIBODY PRODUCING NON-HUMAN MAMMALS

(57) Abstract: The invention provides transgenic, non-human animals comprising a nucleic acid encoding an immunoglobulin light chain, whereby the immunoglobulin light chain is human, human-like, or humanized. The nucleic acid is provided with a means that renders it resistant to DNA rearrangements and/or somatic hypermutations. In one embodiment, the nucleic acid comprises an expression cassette for the expression of a desired molecule in cells during a certain stage of development in cells developing into mature B cells. The invention further provides methods for producing an immunoglobulin from the transgenic, non-human animal.



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Title: Antibody producing non-human mammals.

TECHNICAL FIELD

5 The invention relates to the production and use of non-human animals capable of producing antibodies or derivatives thereof, which are expressed from at least partially exogenous nucleic acids (transgenes). Transgenes to produce such transgenic animals and methods to produce such heterologous antibodies; methods and vectors for producing such
10 transgenic animals are disclosed.

BACKGROUND OF THE INVENTION

B cells mediate humoral immunity by producing specific antibodies.
15 The basic structural subunit of an antibody (Ab) is an immunoglobulin (Ig) molecule. Ig molecules consist of a complex of two identical heavy (H) and two identical light (L) polypeptide chains. At the amino terminus of each H chain and L chain is a region that varies in amino acid sequence named the variable (V) region. The remaining portion of the H and L chains is
20 relatively constant in amino acid sequence and is named the constant (C) region. In an Ig molecule, the H and L chain V regions (VH and VL) are juxtaposed to form the potential antigen-binding site. The genes that encode H and L chain V regions are assembled somatically from segments of germline DNA during precursor B (pre-B) cell differentiation: V, D and J
25 gene segments for the H chain and V and J gene segments for the L chain. Within Ig V regions are three regions of greatest amino acid sequence variability that interact to form the antigen-recognition site and are thus referred to as complementarity determining regions (CDRs).

30 The V gene segment encodes the bulk of the V region domain, including CDR1 and CDR2. Diversity in CDR1 and CDR2 derives from sequence heterogeneity among multiple different germline-encoded V

segments. CDR3 is encoded by sequences that are formed by the joining of H chain V, D, and J gene segments and L chain V and J segments and by mechanisms that create nucleotide sequence heterogeneity where these segments are combined. Additional diversity may be derived from pairing of different H and L chain V regions. Collectively these processes yield a primary repertoire of antibodies encoded by germline gene segments and expressed by newly-formed B cells.

An additional source of antibody diversity is imposed on top of the diversity generated by recombination of Ig gene segments. B cells are able to introduce mutations into the antibody V regions that they express, a process called somatic hypermutation. Thus, when an animal first encounters an antigen, the antigen binds to a specific B cell which happens to carry antibodies which have a V domain which binds the antigen. This primary response may activate this B cell to go on to secrete the cognate antibody. These activated B cells can also now target a somatic mutation process to their rearranged antibody gene segments and thus allow the production of daughter cells which make variants of the antibodies of the primary response. A selection process amplifies those variant B cell descendants which make an antibody of improved affinity of the antigen. In B cells, somatic hypermutations are targeted to a restricted genomic region including both the rearranged VH and VL genes. Thus somatic mutation allows affinity maturation - the production and selection of high affinity antibodies. Therefore, somatic mutation is important for the generation of high affinity antibodies.

The exquisite specificity and high affinity of antibodies and the discovery of hybridoma technology allowing the generation of monoclonal antibodies (mAbs) has generated great expectations for their utilization as targeted therapeutics for human diseases. MAb are identical because they are produced by a single B cell and its progeny. MAb are made by fusing the spleen cells from a mouse that has been immunized with the desired

antigen with myeloma cells to generate immortalized hybridomas. One of the major impediments facing the development of in vivo applications for mAbs in humans is the intrinsic immunogenicity of non-human Igs.

Patients respond to therapeutic doses of mouse mAbs by making antibodies
5 against the mouse Ig sequences (Human Anti Mouse Antibodies; HAMA), causing acute toxicity, alter their biodistribution and accelerate clearance, thus reducing the efficacy of subsequent administrations (Mirick, et al., (2004) Q. Nucl. Med. Mol. Imaging 48, 251–257).

10 To circumvent the generation of HAMA, antibody humanization methods have been developed in an attempt to produce mAbs with decreased immunogenicity when applied to humans. These endeavors have yielded various recombinant DNA-based approaches aimed at increasing the content of human amino acid sequences in mAbs while retaining the
15 specificity and affinity of the parental non-human antibody. Humanization began with the construction of mouse-human chimeric mAbs (Morrison, S. L., et al., (1984). Proc. Natl. Acad. Sci. USA., 81, 6851-5), in which the Ig C regions in murine mAbs were replaced by human C regions. Chimeric mAbs contain 60-70% of human amino acid sequences and are considerably less
20 immunogenic than their murine counterparts when injected into humans, albeit that a human anti-chimeric antibody response was still observed (Hwang, W. Y., et al. (2005). Methods, 36, 3-10).

In attempts to further humanize murine mAbs, CDR grafting was
25 developed. In CDR grafting, murine antibodies are humanized by grafting their CDRs onto the VL and VH frameworks of human Ig molecules, while retaining those murine framework residues deemed essential for specificity and affinity (Jones, P.T., et al., (1986). Nature, 321, 522). Overall, CDR-grafted antibodies consist of more than 80% human amino acid sequences
30 (Queen, C. et al. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 10029; Carter, P. et al. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4285). Despite these efforts, CDR-grafted, humanized antibodies were shown to still evoke an antibody

response against the grafted V region (Hwang, W. Y., et al. (2005). *Methods*, 36, 3).

Subsequently to CDR grafting, humanization methods based on
5 different paradigms such as resurfacing (Padlan, E. A., et al., (1991). *Mol. Immunol.*, 28, 489), superhumanization (Tan, P., D. A., et al., (2002) *J. Immunol.*, 169, 1119), human string content optimization (Lazar, G. A., et al., (2007). *Mol. Immunol.*, 44, 1986) and humaneering have been developed in an attempt to further decrease the content of non-human sequences in
10 therapeutic mAbs (Almagro, J. C., et al., (2008). *Frontiers in Bioscience* 13, 1619). As in CDR grafting approaches, these methods rely on analyses of the antibody structure and sequence comparison of the non-human and human mAbs in order to evaluate the impact of the humanization process into immunogenicity of the final product. When comparing the
15 immunogenicity of chimeric and humanized antibodies, humanization of variable regions appears to decrease immunogenicity further (Hwang, W. Y., et al. (2005). *Methods*, 36, 3-10).

De-immunization is another approach developed to reduce the
20 immunogenicity of chimeric or mouse antibodies. It involves the identification of linear T-cell epitopes in the antibody of interest, using bioinformatics, and their subsequent replacement by site-directed mutagenesis to human or non-immunogenic sequences (WO09852976A1). Although de-immunized antibodies exhibited reduced immunogenicity in
25 primates, compared with their chimeric counterparts, some loss of binding affinity was observed (Jain, M., et al., (2007). *Trends in Biotechnol.* 25, 307).

The development of phage display technology complemented and
30 extended humanization approaches in attempts to obtain less immunogenic mAbs for therapy in humans. In phage display, large collections ('libraries') of human antibody VH and VL regions are expressed on the surface of

filamentous bacteriophage particles. From these libraries, rare phages are selected through binding interaction with antigen; soluble antibody fragments are expressed from infected bacteria and the affinity of binding of selected antibodies is improved by mutation (Winter, G., et al. (1994). *Annu. Rev. Immunol.* 12, 433). The process mimics immune selection, and antibodies with many different bindings specificities have been isolated using this approach (Hoogenboom, H. R., et al. (2005). *Nat. Biotechnol.*, 23, 1105). Various sources of H and L chain V regions have been used to construct phage display libraries including those isolated from non-immune or immune donors. In addition, phage display libraries have been constructed of V regions that contain artificially randomized synthetic CDR regions in order to create additional diversity. Often, antibodies obtained from phage display libraries are subjected to in vitro affinity maturation to obtain high affinity antibodies (Hoogenboom, H. R., et al. (2005). *Nat. Biotechnol.*, 23, 1105).

The creation of transgenic mouse strains producing human antibodies in the absence of mouse antibodies has provided another technology platform for the generation of specific and high affinity human mAbs for application in humans. In these transgenic animals, the endogenous mouse antibody machinery is inactivated and replaced by human Ig loci to substantially reproduce the human humoral immune system in mice (Jakobovits, A., et al. (2007). *Nat. Biotechnol.* 25, 1134. Lonberg, N. (2005). *Nat. Biotechnol.* 23, 1117). B cell development as well as Ig diversification by recombination of gene segments is faithfully reproduced in these mice, leading to a diverse repertoire of murine B cells expressing human Igs. By immunizing these mice with antigens, it was further demonstrated that these transgenic animals accumulated somatic mutations in the V regions of both heavy and light chains to produce a wide diversity of high-affinity human mAbs (Lonberg, N. (2005). *Nat. Biotechnol.* 23, 1117).

The question, whether “fully human” mAbs such as derived from phage display libraries or transgenic mice are less immunogenic than humanized mAbs cannot be answered yet, because full immunogenicity data are available for just two human mAbs. An anti-tumor necrosis factor mAb, developed from phage-displayed human libraries induced antibody responses in 12% of patients - at the higher end of the incidence of anti-antibody responses of the humanized antibodies (Hwang, W. Y., et al. (2005). *Methods*, 36, 3-10).

10 Evaluation of the immunogenicity of the first registered human mAb generated by the transgenic approach demonstrated that mAb treatment resulted in the generation of antibodies in approximately 5.5% of treated cancer patients (Jakobovits, A., et al. (2007). *Nat. Biotechnol.* 25, 1134., Lofgren, J. A., et al. (2007). *J. Immunol.* 178, 7467).

15 A need therefore remains for a method and means for producing antibodies that are specific for their targets, but are less immunogenic. According to the invention the reduction of immunogenicity is at least partially achieved by providing a transgenic non-human mammal comprising, at least in its B cell lineage, a nucleic acid encoding at least an immunoglobulin light chain or heavy chain, wherein the heavy- or light chain encoding sequence is provided with a means that renders it resistant to DNA rearrangements and/or somatic hypermutations, preferably such a non-human animal is a rodent, more specifically a mouse. The nucleic acid preferably encodes a human, human-like or humanized immunoglobulin chain.

In the remainder of the specification mice are typically used as examples of the non-human mammals. The transgenic non-human mammalian hosts are capable of mounting an immune response to an antigen, where the response produces antibodies having primate, particularly human, variable regions. Various transgenic hosts may be

employed, particularly murine, lagomorpha, ovine, avine, porcine, equine, canine, feline, or the like. Mice have been used for the production of B-lymphocytes for immortalization for the production of antibodies. Since mice are easy to handle, can be bred in large numbers, and are known to
5 have an extensive immune repertoire, mice will usually be the animal of choice. Therefore, in the following discussion, the discussion will refer to mice, but it should be understood that other animals, particularly non-primate mammals, may be readily substituted for the mice, following the same procedures.

10

The reason for preventing rearrangements and hypermutation is that in this manner a non-immunogenic polypeptide can be chosen beforehand knowing that this polypeptide chain will remain non-immunogenic. At least one of the chains of the resulting immunoglobulin is thus less immunogenic.
15 The resulting antibody needs to have (usually) both a light- and a heavy chain. The non-immunogenic chain must therefore be capable of pairing with the other chain. The other chain may be an endogenous chain, an exogenous chain or a hybrid of both. For human therapy, the non-immunogenic chain should be as close to human as possible.

20

A means for rendering a gene encoding an immunoglobulin chain (or chains) resistant to DNA rearrangement and/or mutation is of course removal of all genetic elements responsible for said rearrangement and/or mutation. The drawback thereof is that the variability of the two chains is
25 eliminated, whereas the invention preferably retains the variability in one chain (preferably the heavy chain) and inhibits and/or prevents the rearrangement-mutation of the other chain (preferably the light chain).

The elements for rearrangement and/or hypermutation characterized
30 so far are located within the loci for immunoglobulins. Therefore the means for rendering the immunoglobulin encoding sequence resistant to DNA

rearrangement and/or mutation is inserting the gene in a locus outside the immunoglobulin loci.

Thus according to the invention a transgenic non-human mammal is provided wherein the light/heavy chain encoding sequence is integrated in the genome of the non-human mammal in a locus outside the immunoglobulin loci. Preferably the insertion is in a locus that is resistant to gene silencing. According to the invention, the integration is in the Rosa-locus or a comparable locus.

It is preferred to provide an expression cassette which can be inserted in a Rosa locus or comparable locus with a means that allows expression of the immunoglobulin chain(s) essentially limited to cells of B cell lineage, preferably with a means that allows expression of the light chain encoding nucleic acid during a certain stage of the development of B cells. The term "essentially limited expression" indicates that expression is predominantly in cells of the B-cell lineage, but that lower levels of expression in other cells, as compared to the level of expression in B-cells, is possible. In a preferred embodiment, the term "essentially limited expression" indicates that the expression is exclusively present in cells of the B-cell lineage. Such means typically and preferably include B cell (developmental stage) specific promoters such as CD19, CD20, μ HC (all V-genes), VpreB1, VpreB2, VpreB3, λ 5, Ig α , Ig β , κ LC (all genes), λ LC (all genes), BSAP (Pax5). Although it is very well possible to direct the expression of the DNA rearrangement and/or mutation resistant chain by such promoters, they are relatively weak. A strong promoter will typically be required to ensure adequate surface expression of the B cell receptor (made up of the membrane attached Ig H and L chain) and to compete with the expression and pairing of endogenous chains (if present) through allelic exclusion. Such a promoter, however is usually not tissue specific. To confer tissue specificity, an indirect system employing Cre/lox or the like is preferred. The desired chain is put under control of a strong promoter

inhibited by an element that can be removed by the action of a Cre-protein, leading to activation of the desired immunoglobulin encoding gene. This system is described in detail in Wunderlich F. T. (2004), "Generation of inducible Cre systems for conditional gene inactivation in mice",

- 5 Inauguraldissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultät der Universität zu Köln;
http://deposit.ddb.de/cgi-bin/dokserv?idn=97557230x&dok_var=d1&dok_ext=pdf&filename=97557230x.pdf.

- 10 Preferably the immunoglobulin chain produced in a manner resistant to rearrangements and hypermutation is a light chain capable of pairing with different heavy chains encoded by the non-human mammal. The light chain will then be the same (and less immunogenic) in all antibodies, but variety in specificity is retained through rearrangements and
- 15 hypermutations in the heavy chains. It may in that case be preferable to silence at least one of the endogenous loci encoding a light chain, although allelic exclusion may render this unnecessary.

- According to this embodiment, preferably the endogenous kappa (κ)
- 20 light chain locus is functionally silenced.

- If the endogenous κ light chain locus is silenced, but also for other reasons, it is preferred that the resistant light chain is a κ light chain, preferably a light chain that has a germline-like sequence. According to the
- 25 invention such a light chain would lead to an antibody with reduced immunogenicity. The preferred germline sequence is based on the human IGKV1-39 (O12) as this light chain is very frequently observed in the human repertoire (de Wildt et al. 1999. J. Mol. Biol. 285(3):895) and has superior thermodynamic stability, yield and solubility (Ewert et al. 2003. J.
- 30 Mol. Biol. 325(3):531).

The following gives more specific embodiments of the expression cassette with which the non-human animal can be provided according to the invention. Although this is typically advantageous for immunoglobulins, other genes of interest are also contemplated.

5

Thus the invention provides in a specific embodiment a transgenic non-human mammal wherein the light chain encoding nucleic acid comprises in 5'-3' direction: a B cell specific promoter, a leader, a rearranged human V gene, optionally a MoEki enhancer, a constant region (κ) and optionally a (truncated) MoEk3' enhancer. Neuberger identified and examined a novel B-cell specific enhancer located downstream of the kappa constant region (EP004690251). This enhancer has been shown to play a crucial role in the expression of kappa genes as removal of the 808bp enhancer strongly reduced expression. Deletion of the 3' kappa enhancer also strongly reduced the level of somatic hypermutations (SHM). In transgenic and cell expression studies it has been revealed that reduced, mutated or deleted 3' kappa enhancers not only lowered expression levels but also decreased the level of somatic hypermutations. Currently, it can not be determined whether the 3' kappa enhancer is involved in SHM processes, expression regulation or both (review Odegard, V. H., et al. (2006). Nat. Rev. Immunol. 6, 573; Inlay, M., et al. (2002). Nat. Immunol. 3, 463.).

Detailed expression studies using engineered variants of the 3' kappa enhancer indicated that a 50 nucleotide region is sufficient to drive expression. However for proper expression a reduced sequence of 145 nucleotides is preferred (EP04690251; Meyer, K. B., et al. (1990) Nucleic Acids Res. 18(19):5609-15)

Thus the invention in one aspect provides a nucleic acid for insertion into the genome of a non human animal that is an expression cassette for the expression of a desired proteinaceous molecule in cells developing into

30

mature B cells during a certain stage of development, said cassette comprising means for preventing silencing of expression of the desired proteinaceous molecule after introduction into a host cell, and means for timing expression of the desired proteinaceous molecule with the desired
5 developmental stage of the host cell.

An expression cassette is defined as a nucleic acid that has been provided with means for introduction into the genome of a host cell, such as sequences which allow for homologous recombination with a certain site in
10 the genome. Usually the nucleic acid will be DNA, typically double stranded. Typically the expression cassette will be provided to the cell in a vector from which it is transferred to the genome of the cell. The expression cassette further comprises all elements necessary for expression of the gene in a host cell, although in certain embodiments some of such elements may
15 be present on a second nucleic acid to be introduced, whereby these elements act in trans. Elements necessary for expression in a host cell include promoters, enhancers and other regulatory elements. Only those elements are necessary that are not provided by the host cell.

20 According to the invention it is important that the expression of the gene of interest is not silenced in the genome of the host cell, especially not in the development stage where expression is required. This can be done by various means, such as insertion into the endogenous locus or by providing the cassette with nucleic acid elements that prevent
25 silencing (Kwaks et al. (2006) Trends Biotechnol. 24(3), p.137-142; which is incorporated herein by reference). It is preferred that the expression cassette is inserted in a locus that is not silenced in the host cells (EP 01439234; which is incorporated herein by reference).

30 Said means for prevention of silencing comprise STabilizing Anti-Repression-sequences (STAR®-sequences) and Matrix Attachment Regions (MARs). A STAR sequence is a nucleic acid sequence that comprises a

capacity to influence transcription of genes in cis. Typically, although not necessarily, a STAR sequence does not code by itself for a functional protein element. In one embodiment one STAR element is used. Preferably, however, more than one STAR element is used. In a particularly preferred
5 embodiment an expression cassette according to the invention is provided with two STAR sequences; one STAR sequence at the 5' side of the coding sequence of the immunoglobulin gene and one STAR sequence at the 3' side of the coding sequence of the immunoglobulin gene. MARs are DNA
10 sequences that are involved in anchoring DNA/chromatin to the nuclear matrix and they have been described in both mammalian and plant species. MARs possess a number of features that facilitate the opening and maintenance of euchromatin. MARs can increase transgene expression and limit position-effects.

15 According to the invention it is important that expression from the cassette only occurs during a certain period in the development of a cell, in particular a developing B cell, more in particular a B cell in a transgenic non-human animal, in particular a mouse. In this particular case the developmental period is chosen such that the expression of the gene from
20 the cassette (typically a light- or heavy chain-like polypeptide) does not significantly interfere with the normal differentiation and/or maturation of the cell and when applicable, allows for pairing of the polypeptide chain produced with its counterpart.

25 According to the invention this may, in one embodiment, be achieved by providing a nucleic acid according to the invention, wherein said means for timing expression is a promoter of which the activity is essentially limited to the certain stage of development. In a developing B cell, which, e.g. after immunization is maturing and/or differentiating, the expression of
30 the gene of interest, when it is one of the polypeptide chains of an immunoglobulin, must not interfere (significantly) with said maturation and/or differentiation and it needs to be timed such that the resulting

polypeptide can pair with its counterparts. Therefore the invention provides a nucleic acid according to the invention wherein said certain stage starts at a stage immediately preceding or coinciding with the onset of the expression of light chain molecules by said cells at a certain stage of development into
5 a mature B cell.

This may be achieved by selecting a promoter which is active only during said suitable period. Such a promoter may be a CD19 promoter, the Ig- α promoter, the Ig- β promoter, the μ hc (all genes) promoter, the Vk
10 promoter or analogues or homologues thereof.

In a specific embodiment of the present invention the promoter as disclosed above does not drive the expression of the gene of interest directly. Instead it drives the expression of a gene of which the product activates in
15 trans the expression of the gene of interest. Such an activating gene may be a gene encoding a so-called Cre recombinase or Cre-like protein. The expression cassette for the gene of interest may e.g. be provided with a sequence that inhibits expression of the gene of interest. Said sequence can be removed by the action of the Cre recombinase, which is under control of
20 the desired promoter (active during the proper stage of development). In this embodiment a set of expression cassettes is required.

Therefore the invention provides a set of nucleic acids that are expression cassettes, wherein one nucleic acid comprises an expression
25 cassette encoding a Cre-like protein under control of a promoter active during the desired stage of development of the host cell and the second nucleic acid comprises a sequence encoding a desired proteinaceous molecule under control of a constitutive promoter which can be activated by the action of a Cre-like protein. Said activation is preferably achieved by
30 removal of a stop sequence flanked by loxP sites. The Cre/lox system is described in detail in Rajewsky et al. (1996) J. Clin. Invest. 98, p.600-603, which is incorporated herein by reference. Such systems are reviewed in

Wunderlich F. T. (2004), "Generation of inducible Cre systems for conditional gene inactivation in mice", Inauguraldissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultät der Universität zu Köln; http://deposit.ddb.de/cgi-bin/dokserv?idn=97557230x&dok_var=d1&dok_ext=pdf&filename=97557230x.pdf, which is incorporated herein by reference.

The invention further provides a transgenic non-human animal which has been provided with an expression cassette according to invention, wherein the desired proteinaceous molecule is a polypeptide chain of an immunoglobulin. A preferred polypeptide chain is a light chain. A more preferred polypeptide is a germline or germline-like light chain. A most preferred polypeptide is O12, preferably the rearranged germline kappa light chain IGKV1-39*01/ IGKJ1*01 (nomenclature according to the IMGT database, <http://www.imgt.org>).

It is further preferred that the polypeptide chain is rendered essentially incapable of rearrangement and/or of excluded of any sequence modification such as normally operating on Ig during the process of B cell affinity maturation. Therefore, the invention provides a transgenic non-human animal which has been provided with an expression cassette according to the invention, wherein said re-arrangement and/or sequence modifications are prevented by the absence of elements at least partially responsible for somatic hypermutation such as, for example, the MoEki enhancer.

A preferred expression cassette according to the invention comprises means for prevention of silencing. In one embodiment, said means for prevention of silencing are means for insertion into a locus in the genome of the host cell that is resistant to silencing. Said means for insertion are preferably means for homologous recombination into said site resistant to

silencing. A preferred locus when the non-human animal is a mouse is the rosa-locus.

A further preferred expression cassette according to the invention
5 comprises in 5'-3' direction: a V_k promoter, a mouse leader, a human V gene, optionally a MoE_ki enhancer, a rat constant region (C_k) and optionally a (truncated) MoE_k3' enhancer.

Yet a further preferred expression cassette according to the invention
10 comprises in 5'-3' direction: a V_k promoter, a human leader, a human V gene, optionally a MoE_ki enhancer, a rat constant region (C_k) and optionally a (truncated) MoE_k3' enhancer.

Of course the ultimate goal of the invention is to produce antibodies
15 to be used in human therapeutics. Thus the invention provides a method for producing a desired antibody comprising exposing a non-human mammal according to the invention to an antigen such that an antibody response is induced and isolating the antibodies specific for the antigen.

20 In an alternative embodiment, the invention provides a method for producing a desired antibody comprising exposing a non-human mammal according to the invention to an antigen such that an antibody response is induced and isolating cells producing such antibodies, culturing and optionally immortalizing said cells and harvesting said antibodies.

25 In a further embodiment, the invention provides a method for producing a desired antibody comprising exposing a non-human mammal according to the invention to an antigen such that an antibody response is induced and isolating a nucleic acid encoding at least part of such an
30 antibody, inserting said nucleic acid or a copy or a derivative thereof in an expression cassette and expressing said antibody in a host cell.

The methods for producing antibodies from transgenic mice are known to a person skilled in the art. Particularly preferred are methods for production of mixtures of antibodies from one cell, whereby the nucleic acids encoding these antibodies have been derived from mice according to
5 the invention.

These so-called oligoclonics are disclosed in WO04106375 and WO05068622, which are incorporated herein by reference.

10 The present invention provides transgenic non-human mammals, preferably mice, capable of generating specific and high affinity hybrid mouse-human antibodies with preferably human immunoglobulin light chain variable (VL) regions in or near germline configuration and preferably murine immunoglobulin heavy chain variable (VH) regions that
15 may have accumulated somatic mutations during the process of antigen-driven affinity maturation. It is envisaged that the murine VH regions of the hybrid antibodies may be subjected to humanization procedures to yield mAbs that have reduced immunogenicity when applied in humans based on germline or near-germline VL regions and murine VH regions that have
20 been humanized.

In particular, it has been shown in the present invention that transgenic mice that harbor a DNA expression construct that encodes a rearranged human VL region under the control of cis-acting genetic
25 elements that provide timely and regulated expression of the transgene on a significant proportion of B cells during B cell development, yet lack elements that direct the somatic hypermutation machinery to the transgene, are capable of generating specific and high affinity mouse-human hybrid antibodies with essentially unmutated L chains. It is shown
30 that the rearranged human transgene is capable of pairing with a diversity of endogenous murine immunoglobulin H chains to form mouse-human hybrid immunoglobulins expressed on the surface of B cells and to

sufficiently facilitate murine B cell development to obtain a sizeable and diverse peripheral B cell compartment.

In a preferred embodiment, the transgene expression construct
5 harbors the coding sequences of a human rearranged L chain V region under the control of a human VL promoter to direct B-cell specific expression. In addition, the construct harbors the murine 3' Ck enhancer sequence for B cell specific and inducible and high level expression of the transgene. Furthermore, the construct is designed to lack regulatory
10 elements that facilitate the recruitment of the somatic hypermutation machinery to the transgene, such as the intron enhancer and the 3' C-kappa enhancer.

In a related embodiment, the rearranged human VL gene is inserted
15 in the murine Rosa26 locus by site-specific integration. The Rosa26 locus is useful in the context of the "targeted transgenesis" approach for efficient generation of transgenic organisms (such as mice) with a predictable transgene expression pattern.

20 In a preferred embodiment, the rearranged human VL region is selected for its capacity to pair with many different murine VH genes so as to ensure the generation of a population of B cells with a diverse VH gene repertoire. A method of obtaining such VL regions comprises amplifying a repertoire of rearranged VH genes from the B cells of mice and a repertoire
25 of human rearranged germline VL regions from the B cells of humans and cloning them into phagemid display vectors to prepare diverse libraries of hybrid immunoglobulins in bacteria. By nucleotide sequence analysis of collections of unselected and antigen-selected VH/VL pairs, human germline VL genes that pair with many different murine VH genes are
30 identified. A collection of human germline VL genes with this capacity is described.

In one embodiment, it is shown that upon immunization with antigen, the B cells are capable of mounting an immune response, leading to the generation of B cells that secrete hybrid antibodies with high specificity and affinity. The V regions encoding these antibodies are
5 characterized by the human transgenic light chain that harbors no or very few mutations and a murine heavy chain that harbors a variable number of mutations introduced by the somatic hypermutation machinery.

In a related embodiment, strategies to obtain high affinity hybrid
10 monoclonal antibodies from the transgenic mice by hybridoma and display technologies are contemplated as well as procedures to humanize the murine VH regions to obtain less immunogenic antibodies for application in humans.

15 In one embodiment, the invention provides an immunoglobulin L chain transgene construct comprising DNA sequences that encode a human immunoglobulin VL region in combination with a light chain constant region (CL) of an animal immunoglobulin protein, which sequences are operably linked to transcription regulatory sequences that, when integrated
20 in a non-human transgenic animal, produce an Ig VL-CL polypeptide with a human VL region that is not or marginally subject to somatic hypermutation. The Ig VL is capable of pairing with rearranged VH-CH polypeptides that are generated during B cell development in the non-human transgenic animal, with said VH-CH polypeptides retaining the
25 capacity to undergo somatic hypermutation upon stimulation. The CL region may be of any animal species and is generally capable of pairing with the CH regions of the non-human transgenic animal.

The invention also includes the use of a transgene construct as above
30 in producing a transgenic non-human animal capable of the production of hybrid antibodies consisting of VL-CL polypeptides and VH-CH polypeptides in which the VL region is of human origin and the CL, VH and

CH may be of any animal species, including human. Upon immunization, these transgenic animals are capable of generating high affinity antibodies encoded by somatically hypermutated VH genes and essentially non-mutated VL genes encoded by the transgene.

5

In another aspect, the invention provides a process for the production of a transgenic non-human animal capable of the production of hybrid antibodies in response to antigenic challenge, comprising functionally disrupting the endogenous immunoglobulin light chain locus and inserting
10 into the animal genome a transgene construct of the invention.

The invention includes the use of animals obtainable by this process in the production of B cells that produce immunoglobulin having human VL light chain. In another aspect of the invention there is provided a process
15 for the production of B cells that produce immunoglobulin having human VL and binding to a selected antigen, comprising challenging an animal obtainable by a process as above with said antigen and screening for B cells from said animal that bind said antigen. The invention further includes B cells obtainable by this process and hybridomas obtainable by
20 immortalizing such B cells, e.g. hybridomas obtained by fusing B cells as above with myeloma cells. The invention also includes a process for producing monoclonal antibody comprising cultivating such a hybridoma. In yet a further aspect, the invention provides the use of the above B cells in producing a hybridoma or corresponding monoclonal antibody.

25

Yet a further aspect of the invention is a process for the production of immunoglobulin having human VL chain and binding to a selected antigen, comprising challenging an animal obtainable as above with said antigen and obtaining immunoglobulin there from.

30

In one strategy, as an individual step, a rearranged VL region encoded by human germline V and J gene segments and a light chain

constant region of any animal species but preferably a murine constant region is introduced into the mouse germ line. The transgene DNA may be introduced into the pronuclei of fertilized oocytes or embryonic stem cells. The integration may be random or homologous depending on the particular strategy to be employed. For example, the VL transgene may be introduced by random insertion, resulting in mice that bear one or multiple copies of the transgene in the genome. Alternatively, the human VL transgene may be targeted to a specific genomic locus using site-specific recombination as described in the art.

10

In one preferred embodiment, the VL transgene is targeted to the murine ROSA26 locus which is a suitable integration site allowing strong and predictable expression of inserted transgenes (EP 1439234). The targeting vector allows insertion of a single copy of a gene expression cassette, thus avoiding modulation of transgene expression by the arrangement of multiple copies. By choosing the autosomal Rosa26 locus as insertion site, the expression pattern of the inserted transgene in the non-human animal is predictable. Furthermore, random X inactivation and/or modulation by chromosomal position effects are avoided. This also eliminates the need to generate and analyse multiple transgenic strains for any given transgene. Finally, the Rosa26 targeting vector for the site-specific integration can be used for multiple gene expression cassettes. Thus, it may be envisaged that 2 or more different rearranged germline human VL regions are inserted into the Rosa26 locus to further increase the diversity of the repertoire of hybrid or human antibodies.

25

In another embodiment, a rearranged human VL region may be targeted to the murine Ig kappa or lambda light chain locus so as to functionally inactivate the endogenous locus or mice containing the rearranged human VL region may be bred with mice that lack functional kappa or lambda Ig loci or both. Thus, by using transformation, using repetitive steps or in combination with breeding, transgenic animals may be

30

obtained which are able to produce antibodies harboring the human VL transgene in the substantial absence of endogenous host immunoglobulin light chains.

In one embodiment, a human VL transgene is selected for its capacity to pair with a substantial portion of murine VH regions to form a diverse repertoire of functional mouse-human hybrid antibodies expressed on the surface of B cells. By a substantial portion of murine VH regions is meant that the human VL pairs with at least with 0.1% of the murine VH regions generated during B cell development, more preferably with at least 1% and most preferably with at least 10%. Methods to identify human VL genes with this characteristic include randomly pairing a repertoire of human VL regions with a repertoire of murine VH regions, co-expression of VH and VL regions in appropriate eukaryotic or prokaryotic expression vectors and screening for human VL regions that pair with a substantial portion of murine VH regions. In one embodiment, phagemid vectors may be used to direct expression of mouse-human antibody fragments in bacterial cells or to the surface of filamentous phage and analysis of binding capacity of antibody fragments by methods known in the art.

In another embodiment, a human VL transgene is selected for its capacity to pair with a substantial portion of human VH regions to form a diverse repertoire of human antibodies expressed on the surface of B cells. By a substantial portion of human VH regions is meant that the human VL pairs with at least with 0.1% of the human VH regions generated during B cell development, more preferably with at least 1% and most preferably with at least 10%.

In the latter embodiment, the human VL transgenic mice are crossed with mice that harbor functional rearranged or non-rearranged human H chain immunoglobulin loci and functionally inactivated endogenous H chain Ig loci as described in the art. The functional inactivation of the two copies of each of the three host Ig loci (heavy chain, kappa and lambda light

chain), where the host contains the human IgH and the rearranged human VL transgene would allow for the production of purely human antibody molecules without the production of host or host human chimeric antibodies. Such a host strain, by immunization with specific antigens, would respond by the production of mouse B-cells producing specific human antibodies, which B-cells are subsequently fused with mouse myeloma cells or are immortalized in any other manner for the continuous stable production of human monoclonal antibodies. Alternatively, said population of B cells is used as a source of VH regions that can be obtained by constructing cDNA libraries or by PCR amplification using primers for human VH regions as is known in the art.

A human rearranged VL gene is reconstructed in an appropriate eukaryotic or prokaryotic microorganism and the resulting DNA fragments can be introduced into pronuclei of fertilized mouse oocytes or embryonic stem cells. Various constructs that direct B cell specific expression of VL transgenes have been described in the art and have the following general format: a leader sequence and relevant upstream sequences to direct B cell specific expression of the transgene, a coding sequence of a human VL transgene, an enhancer sequence that directs B cell specific and high level expression of the transgene and a murine constant region gene. In a preferred format, the enhancer is the C-kappa 3' enhancer because it directs high level expression in B-lineage cells, but does not recruit somatic hypermutation when used in transgene constructs.

25

In one embodiment, animals, preferably mice, comprising one or multiple copies of the transgene in the genome are isolated and analyzed for stable expression. Animals are selected that show stable expression of the transgene over longer periods of time, preferably in B-cells. If required, different animal lines comprising independent insertions of one or multiple copies of the transgene, preferably on different chromosomes, are crossed to obtain animals with different insertions of one or multiple copies of the

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transgene to increase expression of the transgene in animals, preferably in B-cells.

5 The invention further provides progeny of a transgenic non-human animal according to the invention, the progeny comprising, at least in its B-cell lineage, a heavy- or light chain encoding sequence together with a means that renders the sequence resistant to DNA rearrangements and/or somatic hypermutations.

10 The invention further provides progeny of a transgenic non-human animal according to the invention, the progeny comprising an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B cells.

15 The invention in addition provides a cell that is isolated from a transgenic non-human animal according to the invention, the cell comprising a heavy- or light chain encoding sequence together with a means that renders the sequence resistant to DNA rearrangements and/or somatic hypermutations. The invention in addition provides a cell that is isolated from a transgenic non-human animal according to the invention, the cell comprising an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B cells. A cell according to the invention, preferably an antibody-producing B-cell or a cell that is capable of differentiating or maturing into an antibody-producing B-cell, can be used for *in vitro* production of antibodies, as is known to the skilled person, for example from Gascan et al. 1991. J. Exp. Med. 173: 747-750. Methods for immortalization of a cell according to the invention are known in the art and include the generation of hybridomas, for example by fusion with a myeloma cell, transformation with Epstein Barr Virus; expression of the signal transducer of activation and transcription (STAT), activation via

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CD40 and IL4 receptor signaling, and/or expression of Bcl6 (Shvarts et al. 2002. Genes Dev 16: 681–686).

In a separate step the mouse endogenous Kappa and Lambda light
5 chain loci are rendered essentially non-functional such that at least the
majority of B cells in the transgenic mice bear Ig receptors that contain the
transgenic human VL region. Inactivation of the endogenous mouse
immunoglobulin loci is achieved by targeted disruption of the appropriate
loci by homologous recombination in mouse embryonic stem cells. Said
10 targeted disruption comprises alteration of the genomic sequence such that
substantially no functional endogenous mouse immunoglobulin Kappa
and/or Lambda light chain is produced. The term "substantially no
functional endogenous mouse immunoglobulin" indicates that the
endogenous Kappa and/or Lambda light chain loci are functionally silenced
15 such that the level of functional protein expression of the endogenous
Kappa and/or Lambda light chain loci, preferably the endogenous Kappa
light chain locus, is reduced to about 20% of the level of expression in a
reference mouse, more preferred to about 10%, more preferred to about 5%,
more preferred to about 2% and more preferred to about 1%. In a most
20 preferred embodiment, the level of functional protein expression of the
endogenous Kappa and/or Lambda light chain loci is reduced to 0%. The
level of functional protein expression can be determined by means known to
the skilled person, including western blotting and pairing with a mouse
heavy chain. Said reference mouse is a mouse in which the endogenous
25 Kappa and/or Lambda light chain loci is not disrupted. Said alteration
comprises mutation and/or deletion of gene sequences that are required for
functional expression of the endogenous immunoglobulin genes.
Alternatively, said alteration comprises insertion of a nucleic acid into the
endogenous mouse immunoglobulin Kappa and/or Lambda light chain loci
30 such that the functional expression of the endogenous immunoglobulin
genes is reduced. In one embodiment, said nucleic acid comprises a
silencing element resulting in transcriptional silencing of the endogenous

immunoglobulin gene. In a further embodiment, or in addition, said nucleic acid comprises a sequence that disrupts splicing and/or translation of the endogenous immunoglobulin gene, for example by introducing an exon that renders a frame shift in the coding sequence, or that comprises a premature
5 stop codon. In each case chimeric animals are generated which are derived in part from the modified embryonic stem cells and are capable of transmitting the genetic modifications through the germ line. The mating of mouse strains with human immunoglobulin loci to strains with inactivated mouse loci yields animals which produce antibodies comprising essentially
10 only human light chains.

A construct for homologous recombination is prepared by means known in the art and any undesirable sequences are removed, e.g., procaryotic sequences. Any convenient technique for introducing a construct
15 for homologous recombination into a target cell may be employed. These techniques include spheroplast fusion, lipofection, electroporation, calcium phosphate-mediated DNA transfer or direct microinjection. After transformation or transfection of the target cells, target cells are selected by means of positive and/or negative markers, for example by neomycin
20 resistance and/or acyclovir and/or gancyclovir resistance. Those cells which show the desired phenotype may then be further analyzed by restriction analysis, electrophoresis, Southern analysis, PCR, or the like. By identifying fragments which show the presence of the lesion(s) at the target locus, cells in which homologous recombination has occurred to inactivate a
25 copy of the target locus are identified.

Furthermore, it is shown that upon immunization, the murine and human VH regions in the afore-mentioned transgenic mice but not the VL regions are capable of undergoing somatic hypermutations to generate high
30 affinity antibodies. Advantageously, these antibodies encoded by germline VL regions are predicted to contribute to lower immunogenicity when

applied in humans and result in more stable antibodies that are less prone to aggregation and thus safer for therapeutic use in humans.

MAbs derived from the afore-mentioned non-human transgenic
5 animals or cells all share the same identical human VL regions. It has been described that mAbs that share the same identical VL region may be co-expressed in a single clonal cell for the production of mixtures of recombinant antibodies with functional binding sites (see WO04106375 and WO05068622). Thus, the invention provides a platform for the generation of
10 specific and high affinity mAbs that constitute the basis for mixtures of mAbs produced by clonal cells.

It is preferred that mAbs derived from the afore-mentioned non-human transgenic animals or cells are directed against cellular targets.
15 Preferred targets are human surface-expressed or soluble proteins or carbohydrate molecules. Further preferred targets are surface-expressed proteins or carbohydrate molecules that are expressed on the surface of bacteria, viruses, and other pathogens, especially of humans.

20 More specifically, preferred targets include cytokines and chemokines, including but not limited to InterLeukin 1beta (IL1beta), IL2, IL4, IL5, IL7, IL8, IL12, IL13, IL15, IL18, IL21, IL23 and chemokines such as, for example, CXC chemokines, CC chemokines, C chemokines (or γ chemokines) such as XCL1 (lymphotactin- α) and XCL2 (lymphotactin- β),
25 and CX3C chemokines. Further included as preferred targets are receptor molecules of the cytokines and chemokines, including type I cytokine receptors such as, for example, the IL-2 receptor, type II cytokine receptors such as, for example interferon receptors, immunoglobulin (Ig) superfamily receptors, tumor necrosis factor receptor family including receptors for
30 CD40, CD27 and CD30, serine/threonine-protein kinase receptors such as TGF beta receptors, G-protein coupled receptors such as CXCR1-CXCR7, and tyrosine kinase receptors such as fibroblast growth factor receptor

(FGFR) family members, EGF receptor family members including erbB1 (EGF-R; HER1), erbB2, (HER2), erbB3 (HER3), and erbB4 (HER4), insulin receptor family members including IGF-R1 and IGF-RII, PDGF receptor family members, Hepatocyte growth factor receptor family members including c-Met (HGF-R), Trk receptor family members, AXL receptor family members, LTK receptor family members, TIE receptor family members, ROR receptor family members, DDR receptor family members, KLG receptor family members, RYK receptor family members, MuSK receptor family members, and vascular endothelial growth factor receptor (VEGFR) family members.

Further preferred targets are targets that are over-expressed or selectively expressed in tumors such as, for example, VEGF, CD20, CD38, CD33, CEA, EpCAM, PSMA, CD54, Lewis Y, CD52, CD40, CD22, CD51/CD61, CD74, MUC-1, CD38, CD19, CD262 (TRAIL-R2), RANKL, CTLA4, and CD30; targets that are involved in chronic inflammation such as, for example, CD25, CD11a, TNF, CD4, CD80, CD23, CD3, CD14, IFNgamma, CD40L, CD50, CD122, TGFbeta and TGFalpha.

Preferred surface-expressed proteins or carbohydrate molecules that are expressed on the surface of bacteria, viruses, and other parasitic pathogens, especially of humans, include surface markers of influenza A and B viruses such as hemagglutinin (HA) and neuraminidase (NA), filoviruses such as Ebola virus, rabies, measles, rubella, mumps, flaviviruses such as Dengue virus types 1-4, tick-borne encephalitis virus, West Nile virus, Japanese encephalitis virus, and Yellow fever virus, Paramyxoviruses including Paramyxovirus such as Parainfluenza 1, 3, Rubulavirus such as Mumpsvirus and Parainfluenza 2, 4, Morbillivirus, and Pneumovirus such as Respiratory syncytial virus, Vaccinia, small pox, coronaviruses, including Severe Acute Respiratory Syndrome (SARS) virus, hepatitis virus A, B and C, Human Immunodeficiency Virus, Herpes viruses, including cytomegalovirus, Epstein Barr virus, Herpes simplex

virus, and Varicella zoster virus, parvoviruses such as, for example, B19; Legionella pneumophila; Listeria monocytogenes; Campylobacter jejuni; Staphylococcus aureus; E. coli O157:H7; Borrelia burgdorferi; Helicobacter pylori; Ehrlichia chaffeensis; Clostridium difficile; Vibrio cholera;

5 Salmonella enterica Serotype Typhimurium; Bartonella henselae; Streptococcus pyogenes (Group A Strep); Streptococcus agalactiae (Group B Strep); Multiple drug resistant S. aureus (e.g. MRSA); Chlamydia pneumoniae; Clostridium botulinum; Vibrio vulnificus; Parachlamydia pneumonia; Corynebacterium amycolatum; Klebsiella pneumonia;

10 Linezolid-resistant enterococci (E. faecalis and E. faecium); and Multiple drug resistant Acinetobacter baumannii.

Most preferred targets are IL-6 and its receptor, IL-6Ralpha, glycoprotein-denominated gp130, RSV, especially the surface proteins F, G

15 and SH and non-structural proteins such as N and M, and receptor tyrosine kinases, in particular erbB1 (EGF-R; HER1), erbB2, (HER2), erbB3 (HER3), erbB4 (HER4), IGF-R1 and IGF-R2, c-Met (HGF-R).

Therefore, the invention provides a platform for the generation of

20 specific and high affinity mAbs against the above mentioned targets that constitute the basis for mixtures of mAbs produced by clonal cells. In a preferred embodiment, said specific and high affinity mAbs comprise mAbs that are directed against different epitopes on at least one of the targets. In a further preferred embodiment, said specific and high affinity mAbs

25 comprise mAbs that are directed against different targets, such as, for example, one or more members of the EGF-receptor family, including erbB1 (EGF-R; HER1), erbB2, (HER2), erbB3 (HER3) and erbB4 (HER4).

Unless otherwise defined, scientific and technical terms used in

30 connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and

plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (3rd edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001)), which is incorporated herein by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Figure legends

Figure 1

A topology map of the annealing locations of mouse specific VH primers and the position of required restriction sites that are introduced by overhanging sequences at the 3' end of primers.

Figure 2

PCR amplification steps (Amplification, Intermediate and Site introduction). The location and names of the mouse VH amplification primers (and mixtures of primers) are indicated per step.

Figure 3

Topology of the MV1043 vector. This vector is used for the cloning of human or murine VH fragments. O12 (IGKV1-39) is indicated as the VL gene. Products of this vector in combination with helper phages in E. coli cells allow the generation of phages that display Fab fragments on the surface of the phage particles as a fusion product to the g3 protein and presence of the vector in the phage as the genetic content (F1 ORI).

Figure 4

The topology of the mouse Ckappa locus downstream of the J-segments. Both enhancers and Ckappa region are indicated. The lower arrow indicates the region that is removed in order to silence the locus.

Figure 5

The topology of the mouse C-lambda locus. All three active V-regions are indicated (Igl-V1, V2 and V3) as are the J-segments (Igl-J1, Igl-J2, Igl-J3, Igl-J4 and the pseudo segment Igl-J3p) and constant regions (Igl-C1, Igl-C2, Igl-C3 and Igl-C4). The regions that are deleted in order to silence the locus are indicated by deletion markers. These deletions include all active V genes (1, 2 and 3) and the intergenic segment between V2 and V3.

Figure 6

Construct topology of IGKV1-39/J-Ck with an intron located in the leader open reading frame (ORF).

5

Figure 7

Construct topology of IGLV2-14/J-Ck with an intron located in the leader open reading frame (ORF).

10 Figure 8

Construct topology of VkP-IGKV1-39/J-Ck (VkP-O12). The promoter originates from the IGKV1-39 gene and is placed directly in front of the required elements for efficient transcription and translation. Intergenic sequences (including the enhancers) are derived from mice and obtained from BAC clones. The C-kappa sequence codes for the kappa constant region of rat.

Figure 9

Construct topology of VkP-IGLV2-14/J-Ck (VkP-2a2). The promoter originates from the IGKV1-39 gene and is placed directly in front of the required elements for efficient transcription and translation. Intergenic sequences (including the enhancers) are derived from mice and obtained from BAC clones. The C-kappa sequence codes for the kappa constant region of rat.

25

Figure 10

Construct topology of VkP-IGKV1-39/J-Ck-Δ1 (VkP-O12-del1) is identical to VkP-IGKV1-39/J-Ck from figure 9 except that the intron enhancer region is removed.

30

Figure 11

Construct topology of VkP-IGKV1-39/J-Ck- Δ 2 VkP-O12-del2) is identical to VkP-IGKV1-39/J-Ck- Δ 1 from figure 10 except that a large piece of the intergenic region between the Ck gene and 3'enhancer is deleted. In addition, the 3' enhancer is reduced in size from 809 bp to 125 bp.

Figure 12

Overview of the sequences used or referred to in this application.

10 Figure 13

Generation of Rosa26-IgVk1-39 KI allele. (A) Schematic drawing of the pCAGGS-IgVK1-39 targeting vector. (B) Nucleotide sequence of the pCAGGS-IgVK1-39 targeting vector. (C) Targeting strategy.

15 Figure 14

(A) Southern blot analysis of genomic DNA of ES clones comprising an insertion of the pCAGGS-IgVK1-39 targeting vector. Genomic DNA of 4 independent clones was digested with AseI and probed with 5e1 indicating the 5'-border of the targeting vector. All clones comprise a correct insertion of the targeting vector at the 5' end.

(B) Southern blot analysis of genomic DNA of ES clones comprising an insertion of the pCAGGS-IgVK1-39 targeting vector. Genomic DNA of 4 independent clones was digested with MscI and probed with 3e1 indicating the 3'-border of the targeting vector. All clones comprise a correct insertion of the targeting vector at the 3' end.

(C) Southern blot analysis of genomic DNA of ES clones comprising an insertion of the pCAGGS-IgVK1-39 targeting vector. Genomic DNA of 4 independent clones was digested with BamHI and probed with an internal Neo probe indicating the 5'-border of the targeting vector. All clones comprise a correct, single insertion of the targeting vector.

Figure 15

5 Generation of Rosa26-IgVL2-14 KI allele. (A) Schematic drawing of the pCAGGS-IgVL2-14 targeting vector. (B) Nucleotide sequence of the pCAGGS-IgVL2-14 targeting vector containing the CAGGS expression insert based on the rearranged germline IGLV2-14/J V lambda region (IGLV2-14/J-Ck). (C) Targeting strategy.

10

Figure 16

 Epibase[®] profile of IGKV1-39 residues 1-107. Sub-figure A displays the binding strength for DRB1 allotypes, while C displays the binding strength for DRB3/4/5, DQ and DP allotypes. The values in the figure represent dissociation constants (Kd's) and are plotted on a logarithmic scale in the range 0.01 μ M - 0.1 μ M (very strong binders may have run off the plot). For medium binding peptides, qualitative values are given only, and weak and non-binders are not shown. Values are plotted on the first residue of the peptide in the target sequence (the peptide itself extends by another 9 residues). Importantly, only the strongest binding receptor for each peptide is shown: cross-reacting allotypes with lower affinity are not visible in this plot. The strongest binding receptor is indicated by its serotypic name. Finally, any germline-filtered peptides are plotted with a lighter color in the epitope map (in this case, no non-self epitopes were found). Sub-figure B shows the HLA binding promiscuity for every decameric peptide (Y-axis: the number of HLA allotypes recognizing critical epitopes in each of the peptides starting at the indicated residue shown on the X-axis). The promiscuity is measured as the number of allotypes out of the total of 47 for which the peptide is a critical binder. White columns refer to self-peptides, and black columns (absent here) to non-self peptides.

Figure 17

Epitope map of IGKV1-39 showing the presence of peptide binders predicted in the sequence of IGKV1-39 by serotype in the 15-mer format. Each 15-mer is numbered as indicated in the top of the figure. The full
 5 sequence of the corresponding 15-mer is listed in Table 7. Black boxes indicate the presence of one or more critical self-epitopes in the 15-mer for the serotype listed on the left. Critical epitopes are operationally defined as strong or medium DRB1 binders and strong DRB3/4/5 or DP or DQ binders.

10 Figure 18

Constitutive knock-out (KO) of the Ig kappa locus. (A) Targeting strategy. (B) Schematic drawing of the pIgKappa targeting vector.

Figure 19

15 Constitutive KO of the Ig lambda locus. (A) First step of the targeting strategy. (B) Second step of the targeting strategy.

Figure 20

20 Schematic drawing of targeting vectors. (A) pVkP-O12 (VkP-IGKV1-39/J-Ck); (B) pVkP-O12-del1 (VkP-IGKV1-39/J-Ck-Δ1); (C) pVkP-O12-del2 (VkP-IGKV1-39/J-Ck-Δ2).

Figure 21

25 Targeting strategies for insertion of transgene into the Rosa26 locus by targeted transgenesis using RMCE. (A) VkP-O12 (VkP-IGKV1-39/J-Ck); (B) VkP-O12-del1 (VkP-IGKV1-39/J-Ck-Δ1); (C) VkP-O12-del2 (VkP-IGKV1-39/J-Ck-Δ2).

Figure 22

30 Topology of the MV1057 vector. Replacing the indicated stuffer fragment with a VH fragment yields an expression vector that can be

transfected to eukaryotic cells for the production of IgG1 antibodies with light chains containing an O12 (IGKV1-39) VL gene.

Figure 23

5 Lack of transgenic human Vk1 light chain expression in non-B cell populations of the spleen.

Figure 24

10 Transgenic human Vk1 light chain is expressed in all B cell populations of the spleen.

Figure 25

15 Transgenic human Vk1 light chain is expressed in B1 cells of the peritoneal cavity.

Figure 26

20 Transgenic human Vk1 light chain is not expressed in pro- and pre-B cells but in the immature and recirculating populations B cells in the bone marrow. (A) Gating of bone marrow cells. (B) Histograms of transgene expression with overlay from one WT control.

Figure 27

25 Transgenic human Vk1 light chain is directly correlated with endogenous light chain and IgM expression in circulating B cells in the blood.

Examples.

Example 1

Human light chain V-gene clones

5 This example describes the rationale behind the choice of two human light chain V- genes, one gene of the kappa type and one gene of the lambda type, that are used as a proof of concept for light chain expressing transgenic mice. De Wildt et al. 1999 (de Wildt et al. (1999) J. Mol. Biol. 285(3):895) analysed the expression of human light chains in peripheral
10 IgG-positive B-cells. Based on these data, IGKV1-39 (O12) and IGLV2-14 (2a2) were chosen as light chains as they were well represented in the B-cell repertoire. The J-segment sequence of the light chains has been chosen based upon sequences as presented in GenBank ABA26122 for IGKV1-39 (Rabquer,B.J., Smithson,S.L., Shriner,A.K. and Westerink,M.A.J.) and
15 GenBank AAF20450 for IGLV2-14 (Ignatovich,O., Tomlinson,I.M., Popov,A.V., Bruggemann,M. and Winter,G. J. Mol. Biol. 294 (2), 457-465 (1999)).

All framework segments are converted into germline amino acid
20 sequences to provide the lowest immunogenicity possible in potential clinical applications.

Example 2

Obtaining mouse heavy chain V-genes that pair with human IGKV1-
25 39 gene segment to form functional antibody binding sites

This example describes the identification of mouse heavy chain V- genes that are capable of pairing with a single, rearranged human germline IGKV1-39/J region. A spleen VH repertoire from mice that were immunized with tetanus toxoid was cloned in a phage display Fab vector with a single
30 human IGKV1-39-C kappa light chain and subjected to panning against tetanus toxoid. Clones obtained after a single round of panning were analyzed for their binding specificity. The murine VH genes encoding

tetanus toxoid-specific Fab fragments were subjected to sequence analysis to identify unique clones and assign VH, DH and JH utilization.

Many of the protocols described here are standard protocols for the construction of phage display libraries and the panning of phages for
5 binding to an antigen of interest and described in Antibody Phage Display: Methods and Protocols (editor(s): Philippa M. O'Brien, Robert Aitken).

Immunizations

BALB/c mice received one immunization with tetanus toxoid and
10 were boosted after 6 weeks with tetanus toxoid.

Splenocyte isolation

Preparation of spleen cell suspension. After dissection, the spleen was washed with PBS and transferred to a 60 mm petridish with 20 ml
15 PBS. A syringe capped with 20 ml PBS and a G20 needle was used to repeatedly flush the spleen. After washing the flushed cells with PBS, the cells were carefully brought into suspension using 20 ml PBS and left on a bench for 5 minutes to separate the splenocytes from the debris and cell clusters. The splenocytes suspension was transferred on top of a Ficoll-
20 Paque™ PLUS-filled tube and processed according to the manufacturer's procedures for lymphocyte isolation (Amersham Biosciences).

RNA isolation and cDNA synthesis

After isolation and pelleting of lymphocytes, the cells were suspended
25 in TRIzol LS Reagent (Invitrogen) for the isolation of total RNA according to the accompanying manufacturer's protocol and subjected to reverse transcription reaction using 1 microgram of RNA, Superscript III RT in combination with dT20 according to manufacturer's procedures (Invitrogen).

PCR amplification of cDNA

The cDNA was amplified in a PCR reaction using primer combinations that allow the amplification of approximately 110 different murine V-genes belonging to 15 VH families (table 1; RefSeq NG_005838; Thiebe et al., 1999. European Journal of Immunology 29: 2072 - 2081). In the first round, primer combinations that bind to the 5' end of the V-genes and 3' end of the J regions were used. In the second round, PCR products that were generated with the MJH-Rev2 primer were amplified in order to introduce modifications in the 3' region to enable efficient cloning of the products. In the last round of amplification, all PCR products were amplified using primers that introduce a SfiI restriction site at the 5' end and a BstEII restriction site at the 3' end (see figures 1 and 2, and table 1).

Reaction conditions for 1st round PCR: 4 different reactions

combining all 25 forward primers (MVH1 to MVH25, table 1 and figure 2) and 1 reverse primer per reaction (MJH-Rev1, MJH-Rev2, MJH-Rev3 or MJH-Rev4; see table 1 and figure 2). 50 microliter PCR volumes were composed of 2 microliter cDNA (from RT reactions), 10 microliter 5* Phusion polymerase HF buffer, 40 nM of each of the 25 forward primers (total concentration of 1 micromolar), 1 micromolar reverse primer, 1 microliter 10 mM dNTP stock, 1.25 unit Phusion polymerase and sterile MQ water. The thermocycler program consisted of a touch down program: 1 cycle 98°C for 30 seconds, 30 cycles 98°C for 10 seconds, 58°C decreasing 0.2°C per cycle 10 seconds, 72°C 20 seconds and 1 cycle 72°C for 3 minutes. The second round PCR program was set up only for the products of the 1st PCR that contain the MJH-Rev2 primer: 2 different reactions combining either the ExtMVH-1 or ExtMVH-2 primers (table 1 and figure 2) in combination with the reverse primer ExtMJH-Rev2int (table 1 and figure 2). 50 microliter PCR volumes were composed of 50 ng PCR product (from first PCR round), 10 microliter 5* Phusion polymerase HF buffer, 500 nM of each forward primer, 1 micromolar reverse primer, 1 microliter 10 mM dNTP stock, 1.25 unit Phusion polymerase and sterile MQ water. The

thermocycler program consisted of a touch down program followed by a regular amplification step: 1 cycle 98°C for 30 seconds, 10 cycles 98°C for 10 seconds, 65°C decreasing 1.5°C per cycle 10 seconds, 72°C 20 seconds, 10 cycles 98°C for 10 seconds, 55°C 10 seconds, 72°C 20 seconds and 1 cycle 72°C for 3 minutes. The third round PCR program was setup as described in figure 2. 50 microliter PCR volumes were composed of 50 ng PCR product (from earlier PCR rounds, figure 2), 10 microliter 5* Phusion polymerase HF buffer, 1 micromolar forward primer (table 1 and figure 2), 1 micromolar reverse primer, 1 microliter 10 mM dNTP stock, 1.25 unit Phusion polymerase and sterile MQ water. The program consists of a touch down program followed by a regular amplification step: 1 cycle 98°C for 30 seconds, 10 cycles 98°C for 10 seconds, 65°C decreasing 1.5°C per cycle 10 seconds, 72°C 20 seconds, 10 cycles 98°C for 10 seconds, 55°C 10 seconds, 72°C 20 seconds and 1 cycle 72°C for 3 minutes. After PCR amplifications, all PCR products were gel purified using Qiaex II according to the manufacturer's protocols.

Restriction enzyme digestions

Purified products were digested with BstEII and SfiI in two steps. First 1 microgram of DNA was digested in 100 microliter reactions consisting of 10 microliters of 10* NEB buffer 3 (New England Biolabs), 1 microliter 100* BSA, 12.5 unit BstEII and sterile water for 6 hours at 60°C in a stove. The products were purified using Qiaquick PCR Purification kit from Qiagen according to the manual instructions and eluted in 40 microliter water. Next all products were further digested with SfiI in 100 microliter reactions consisting of 10 microliters of 10* NEB buffer 2 (New England Biolabs), 1 microliter 100* BSA, 12.5 unit SfiI and sterile water for 12 hours at 50°C in a stove. The digested fragments were purified by Qiaquick Gel Extraction kit following gel separation on a 20 cm 1.5% agarose TBE plus ethidium bromide gel at 80 V. 100 microgram of the acceptor vector (MV1043, figures 3 and 12) was digested with 50 units Eco91I in 600 microliter under standard conditions (Tango buffer) and next

purified on a 0.9% agarose gel. After a second digestion step under prescribed conditions with 400 units SfiI in 500 microliter for 12 hours, 100 units BsrGI were added for 3 hours at 50°C.

5 Ligations

Each PCR product was ligated separately according to the following scheme: 70 ng digested PCR products, 300 ng digested acceptor vector, 100 units T4 Ligase (NEB), 1* ligase buffer in 30 microliters for 16 hours at 12°C. The ligation reactions were purified with phenol/chloroform/isoamyl
10 alcohol extractions followed by glycogen precipitations (Sigma-Aldrich #G1767) according to the manufacturer's protocol and finally dissolved in 25 microliter sterile water.

Transformations and library storage

15 The purified ligation products were transformed by electroporation using 1200 microliter TG1 electrocompetent bacteria (Stratagene #200123) per ligation batch and plated on LB carbenicillin plates containing 4% glucose. Libraries were harvested by scraping the bacteria in 50 ml LB carbenicillin. After centrifugation at 2000g for 20 minutes at 4°C, the
20 bacterial pellets were resuspended carefully in 2 ml ice cold 2*TY/30% glycerol on ice water and frozen on dry-ice/ethanol before storage at -80°C.

Library amplification

Libraries were grown and harvested according to procedures as
25 described by Kramer et al. 2003 (Kramer et al. 2003. Nucleic Acids Res. 31(11): e59) using VCSM13 (Stratagene) as helper phage strain.

Selection of phages on coated immunotubes

Tetanus toxoid was dissolved in PBS in a concentration of 2 µg/ml
30 and coated to MaxiSorp Nunc-Immuno Tube (Nunc 444474) overnight at 4°C. After discarding the coating solution, the tubes were blocked with 2% skim milk (ELK) in PBS (blocking buffer) for 1 hour at RT. In parallel, 0.5

ml of the phage library was mixed with 1 ml blocking buffer and incubated for 20 minutes at room temperature. After blocking the phages, the phage solution was added to the tetanus toxoid coated tubes and incubated for 2 hours at RT on a slowly rotating platform to allow binding. Next, the tubes
5 were washed 10 times with PBS/0.05% Tween-20 followed by phage elution by an incubation with 1 ml 50 mM glycine-HCl pH 2.2 10 min at RT on rotating wheel and directly followed by neutralization of the harvested eluent with 0.5 ml 1 M Tris-HCl pH 7.5.

10

Harvesting phage clones

5 ml XL1-Blue MRF (Stratagene) culture at O.D. 0.4 was added to the harvested phage solution and incubated for 30 minutes at 37°C without shaking to allow infection of the phages. Bacteria were plated on
15 Carbenicillin/Tetracycline 4% glucose 2*TY plates and grown overnight at 37°C.

Phage production

Phages were grown and processed as described by Kramer et al. 2003
20 (Kramer et al. 2003. Nucleic Acids Res. 31(11): e59) using VCSM13 as helper phage strain.

Phage ELISA

ELISA plates were coated with 100 microliter tetanus toxoid per well
25 at a concentration of 2 microgram/ml in PBS overnight at 4°C. Plates coated with 100 microliter thyroglobulin at a concentration of 2 microgram/ml in PBS were used as a negative control. Wells were emptied, dried by tapping on a paper towel, filled completely with PBS-4% skimmed milk (ELK) and incubated for 1 hour at room temperature to block the wells. After
30 discarding the block solution, phage minipreps pre-mixed with 50 µl blocking solution were added and incubated for 1 hour at RT. Next 5 washing steps with PBS-0.05% Tween-20 removed unbound phages. Bound

phages were detected by incubating the wells with 100 microliter anti-M13-HRP antibody conjugate (diluted 1/5000 in blocking buffer) for 1 hour at room temperature. Free antibody was removed by repeating the washing steps as described above, followed by TMB substrate incubation until color development was visible. The reaction was stopped by adding 100 microliter of 2 M H₂SO₄ per well and analyzed on an ELISA reader at 450 nm emission wavelength (Table 2). Higher numbers indicate stronger signals and thus higher incidence of specific binding of the phage-Fab complex.

10 Sequencing

Clones that gave signals at least 3 times above the background signal (Table 2) were propagated, used for DNA miniprep procedures (see procedures Qiagen miniPrep manual) and subjected to nucleotide sequence analysis. Sequencing was performed according to the Big Dye 1.1 kit accompanying manual (Applied Biosystems) using a reverse primer (CH1_Rev1, table 1) recognizing a 5' sequence of the CH1 region of the human IgG1 heavy chain (present in the Fab display vector MV1043, figures 3 and 12). Mouse VH sequences of 28 tetanus toxoid binding clones are depicted in Table 3. The results show that the selected murine VH genes belong to different gene families, and different individual members from these gene families are able to pair with the rearranged human IGKV1-39/J VH region to form functional tetanus toxoid-specific antibody binding sites. From the sequence analyses, it was concluded that the murine VH regions utilize a diversity of DH and JH gene segments.

25

Example 3

Silencing of the mouse kappa light chain locus

This example describes the silencing of the mouse endogenous kappa light chain locus. The endogenous kappa locus is modified by homologous recombination in ES cells, followed by the introduction of genetically modified ES cells in mouse embryos to obtain genetically adapted offspring.

30

A vector that contains an assembled nucleotide sequence consisting of a part comprising the J-region to 338 bp downstream of the J5 gene segment fused to a sequence ending 3' of the 3' CK enhancer is used for homologous recombination in ES cells. The assembled sequence is used to
5 delete a genomic DNA fragment spanning from 3' of the JK region to just 3' of the 3' CK enhancer. As a consequence of this procedure, the CK constant gene, the 3' enhancer and some intergenic regions are removed (see figures 4 and 18).

10 Construction of the targeting vector

A vector that received 4.5-8 kb flanking arms on the 3' and 5' end fused to the deletion segment was used for targeted homologous recombination in an ES cell line. Both arms were obtained by PCR means ensuring maximum homology. The targeting strategy allows generation of
15 constitutive KO allele. The mouse genomic sequence encompassing the Igk intronic enhancer, Igk constant region and the Igk 3' enhancer was replaced with a PuroR cassette, which was flanked by F3 sites and inserted downstream of the Jk elements. Flp-mediated removal of the selection marker resulted in a constitutive KO allele. The replacement of the Igk
20 MiEk-Igk C-Igk 3'E genomic region (approx. 10 kb) with a F3-Puro cassette (approx. 3 kb) was likely to decrease the efficiency of homologous recombination. Therefore, the arms of homology were extended accordingly and more ES cell colonies were analysed after transfection in order to identify homologous recombinant clones.

25

Generation of ES cells bearing the deleted kappa fragment

The generation of genetically modified ES cells was essentially performed as described (Seibler et al. Nucleic Acids Res. 2003 Feb
15;31(4):e12). See also example 14 for a detailed description.

30

Generation of ES mice by tetraploid embryo complementation.

The production of mice by tetraploid embryo complementation using genetically modified ES cells was essentially performed as described (Eggan et al., PNAS 98, 6209-6214; Seibler J, et al. Nucleic Acids Res. 2003 Feb 15;31(4):e12; Hogan et al., (Summary of mouse development. Manipulating the Mouse Embryo, (1994) Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.), pp. 253-289.)).

Example 4

Silencing of the mouse lambda light chain locus

10 This example describes the silencing of the mouse endogenous lambda light chain locus. The endogenous lambda locus is modified by homologous recombination in ES cells followed by the introduction of genetically modified ES cells in mouse embryos to obtain genetically adapted offspring.

15 Two regions of the murine lambda locus that together contain all functional lambda V regions are subject to deletion.

The first region targeted for homologous recombination-based deletion is a region that is located 408 bp upstream of the start site of the IGLV2 gene segment and ends 215 bp downstream of IGLV3 gene segment ,
20 including the intergenic sequence stretch between these IGLV gene segments. The second region that is subject to a deletion involves the IGLV1 gene segment consisting of a fragment spanning from 392 bp upstream to 171 bp downstream of the IGLV1 gene segment. As a consequence of these two deletion steps, all functional V-lambda genes
25 segments are deleted, rendering the locus functionally inactive (figures 5 and 19).

Construction of the targeting vectors

Vectors that received 3-9.6 kb flanking arms on the 3' and 5' end
30 fused to the deletion segment were used for targeted homologous recombination in an ES cell line. Both arms were obtained by PCR means ensuring maximum homology. In a first step, the mouse genomic sequence

encompassing the Igl V2-V3 regions were replaced with a PuroR cassette flanked by F3 sites, which yields a constitutive KO allele after Flp-mediated removal of selection marker (see figure 19A). In a second step, the mouse genomic sequence encompassing the Igl V1 region was replaced with a Neo cassette in ES cell clones which already carried a deletion of the Igl V2-V3 regions (see figure 19B). The selection marker (NeoR) was flanked by FRT sites. A constitutive KO allele was obtained after Flp-mediated removal of selection markers.

10 Generation of ES cells bearing the deleted lambda fragment

The generation of genetically modified ES cells was essentially performed as described (Seibler J, Zevnik B, Küter-Luks B, Andreas S, Kern H, Hennek T, Rode A, Heimann C, Faust N, Kauselmann G, Schoor M, Jaenisch R, Rajewsky K, Kühn R, Schwenk F. Nucleic Acids Res. 2003 Feb 15;31(4):e12). See also example 14 for a detailed description. To show that both targeting events occurred on the same chromosome several double targeted clones were selected for the in vitro deletion with pCMV C31deltaCpG. The clones were expanded under antibiotic pressure on a mitotically inactivated feeder layer comprised of mouse embryonic fibroblasts in DMEM High Glucose medium containing 20% FCS (PAN) and 1200 u/mL Leukemia Inhibitory Factor (Millipore ESG 1107). 1x10⁷ cells from each clone were electroporated with 20 µg of circular pCMV C31deltaCpG at 240 V and 500 µF and plated on four 10 cm dishes each. 2 - 3 days after electroporation cells were harvested and analysed by PCR.

25 Primers used were:

2005_5: CCCTTTCCAATCTTTATGGG

2005_7: AGGTGGATTGGTGTCTTTTTCTC

2005_9: GTCATGTCGGCGACCCTACGCC

PCR reactions were performed in mixtures comprising 5 µl PCR Buffer 10x (Invitrogen), 2 µl MgCl₂ (50 mM), 1 µl dNTPs (10 mM), 1 µl first primer (5 µM), 1 µl second primer (5 µM), 0,4 µl Taq (5 U/ul, Invitrogen), 37,6µl H₂O,

and 2 µl DNA. The program used was 95°C 5'; followed by 35 cycles of 95°C 30"; 60°C 30"; 72°C 1'; followed by 72°C 10'.

Generation of ES mice by tetraploid embryo complementation.

5 The production of mice by tetraploid embryo complementation using genetically modified ES cells was essentially performed as described (Eggan et al., PNAS 98, 6209-6214; Seibler J, Zevnik B, Küter-Luks B, Andreas S, Kern H, Hennek T, Rode A, Heimann C, Faust N, Kauselmann G, Schoor M, Jaenisch R, Rajewsky K, Kühn R, Schwenk F. Nucleic Acids Res. 2003 Feb 10 15;31(4):e12; Hogan et al., (Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY.), pp. 253-289).

Example 5

Construction of the CAGGS expression insert based on a rearranged
15 human germline IGKV1-39/J-Ck gene (IGKV1-39/J-Ck)

This example describes the construction of a CAGGS expression cassette incorporating the rearranged human germline IGKV1-39/J region. This insert expression cassette encompasses cloning sites, a Kozak sequence, a leader sequence containing an intron, an open reading frame of
20 the rearranged IGKV1-39 region, a rat CK constant region from allele a and a translational stop sequence (IGKV1-39/J-Ck; figure 6). The primary construct consists of naturally occurring sequences and has been analyzed and optimized by removing undesired cis acting elements like internal TATA-boxes, poly adenylation signals, chi-sites, ribosomal entry sites, AT-
25 rich or GC-rich sequence stretches, ARE-, INS- and CRS sequence elements, repeat sequences, RNA secondary structures, (cryptic) splice donor and acceptor sites and splice branch points (GeneArt GmbH). In addition, the codon usage in the open reading frame regions is optimized for expression in mice. The intron sequence is unchanged and thus represents
30 the sequence identical to the coding part of the human IGKV1-39 leader intron.

At the 5' end of the expression cassette, a NotI site was introduced and on the 3' site a NheI site. Both sites are used for cloning in the CAGGS expression module. After gene assembly according to methods used by GeneArt, the insert is digested with NotI-NheI and cloned into the expression module containing a CAGGS promoter, a stopper sequence flanked by LoxP sites ('floxed'), a polyadenylation signal sequence and, at the 5' and 3' end, sequences to facilitate homologous recombination into the Rosa26 locus of mouse ES cell lines. Promoter and/or cDNA fragments were amplified by PCR, confirmed by sequencing and/or cloned directly from delivered plasmids into an RMCE exchange vector harbouring the indicated features. A schematic drawing and the confirmed sequence of the final targeting vector pCAGGS-IgVK1-39 are shown in figure 13A and 13B. The targeting strategy is depicted in figure 13C.

Example 6

CAGGS expression insert based on the rearranged germline IGLV2-14/J V lambda region (IGLV2-14/J-Ck)

This example describes the sequence and insertion of an expression cassette incorporating the rearranged germline IGLV2-14/J V lambda region. This insert encompasses cloning sites, a Kozak sequence, a leader sequence containing an intron, an open reading frame of the rearranged IGLV2-14/J region, a rat CK constant region from allele a and a translational stop sequence (IGLV2-14/J-Ck; figure 7). The primary construct consists of naturally-occurring sequences and has been analyzed and optimized by removing undesired cis acting elements like: internal TATA-boxes, poly adenylation signals, chi-sites, ribosomal entry sites, AT-rich or GC-rich sequence stretches, ARE-, INS- and CRS sequence elements, repeat sequences, RNA secondary structures, (cryptic) splice donor and acceptor sites and splice branch points (GeneArt GmbH). In addition, the codon usage in the open reading frame regions was optimized for expression in mice. The intron sequence is unchanged and thus represents the sequence identical to the human IGKV1-39 leader intron.

At the 5' end of the expression cassette, a NotI site was introduced and on the 3' site a NheI site. Both sites are used for cloning in the CAGGS expression module as described by TaconicArtemis. After gene assembly according to methods used by GeneArt, the insert was digested with NotI-
5 NheI and cloned into the expression module containing a CAGGS promoter, a stopper sequence flanked by LoxP sites ('floxed'), a polyadenylation signal sequence and, at the 5' and 3' end, sequences to facilitate homologous recombination into the Rosa26 locus of mouse ES cell lines. To construct the final ROSA26 RMCE targeting vector, promoter and/or cDNA fragments
10 were amplified by PCR. Amplified products were confirmed by sequencing and/or cloned directly from delivered plasmids into an RMCE exchange vector harbouring the indicated features. A schematic drawing and the confirmed sequence of the final targeting vector pCAGGS-IgVL2-14 is shown in figure 15A and 15B. The targeting strategy is depicted in figure
15 15C.

Example 7

Expression of IGKV1-39/J-Ck in HEK293 cell lines (pSELECT-IGKV1-39/J-Ck)

20 This example describes a method to verify that the IGKV1-39/J-Ck constructs described in example 5 enable expression and detection of the IGKV1-39/J-Ck L chain in HEK293 cells. The IGKV1-39/J insert (figure 6) was modified at the 5' end by changing the NotI site into a Sall site. This change is required for cloning of the product into the expression cassette
25 plasmid pSELECT-hygro (InvivoGen). The CAGGS expression insert IGKV1-39/J-Ck and pSELECT-hygro were digested with Sall and NheI, ligated and used to transform competent XL1-Blue cells using standard techniques. Colonies were picked and DNA purified using Qiagen Midi-prep columns according to the manufacturer's procedures. The resulting light
30 chain (LC) expressing vector named 0817676_pSELECT_0815426 was used to transfect HEK293 cells with Fugene6 (Roche) according to the manufacturer's protocols. Supernatants were screened for the presence of

IGKV1-39/J-Ck light chains by ELISA and western blot using anti-rat-Ck antibodies (Beckton Dickinson #550336 and 553871) and protocols used in the art.

5 The VH of anti-tetanus toxoid (TT) IgG MG1494 was cloned into IgG expression vector MV1056 using restriction sites SfiI and BstEII. The resulting clone was sequence verified. HEK293T cells were transfected with five different vector combinations as shown in Table 4 (see example 8 for details of vector 0817678_pSELECT_0815427). Supernatants were
10 harvested and IgG concentrations determined (see Table 4). No IgG could be detected for supernatants A and B containing light chain only as expected (detection antibody recognized Fc part of IgG). IgG concentration in supernatants C and D was comparable to that of positive control supernatant E, indicating correct expression of the light chain constructs.

15

 Binding to TT was analyzed by ELISA to check functionality of the produced antibodies, using hemoglobin as negative control antigen. No TT-specific binding could be detected for supernatants A and B containing light chain only, as expected. TT-specific binding for supernatants C and D was
20 at least as good as for positive control supernatant E, confirming correct expression of the light chain constructs and functional assembly with heavy chain. Antibodies were detected not only using an anti-human IgG secondary antibody, but also an anti-rat Ckappa light chain secondary antibody. The results confirm that the anti-rat Ckappa antibody (BD
25 Pharmingen #553871, clone MRK-1) recognizes the light chain expressed by the pSELECT vectors.

 Supernatants were analyzed by non-reducing SDS-PAGE and Western blot (not shown). Detection using an anti-human IgG heavy chain
30 antibody did not show bands for supernatants A and B containing light chain only, as expected. Results for supernatants C and D were comparable to positive control supernatant E, with a band close to the 170 kD marker

as expected for intact IgG. Additional lower molecular weight bands were observed as well for supernatants C, D and E, which might represent degradation products, IgG fragments resulting from (partial) reduction and/or irrelevant protein bands due to non-specific binding of the detection
5 antibody.

Detection using an anti-rat Ckappa light chain antibody showed a band close to the 26 kD marker for supernatants A and B, as expected for light chain only. This band was much more intense for A compared to B,
10 indicating that the free IGKV1-39 light chain may be better expressed and/or more stable than the free IGLV2-14 light chain. No bands were detected for control supernatant E as expected, since the expressed IgG contains a human Ckappa light chain. For supernatants C and D, expected bands close to the 170 kD marker were observed; lower molecular weight
15 bands were also observed, but to a lesser extent than above using the anti-human IgG antibody.

In conclusion, transfection of the light chain expression constructs combined with the heavy chain of anti-tetanus toxoid (TT) IgG MG1494
20 resulted in IgG production comparable to the positive control construct for both the pSELECT kappa and lambda light chain constructs. Both IgG productions yielded ELISA signals in a TT ELISA that were better than or comparable to the control IgG. SDS-PAGE and Western blot analysis confirmed the presence of intact IgG. The tested anti-rat Ckappa antibody
25 worked efficiently in both ELISA and Western blot. Culture supernatant from cells transfected with light chain constructs only did not result in detectable IgG production nor in detectable TT-specific binding, while free light chain was detected on Western blot.

Example 8

Expression of IGLV2-14/J-Ck in HEK293 cell lines (pSELECT-IGLV2-14/J-Ck)

This example describes a method to verify that the IGLV2-14/J constructs described in example 6 enable expression and detection of the IGLV2-14/J-Ck L chain in HEK293 cells. The IGLV2-14/J-Ck insert (figure 7) was modified at the 5' end by changing the NotI site into a SalI site. This change is required for cloning of the product into the expression cassette plasmid pSELECT-hygro (InvivoGen). The CAGGS expression insert IGLV2-14/J-Ck and pSELECT-hygro were digested with SalI and NheI ligated and used to transform competent XL1-Blue cells using standard techniques. Colonies were picked and DNA purified using Qiagen Midi-prep columns according to the manufacturer's procedures. The resulting light chain (LC) expressing vector named 0817678_pSELECT_0815427 was used to transfect HEK293 cells with Fugene6 (Roche) according to the manufacturer's protocols. Supernatants were screened for the presence of IGLV2-14/J-Ck light chains by ELISA and western blot using anti-rat-Ck antibodies (Becton Dickinson #550336 and 553871) and protocols used in the art. See example 7 for details and results.

20

Example 9

Construction of a VK promoter-driven expression construct containing an IGKV1-39/J insert and multiple enhancer elements derived from the murine CK locus (VkP-IGKV1-39/J-Ck; VkP-O12)

This example describes the construction of an expression cassette that contains relevant elements to enable B-cell and developmental/differentiation stage-specific expression of the rearranged human IGKV1-39 VK region, based on the IGKV1-39 VK promoter region, leader containing an intron, germline V-gene, CDR3, IGKJ segment, mouse intergenic region located between Jk and CK, rat Ck allele a open reading frame, and a mouse intergenic fragment from the 3' end of the mouse CK gene ending just 3' of the 3'CK enhancer.

30

Optimized open reading frames of the leader, IGKV1-39 rearranged gene, and rat CK allele a gene, as described in example 5, was used for the construction of the expression cassette. The VK promoter region was obtained by gene synthesis procedures (GeneArt, GmbH) and is almost identical to the sequence of the human IGKV1-39 region between -500 bp and the ATG (start site) of the gene. The only deviation from the natural sequence is the introduction of a GCCACCATGG Kozak sequence at the ATG (start) site in order to promote translation. A genomic fragment from a mouse BAC clone (TaconicArtemis) is used as the basis for the introduction of individual elements. This fragment is identical to the sequence of the mouse VK locus starting with the intron donor site located directly 3' of the JK5 region and ending just 3' of the 3' CK enhancer and covers approximately 12.5 kb.

The final construct contains from 5' to 3' end the following elements: human genomic IGKV1-39 promoter (500 bp), a Kozak sequence, a human IGKV1-39 leader part 1 (optimized), a human IGKV1-39 leader intron, a human IGKV1-39 leader part 2 (optimized), a human IGKV1-39 germline gene (optimized), a human J-region (optimized), a mouse intergenic region including the intron enhancer element, a rat (*Rattus norvegicus*) kappa constant region (optimized), and a mouse intergenic region including the 3' kappa enhancer. The elements of this expression cassette are shown in figure 8 and named VkP-IGKV1-39/J-Ck (VkP-O12). An outline of the pVkP-O12 vector and the targeting strategy is depicted in figure 20A and 21A. The vector was introduced into ES cells following standard procedures (see example 14).

Example 10

Construction of a VK promoter-driven expression construct containing an IGLV2-14/J clone and multiple CK locus-derived enhancer elements (VkP-IGLV2-14/J-Ck; VkP-2a2).

This example describes the same construct as described in example 9, except that the IGKV1-39 gene and J-region are replaced by the optimized

human IGLV2-14 germline gene including a unique V-J region (VkP-IGLV2-14/J-Ck; VkP-2a2; figure 9).

Example 11

5 Construction of a VK promoter-driven expression construct containing an IGKV1-39 clone lacking the CK intron enhancer element (VkP-IGKV1-39/J-Ck- Δ 1; VkP-O12-del1)

The construct described in example 9 was modified by removing the CK intron enhancer element, located in the intergenic region between the
10 human J region and the rat CK region by standard PCR modification and DNA cloning methodologies (GeneArt, GmBH). The resulting expression cassette is shown in figure 10 and named VkP-IGKV1-39/J-Ck- Δ 1 (VkP-O12-del1).

An outline of the pVkP-O12-del1 vector and the targeting strategy is
15 depicted in figure 20B and 21B. The vector was introduced into ES cells following standard procedures (see example 14).

Example 12

Construction of a VK promoter-driven expression construct
20 containing an IGKV1-39 clone lacking the CK intron enhancer element and a truncated 3' CK enhancer element (VkP-IGKV1-39/J-Ck- Δ 2; VkP-O12-del2)

The construct described in example 11 was modified by truncating the 3' CK enhancer element and deleting part of the intergenic region 3' of
25 the rat Ck gene, to remove potential inhibitory elements. This was achieved by removing the intergenic sequence between an EcoRV site (located 3' of the rat Ck gene) and the NcoI site present in the 3' enhancer (5993 bp) and further removing the sequence between the 3' enhancer BstXI site and the BstXI site 3' of the 3' enhancer (474 bp) using standard methods. The
30 resulting expression cassette is shown in figure 11 and named VkP-IGKV1-39/J-Ck- Δ 2 (VkP-O12-del2).

An outline of the pVkP-O12-del2 vector and the targeting strategy is depicted in figure 20C and 21C. The vector was introduced into ES cells following standard procedures (see example 14).

5 Example 13

 Expression of Vk constructs in cell lines

 The constructs described in example 9-12 are tested for their ability to produce light chain proteins in the myeloma cell lines MPC11 (ATCC CCL167), B-cell lymphoma WEHI231 (ATCC CRL-1702), the T-cell
10 lymphoma EL4 (ATCC TIB-39) and in HEK293 (ATCC CRL1573). The enhancer and promoter elements in the construct enable expression in the B-cell lines but not in cell lines derived from other tissues. After transfection of the cell lines using purified linearized DNA and Fugene6 (Roche) cells are cultured for transient expression. Cells and supernatant
15 are harvested and subjected to SDS-PAGE analysis followed by western blotting using a specific anti-rat-C-kappa antibody. Supernatants are analyzed in ELISA for secreted L chains using the anti-rat CK antibody (Beckton Dickinson #550336).

20 Example 14

 Generation of transgenic ES lines

 All constructs as described in examples 3, 4, 5, 6, 9, 10, 11 and 12 were used to generate individual stable transgenic ES lines by means of homologous recombination. The methods for generation of transgenic ES
25 lines via homologous recombination are known in the field (e.g. Eggan et al., PNAS 98, 6209-6214; Seibler J, Zevnik B, Küter-Luks B, Andreas S, Kern H, Hennek T, Rode A, HeimannC, Faust N, Kauselmann G, Schoor M, Jaenisch R, Rajewsky K, Kühn R, Schwenk F. Nucleic Acids Res. 2003 Feb 15;31(4):e12; Hogan et al. (Cold Spring Harbor Laboratory Press, Cold
30 Spring Harbor NY.), pp. 253-289.).

For all constructs described in examples 5-6, and examples 9-12, the RMCE ES cell line (derived from mouse strain 129S6B6F1-Gt(ROSA)26Sortm10Arte) was grown on a mitotically inactivated feeder layer comprised of mouse embryonic fibroblasts (MEF) in DMEM High Glucose medium containing 15% FBS (PAN 1302-P220821). Leukemia Inhibitory Factor (Chemicon ESG 1107) was added to the medium at a concentration of 900 U/mL. For manipulation, 2×10^5 ES-cells were plated on 3,5 cm dishes in 2 ml medium. Directly before transfection, 2 ml fresh medium was added to the cells. 3 ul Fugene6 Reagent (Roche; Catalog No. 1814 443) was mixed with 100 ul serum free medium (OptiMEM I with Glutamax I; Invitrogen; Catalog No. 51985-035) and incubated for 5 min. 100 ul of the Fugene/OptiMEM solution was added to 2 ug circular vector and 2 ug CAGGS-Flp and incubated for 20 min. This transfection complex was added dropwise to the cells and mixed. Fresh medium was added to the cells the following day. From day 2 onwards the medium was replaced daily with medium containing 250 ug/mL G418 (Geneticin; Invitrogen; Catalog No. 10131-019). Seven days after transfection, single clones were isolated, expanded, and molecular analyzed by Southern blotting according to standard procedures.

20

For each construct, analysis of multiple clones by restriction enzyme digestion of genomic DNA of single clones followed by hybridization with 5' probes, 3' probes, and internal probes resulted in clones that comprised a correct, single insertion at the correct position in the Rosa26 locus. An example is provided in figure 14.

25

Example 15

Generation of transgenic mouse strains

All ES cell lines that were generated and verified for their modifications as described in example 14 were used to generate stable transgenic mice by means of tetraploid recombination. The methods are known in the field. In general, after administration of hormones,

30

superovulated Balb/c females were mated with Balb/c males. Blastocysts were isolated from the uterus at dpc 3.5. For microinjection, blastocysts were placed in a drop of DMEM with 15% FCS under mineral oil. A flat tip, piezo actuated microinjection-pipette with an internal diameter of 12 - 15 micrometer was used to inject 10-15 targeted C57BL/6 N.tac ES cells into each blastocyst. After recovery, injected blastocysts were transferred to each uterine horn of 2.5 days post coitum, pseudopregnant NMRI females. Chimerism was measured in chimeras (G0) by coat colour contribution of ES cells to the Balb/c host (black/white). Highly chimeric mice were bred to strain C57BL/6 females. Depending on the project requirements, the C57BL/6 mating partners are non-mutant (W) or mutant for the presence of a recombinase gene (Flp-Deleter or Cre-deleter or CreER inducible deleter or combination of Flp-deleter/CreER). Germline transmission was identified by the presence of black, strain C57BL/6, offspring (G1).

15

For example, ESC clone IgVK1-39 2683 8 (see examples 5 and 14) was injected in a total of 62 blastocysts in 3 independent experiments. 3 litters were obtained with a total of 6 pups. All pups were chimaeric. 3 heterozygous offspring pups were obtained that were used for further crossing.

20

ESC Clone Kappa 2692 A-C10 (see examples 3 and 14) was injected in a total of 54 blastocysts in 3 independent experiments. 3 litters were obtained with a total of 11 pups, of which 10 were chimaeric. 8 heterozygous offspring pups were obtained that were used for further crossing.

25

ESC Clone Kappa 2692 B-C1 (see examples 3 and 14) was injected in a total of 51 blastocysts in 3 independent experiments. 2 litters were obtained with a total of 6 pups, of which 4 were chimaeric. 3 heterozygous offspring pups were obtained that were used for further crossing.

30

Example 16

Breeding

This example describes the breeding for obtaining mice that contain transgenic expression cassettes as described example 14 and knock out mice in which the endogenous lambda and kappa loci have been silenced. The localization of V-lambda on chromosome 16 and CD19 on chromosome 7 allow standard breeding procedures. The breeding of the co-localized Vk locus and Rosa26 locus on chromosome 6 with a distance of about 24 cM requires special attention during the screening as only a percentage of the offspring shows crossover in a way that both modifications are brought together on one chromosome.

All four loci have to be combined in a single mouse strain that is homo- or heterozygous for CD19-cre (not described) and modified Rosa26 transgene and homozygous for the other loci. Breeding is performed by standard breeding and screening techniques as appropriate and offered by commercial breeding companies (e.g. TaconicArtemis).

Example 17

Immunizations of mice

Primary and booster immunization of mice are performed using standard protocols.

To validate the transgenic expression of human rearranged Vk O12 (IGKV1-39) - rat Ck light chains (see example 5, 14-16) in B cells from CD19-HuVk1 mice and to assess its impact on VH repertoire size, diversity of VH family usage and V(D)J recombination after immunization, the CD19-HuVk1 transgenic mice are immunized with tetanus toxin vaccine (TT vaccine) and VH sequence diversity of randomly-picked clones from CD19-HuVk1 mice are compared with TT-immunized wt mice and CD19-Cre HuVk1 negative littermates. Data on the SHM frequency of the human Vk O12 transgene in the immunized mice are obtained. A diverse collection of at least 40 TT-specific, clonally-unrelated mAbs containing the human Vk O12 are recovered from CD19-HuVk1 mice by phage display.

For this, three adult CD19-HuVk1 mice are vaccinated with TT vaccine using standard immunization procedures. After immunization, serum titers are measured using TT specific ELISA (TT: Statens Serum
5 Institute, Art. no. 2674) and spleen suspensions subjected to cell sorting by the FACS procedure after staining with a rat Ck-specific monoclonal antibody to isolate transgenic B cells (clone RG7/9.1; BD Pharmingen# 553901, Lot# 06548). RNA from rat Ck-positive B cells are extracted and the resulting cDNA material used for library building and SHM analysis.

10

The standard monoclonal mouse anti-rat Ck antibody (clone RG7/9.1; BD Pharmingen# 553901, Lot# 06548) is used in FACS analysis of transgene expressing B cells (Meyer et al., 1996, Int. Immunol., 8: 1561). The clone RG7/9.1 antibody reacts with a monotypic (common) kappa chain
15 determinant. This anti-rat Ck antibody (clone RG7/9.1 (BD Pharmingen# 553901, Lot# 06548) is labeled with R-phycoerythrin (PE) using the LYNX rapid conjugation kit according to the manufacturer's instructions for FACS analysis and sorting. The labeled antibody is firstly tested by flow
cytometry for binding to rat Ck-containing functional light chain proteins
20 produced into transiently transfected HEK-293T cells; the un-conjugated antibody serves as a positive control. Two other antibodies shown to bind to rat Ck by ELISA and Western-blot (see example 7) are tested as well by flow cytometry.

Fab-phage display library building is carried out with a set of
25 optimized degenerate PCR primers designed to amplify C57BL/6 VH genes; the minimal library size is 10^6 clones, and minimal insert frequency is 80%. The vector used, MV1043 (figures 3 and 12), contains the human Vk O12 fused to a human Ck region. The rat Ck is therefore exchanged for the human counterpart in the library generation process.

30 Before selection, VH sequencing of 96 randomly picked clones is performed to validate VH repertoire diversity that is compared to diversity obtained from an unselected library previously generated using the same

procedures from BALB/c mice immunized with TT. A library from C57Bl/6 wt mice that are immunized in the same way allows diversity comparison between two preselected libraries sharing the same vaccine and the same genetic background.

5 Several independent selections are performed on TT coated in immunotubes. Variables that may be included are selections using biotinylated antigens in solution or selections on captured TT. Based on the number and diversity of ELISA-positive clones obtained in the first selections, decisions on additional rounds of selection are made. Clones are
10 considered positive when > 3x positive over a negative control clone. Positive clones are analyzed by ELISA against a panel of negative control antigens to verify antigen specificity. The aim is to identify at least 40 unique VH regions, as based on unique CDR3 sequences and V_HDJ_H rearrangements.

15 Amplification of the cDNA material from rat Ck-positive sorted B cells is performed with a PCR forward primer specific to the human leader sequence and a PCR reverse primer specific to the rat Ck sequence, in a region not redundant with the mouse Ck sequence, as reported in a recent study (Brady et al., 2006, JIM, 315: 61). Primer combinations and annealing
20 temperatures are firstly tested on cDNA from HEK-293T cells transfected with 0817676_pSELECT_0815426 = pSELECT vector with IGKV1-39 DNA cassette (see example 7).

 The amplification products is cloned in pJET-1 vector and after XL1-blue transformation, 96 colonies are sequenced for assessing VL SHM
25 frequency by direct comparison to the V_k O12 (IGKV1-39) germline sequence. The R/S ratio method, as described in our study on human TT-specific antibodies (de Kruif et al., 2009, J. Mol. Biol., 387: 548) allows discrimination between random mutations and antigen-driven mutations that occurred on VL sequences.

Example 18

Immunofluorescent analysis of B cell populations in transgenic mouse lines.

This example describes the use of antibodies and flow cytometry to
5 analyze B cell populations in primary (bone marrow) and secondary (spleen, peritoneal) lymphoid organs and blood. Methods and reagents are described in Middendorp et al. (2002) J. Immunol. 168:2695 and Middendorp et al. (2004) J. Immunol. 172:1371. For analysis of early B cell development in bone marrow, cells were surface stained with combinations of antibodies
10 (Becton Dickinson) specific for B220, CD19, CD25, IgM, IgD, mouse Ckappa, mouse Clambda and rat Ckappa to detect pro-B cells, pre-B cells, large pre-B cells, early and late immature B cells and recirculating B cell populations expressing the transgene on their surface. DAPI staining (Invitrogen) was included to exclude dead cells from the analysis and FC
15 block (Becton Dickinson) to inhibit antibody interaction with Fc receptors on myeloid cells. For analysis of surface transgene expression on B cell populations in peripheral lymphoid organs and blood, cells were stained with combinations of antibodies (Becton Dickinson) specific for B220, CD5, CD19, CD21, CD23, IgM, IgD, mouse Ckappa, mouse Clambda and rat
20 Ckappa. DAPI staining was included to exclude dead cells from the analysis and FC block to inhibit antibody interaction with Fc receptors on myeloid cells. In addition, combinations of antibodies (Becton Dickinson) specific for CD3, CD4, CD11b, CD11c and NK1.1 were included to determine if transgene expression occurred in cell types outside of the B cell
25 compartment.

Three mice heterozygous for the human IGKV1-39/rat Ckappa transgene and heterozygous for the CD19-Cre transgene on a C57BL6 background (HuVk1/CD19-Cre) were analysed. As controls for the FACS analysis, three littermate mice wild type for the human IGKV1-39/rat
30 Ckappa transgene and heterozygous for the CD19-Cre transgene on a C57BL6 background (CD19-Cre) and two C57BL6/NTac mice (Wt) were included. All animals were allowed to acclimatize in the animal facility for 1

week before analysis and all mice were male and 6 weeks of age.

Lymphocytes were isolated from the femurs, spleens, peritoneal cavity and blood of mice using conventional techniques as previously described (Middendorp et al. (2002) J. Immunol. 168:2695 and Middendorp et al.

5 (2004) J. Immunol. 172:1371). Antibodies were pre-combined as shown in Table 10 and staining was carried out in 96 well plates. Incubation with the PE-conjugated anti-rat C kappa (described above) was carried out before staining with the rat anti-murine antibodies to avoid non-specific binding. After completion of cell staining, labeled cells were analysed on a Becton
10 Dickinson LSR II FACS machine and the acquired data analysed with FlowJo software (v6.4.7).

Transgenic mice were similar in weight, appearance and activity to wild type mice. No gross anatomical alterations were observed during the harvesting of tissues. No difference was observed in the numbers of B cells
15 in the bone marrow (BM) and spleen (Table 11) or in the numbers of B cells, T cells and myeloid cells in peripheral organs between transgenic and wild type mice. In addition, the frequency or proportion of the cells in the different lymphocyte developmental pathways was not altered in transgenic mice when compared to wild type mice. Thus in the double transgenic
20 (HuVk1/CD19-Cre) and transgenic (CD19-Cre) mice lymphoid and most importantly B cell development was indistinguishable from wild type mice.

In the peripheral lymphoid organs, staining with the transgene specific antibody (anti-ratCkappa-PE) was only observed in the B cell populations. T cell, myeloid cell and NK cell populations were all negative
25 for surface expression of the transgene in the spleen (Figure 23). In contrast, in cells stained with the pan B cell markers B220 and CD19 all cells were shifted to the right in the FACS plot indicating cell surface expression of the transgene (Figure 24). A similar transgene-specific staining was measured in CD5⁺ B1 cells of the peritoneum, a
30 developmentally distinct population of B cells (Figure 25).

Differentiation of B cells from multilineage precursors to mature B cells occurs in the bone marrow. In the lymphocytes analyzed from the bone

marrow, extracellular and transgene expression was not detectable in the earliest B cell progenitors the pro- and pre-B cell consistent with the pattern of normal light chain expression (Figure 26). Transgene expression first becomes detectable in immature B cells, the developmental stage at which the germline murine light chain undergoes rearrangement and is expressed at the cell surface in the context of the preselected heavy chain (Figure 26). Consistent with the staining in the spleen transgenic light chain expression is also detected on mature recirculating B cells (Figure 26). Thus the CD19-Cre driven expression of the transgene is consistent with the normal pattern of light chain expression. The staining with the endogenous light chain- specific antibody is more intense than that of the transgene-specific light chain antibody. This may indicate a higher expression level of the endogenous light chain, a more sensitive staining with the endogenous light chain-specific antibody or a combination of both. Importantly, the intensity of the surface expression of the transgenic light chain is correlated with both endogenous light chain and IgM surface expression as observed in staining of circulating B cells in the blood (Figure 27).

Thus overall this analysis demonstrates that expression of the human IGKV1-39/Ckappa transgene is restricted to the B cell compartment and the temporal regulation of its expression is similar to the endogenous kappa and lambda light chains resulting in normal development of all B cell populations. The apparent lower level of expression of the transgene could be explained by the strength of the promoter in comparison to the promoter and enhancers present on endogenous light chain genes or by a delay in transgene expression that gives the endogenous light chains a competitive advantage in pairing with the rearranged heavy chain. This is consistent with the observation that as B cells mature the relative intensity of transgene staining increases compared to the endogenous light chains. In addition, the observation that B cells numbers are normal and that every surface Ig+ B cell co-expresses an endogenous and transgenic light chain supports the conclusion that the IGKV1-39 variable region is capable of

pairing with a normal repertoire of different murine heavy chain variable regions. We conclude from this analysis that insertion of the IGKV1-39/rat Ckappa transgene driven by the CD19-Cre activated CAGGS promoter in the Rosa locus facilitates timely and B cell-specific expression of the transgene and that the transgene is capable of pairing with a normal repertoire of murine heavy chains.

Example 19

Epibase® T-cell epitope profile for IGKV1-39.

The protein sequence of IGKV1-39 (figure 12, human germline IGKV1-39/J Protein) was scanned for the presence of putative HLA class II restricted epitopes, also known as T_H-epitopes. For this, Algonomics' Epibase® platform was applied to IGKV1-39. In short, the platform analyzes the HLA binding specificities of all possible 10-mer peptides derived from a target sequence (Desmet et al. Nature 1992, 356:539-542; Desmet et al. FASEB J. 1997, 11:164-172; Desmet et al. Proteins 2002, 48:31-43; Desmet et al. Proteins 2005, 58:53-69). Profiling is done at the allotype level for 20 DRB1, 7 DRB3/4/5, 13 DQ and 7 DP, i.e. 47 HLA class II receptors in total (see Table 5). Epibase® calculates a quantitative estimate of the free energy of binding ΔG_{bind} of a peptide for each of the 47 HLA class II receptors. These data were then further processed as follows:

- Free energies were converted into K_d-values through $\Delta G_{\text{bind}} = RT \ln(K_d)$.
- Peptides were classified as strong (S), medium (M), weak and non (N) binders. The following cutoffs were applied:

S: strong binder: $K_d < 0.1 \mu\text{M}$.

M: medium binder: $0.1 \mu\text{M} \leq K_d < 0.8 \mu\text{M}$.

N: weak and non-binder: $0.8 \mu\text{M} \leq K_d$.

- Peptides corresponding to self-peptides were treated separately. The list of self-peptides was taken from 293 antibody germline sequences. They are referred to as 'germline-filtered' peptides.

S- and M-peptides are mapped onto the target sequence in so-called epitope maps; S-affinities are plotted quantitatively; M-values are presented

qualitatively. As a general overview of the results, Table 6 lists the number of strong and medium binders in the analyzed proteins, for the groups of HLA class II receptors corresponding to the DRB1, DQ, DP and DRB3/4/5 genes. Counting was done separately for strong and medium affinity binders. Peptides binding to multiple allotypes of the same group were counted as one. Values between brackets refer to germline-filtered peptides. In Table 7, the sequence is shown in a format suitable for experimental work. The sequence is broken down in consecutive 15-mers overlapping by 12 residues. For each 15mer, the promiscuity is listed (the number of allotypes out of a total of 47 for which the 15-mer contains a critical binder), as well as the implied serotypes. The Epibase® profile and epitope maps are shown in figure 16 and 17.

It was concluded that IGKV1-39 contains no strong non-self DRB1 binders. Typically, significantly more binders were found for DRB1 than for other HLA genes. This is in agreement with experimental evidence that allotypes belonging to the DRB1 group are more potent peptide binders. Medium strength epitopes for DRB1 allotypes are expected to contribute to the population response, and cannot be disregarded. Again, no non-self DRB1 binders were found in IGKV1-39.

In the humoral response raised against an antigen, the observed T_H cell activation/proliferation is generally interpreted in terms of the DRB1 specificity. However, one cannot ignore the possible contribution of the DRB3/4/5, DQ and DP genes. Given the lower expression levels of these genes as compared to DRB1, the focus was on the class of strong epitopes for DRB3/4/5, DQ and DP. 'Critical epitopes' are those epitopes that are strong binders for any DRB1, DRB3/4/5, DQ or DP allotype or are medium binders for DRB1. IGKV1-39 contains no strong or medium non-self binders for DRB3/4/5, DQ, or DP.

A number of peptides are also present in germline sequences (values between brackets in Table 6). Such peptides may very well bind to HLA but they are assumed to be self and, hence, non-immunogenic. In total, 6 strong

and 16 medium germline-filtered DRB1 binders were found in IGKV1-39. Framework region 1 up to framework region 3 is an exact match for germline V-segment VKI 2-1-(1) O12 (VBase), a.k.a. IGKV1-39*01 (IMGT). Framework region 4 is an exact match for germline J-segment JK1 (V-base) a.k.a. IGKJ1*01(IMGT). It is hardly surprising that these segments do not contain any non-self epitopes.

Example 20

Production characteristics of IGKV1-39

10 There is a great demand for antibody discovery platforms that yield therapeutic antibodies that are thermodynamically stable and give good expression yields. These characteristics are important in ensuring the stability of the drug substance during production and after injection of the drug product into the patient. In addition good expression yields impact
15 directly on the cost of drug manufacture and thus pricing, patient access and profitability. Virtually all therapeutic antibodies in clinical use today are composed of human IgG1 and kappa constant regions but use different heavy and light chain variable regions that confer specificity. Human variable heavy and light chain domains can be divided into families that
20 have greater than 80% sequence divergence. When rearranged examples of these families in germline configuration are combined and compared for stability and yield it is clear that the gene families are not equal in terms of biophysical properties. In particular V_H3, V_H1 and V_H5 have favourable stability for the heavy chains and Vk1 and Vk3 have the best stability and
25 yield of light chains. In addition when mutations are introduced as part of the somatic hypermutation process they can interfere with V_H/V_L pairing. To assess the effect that different light chain genes with different rates of mutation have on the production characteristics of a fixed V_H chain, a Fab phage display library was built of light chains (kappa and lambda) from six
30 naïve healthy donors combined with a panel of 44 TT binding heavy chains from immunized donors. After one round of selection TT binding Fab clones were isolated. Several of these shared the same V_H gene as the TT clone

PG1433 in combination with different light chains. The Fab light chain fragments were recloned into a kappa expression vector and transfected in combination with DNA encoding the heavy chain of PG1433 into 293 cells and specific IgG production measured by ELISA. As demonstrated in table 5 8 the selected clones containing PG1433 V_H combined with different light chains had between 5 and 10 fold lower protein expression PG1433 V_H combined with IGKV1-39. Note that all of the light chains contained amino acid mutations within their coding regions that might disrupt V_H pairing and reduce production stability. Thus, in addition to reducing the chances of 10 unwanted immunogenicity, it is expected that the use of the light chain IGKV1-39 without mutations contributes to improved production stability and yields of various specificity-contributing V_H genes. Indeed stable clones generated by the transfection of different V_H genes all paired with IGKV1-39 are able to be passaged extensively and still retain robust production 15 characteristics as shown in table 9.

Example 21

Generation of mice expressing fully human V_H and V_L regions.

Transgenic mice according to the invention are crossed with mice 20 that already contain a human V_H locus. Examples of appropriate mice comprising a human V_H locus are disclosed in Taylor *et al.* (1992). Nucleic Acids Res 20: 6287-95; Lonberg *et al.* (1994). Nature 368: 856-9; Green *et al.* (1994). Nat Genet 7: 13-21; Dechiara *et al.* (2009). Methods Mol Biol 530: 311-24.). 25 After crossing and selecting for mice that are at least heterozygous for the IGKV1-39 transgene and the human V_H locus, selected mice are immunized with a target. V_H genes are harvested as described hereinabove. This method has the advantage that the V_H genes are already fully human and thus do not require humanization.

Example 22

Isolation, characterization, Oligoclonics formatting and production of antibodies targeting human IL6 for treatment of chronic inflammatory diseases such as rheumatoid arthritis

A spleen VH repertoire from transgenic mice that are immunized with human recombinant IL6 is cloned in a phage display Fab vector with a single human IGKV1-39-C kappa light chain (identical to the mouse transgene) and subjected to panning against the immunogen human IL6. Clones that are obtained after two to four rounds of panning are analyzed for their binding specificity. VH genes encoding IL6-specific Fab fragments are subjected to sequence analysis to identify unique clones and assign VH, DH and JH utilization. The Fab fragments are reformatted as IgG1 molecules and transiently expressed. Unique clones are then grouped based on non-competition in binding assays and subjected to affinity and functional analysis. The most potent anti-IL6 IgG1 mAbs are subsequently expressed as combinations of two, three, four or five heavy chains comprising different VH-regions in the Oligoclonics format, together with one IGKV1-39-C-based kappa light chain and tested in vitro for complex formation with IL-6. The Oligoclonics are also tested *in vivo* for clearance of human IL-6 from mice. An Oligoclonic with the most potent clearance activity is chosen and the murine VH genes humanized according to conventional methods. The humanized IgG1 are transfected into a mammalian cell line to generate a stable clone. An optimal subclone is selected for the generation of a master cell bank and the generation of clinical trial material.

Many of the protocols described here are standard protocols for the construction of phage display libraries and the panning of phages for binding to an antigen of interest and are described, for example, in Antibody Phage Display: Methods and Protocols. 2002. Editor(s): Philippa M. O'Brien, Robert Aitken. Humana Press, Totowa, New Jersey, USA.

Immunizations

Transgenic mice receive three immunizations with human IL6 every
5 two weeks using the adjuvant Sigma titerMax according to manufacturer's
instructions.

RNA isolation and cDNA synthesis

Three days after the last immunization, spleens and lymphnodes
10 from the mice are removed and passed through a 70 micron filter into a
tube containing PBS pH 7.4 to generate a single cell suspension. After
washing and pelleting of lymphocytes, cells are suspended in TRIzol LS
Reagent (Invitrogen) for the isolation of total RNA according to the
manufacturer's protocol and subjected to reverse transcription reaction
15 using 1 microgram of RNA, Superscript III RT in combination with dT20
according to manufacturer's procedures (Invitrogen).

The generation of Fab phage display libraries is carried out as
described in Example 2.

20

Selection of phages on coated immunotubes

Human recombinant IL6 is dissolved in PBS in a concentration of 5
µg/ml and coated to MaxiSorp Nunc-Immuno Tube (Nunc 444474) overnight
at 4 °C. After discarding the coating solution, the tubes are blocked with 2%
25 skim milk (ELK) in PBS (blocking buffer) for 1 hour at Room Temperature
(RT). In parallel, 0.5 ml of the phage library is mixed with 1 ml blocking
buffer and incubated for 20 minutes at room temperature. After blocking
the phages, the phage solution is added to the IL6 coated tubes and
incubated for 2 hours at RT on a slowly rotating platform to allow binding.
30 Next, the tubes are washed 10 times with PBS/0.05% Tween-20 followed by
phage elution by incubating with 1 ml 50 mM glycine-HCl pH 2.2 10 min at

RT on rotating wheel and directly followed by neutralization of the harvested eluent with 0.5 ml 1 M Tris-HCl pH 7.5.

5

Harvesting phage clones

A 5 ml XL1-Blue MRF (Stratagene) culture at O.D. 0.4 is added to the harvested phage solution and incubated for 30 minutes at 37°C without shaking to allow infection of the phages. Bacteria are plated on
10 Carbenicillin/Tetracycline 4% glucose 2*TY plates and grown overnight at 37°C.

Phage production

Phages are grown and processed as described by Kramer et al. 2003
15 (Kramer et al. 2003. Nucleic Acids Res. 31(11): e59) using VCSM13 as helper phage strain.

Phage ELISA

ELISA plates are coated with 100 microliter human recombinant IL6
20 per well at a concentration of 2.5 microgram/ml in PBS overnight at 4°C. Plates coated with 100 microliter thyroglobulin at a concentration of 2 microgram/ml in PBS are used as a negative control. Wells are emptied, dried by tapping on a paper towel, filled completely with PBS-4% skimmed milk (ELK) and incubated for 1 hour at room temperature to block the
25 wells. After discarding the block solution, phage minipreps pre-mixed with 50 µl blocking solution are added and incubated for 1 hour at RT. Unbound phages are subsequently removed by 5 washing steps with PBS-0.05% Tween-20. Bound phages are detected by incubating the wells with 100 microliter anti-M13-HRP antibody conjugate (diluted 1/5000 in blocking
30 buffer) for 1 hour at room temperature. Free antibody is removed by repeating the washing steps as described above, followed by TMB substrate incubation until color development was visible. The reaction is stopped by

adding 100 microliter of 2 M H₂SO₄ per well and analyzed on an ELISA reader at 450 nm emission wavelength.

Sequencing

5 Clones that give signals at least 3 times above the background signal are propagated, used for DNA miniprep procedures (see procedures Qiagen miniPrep manual) and subjected to nucleotide sequence analysis. Sequencing is performed according to the Big Dye 1.1 kit accompanying manual (Applied Biosystems) using a reverse primer (CH1_Rev1, table 1)
10 recognizing a 5' sequence of the CH1 region of the human IgG1 heavy chain (present in the Fab display vector MV1043, figures 3 and 12). The sequences of the murine VH regions are analyzed for diversity of DH and JH gene segments.

15 Construction and expression of chimeric IgG1

Vector MV1057 (figures 12 and 22) was generated by cloning the transgene (IGKV1-39) L chain fragment into a derivative of vector pcDNA3000Neo (Crucell, Leiden, The Netherlands) that contains the human IgG1- and kappa constant regions. VH regions are cloned into
20 MV1057 and nucleotide sequences for all constructs are verified according to standard techniques. The resulting constructs are transiently expressed in HEK293T cells and supernatants containing chimeric IgG1 are obtained and purified using standard procedures as described before (Throsby, M. 2006. J Virol 80: 6982-92).

25

IgG1 binding and competition analysis

IgG1 antibodies are titrated in ELISA using IL6 coated plates as described above and an anti-human IgG peroxidase conjugate. Competition
30 ELISAs to group antibodies based on epitope recognition are performed by incubating Fab phages together with IgG1 or with commercial antibodies against IL6 (e.g. Abcam cat. no. ab9324) in IL6 coated plates, followed by detection of bound Fab phage using an anti-M13 peroxidase conjugate.

IgG1 affinity measurements

The affinities of the antibodies to IL6 are determined with the Quantitative kinetic protocol on the Octet (ForteBio). Antibodies are
5 captured onto an Anti-Human IgG Fc Capture biosensor and exposed to free IL6 and analyzed using proprietary software to calculate the Kd of each antibody.

10 Functional activity of IL6 antibodies

To test the ability of the selected antibodies to inhibit binding between IL6 and IL6 receptor (IL6R), an ELISA based assay is used. Various concentrations of antibody are mixed with a fixed concentration (10 ng/ml) of biotinylated IL6 as described by Naoko *et al.* 2007, Can. Res. 67:
15 817-875. The IL6-antibody immune complex is added to immobilized IL6R. The binding of biotinylated IL6 to IL6R is detected with horseradish peroxidase-conjugated streptavidin. The reduction of ELISA signal is a measurement of inhibition. As positive control for inhibition of binding between IL6 and IL6R either anti-IL6R antibody (Abcam cat. no. ab34351;
20 clone B-R6) or anti IL6 antibody (Abcam cat. no. ab9324) is used.

In vitro blocking activity of the selected anti-IL6 antibodies is measured in a proliferation assay using the IL6 dependent cell line 7TD1. Briefly, cells are incubated with different concentrations of human IL6 with or without the anti-IL6 antibody. The available amount of IL6 determines the degree
25 of proliferation. Thus if an added antibody blocks IL6 binding the proliferation readout is reduced compared to a non binding antibody control. Proliferation is measured by the incorporation of 5-bromo-2'-deoxy-uridine (BrdU) into the DNA using the BrdU proliferation kit (Roche cat. no. 11444611001) according to the manufacturer's instructions.

Generation of anti-IL6 Oligoclonics

The most potent anti-IL6 antibodies are selected from each epitope group. The expression constructs expressing these antibodies are transfected into HEK293T cells in non-competing groups of three in
5 different ratios (1:1:1; 3:1:1; 1:3:1; 1:1:3; 3:3:1; 1:3:3; 3:1:3; 10:1:1; 1:10:1; 1:1:10; 10:10:1; 1:10:10; 10:1:10; 3:10:1; 10:3:1; 1:10:3; 3:1:10; 10:1:3; 1:3:10). Antibody containing supernatants are harvested and purified and characterized as above.

10 Complex formation and *in vivo* clearance of anti-IL6 Oligoclonics

To measure the ability of anti-IL6 Oligoclonics to form immune complexes and to analyze these complexes Size Exclusion Chromatography (SEC) is used according to the approach disclosed by Min-Soo Kim et al. (2007) JMB 374: 1374-1388 to characterize the immune-complexes formed
15 with different antibodies to TNF α . Different molar ratios of the anti-IL6 Oligoclonics are mixed with human IL6 and incubated for 20 hours at 4°C or 25°C. The mixture is analyzed on an HPLC system fitted with a size exclusion column; different elution times are correlated to molecular weight using a molecular weight standards.

20 The ability of antibodies to form complexes with IL6 is correlated with their ability to rapidly clear the cytokine from the circulation *in vivo*. This is confirmed by measuring the clearance of radiolabelled IL6 from mice. Briefly, female, 6- to 8-week-old Balb/c mice are obtained and 18 hrs before the experiment, the animals are injected intravenously (IV) via the lateral
25 tail vein with different doses of purified anti-IL6 Oligoclonics. On day 0 the mice are injected IV with 50 microliters of radiolabeled IL-6 (1x10E7 cpm/mL) under the same conditions. Blood samples (approximately 50 microliters) are collected at several time intervals and stored at 4°C. The samples are
30 centrifuged for 5 minutes at 4000 xg and the radioactivity of the serum determined. All pharmacokinetic experiments are performed simultaneously with three animals for each treatment.

Generation of anti-IL6 Oligoclones stable clones and preclinical development

A lead anti-IL6 Oligoclonic is selected based on the *in vitro* and *in vivo* potency as determined above. The murine VH genes are humanized according to standard methods and combined with the fully human IGKV1-39 light chain in an expression vector as described above. Examples of humanization methods include those based on paradigms such as resurfacing (Padlan, E. A., et al., (1991). Mol. Immunol., 28, 489), superhumanization (Tan, P., D. A., et al., (2002) J. Immunol., 169, 1119) and human string content optimization (Lazar, G. A., et al., (2007). Mol. Immunol., 44, 1986). The three constructs are transfected into PER.C6 cells at the predetermined optimal ratio (described above) under the selective pressure of G418 according to standard methods. A stable high producing anti-IL6 Oligoclonic clone is selected and a working and qualified master cell bank generated.

Table 1 List of primers

DO-	Primer	Sequence
0012	CH1_Rev1	TGCCAGGGGAAGACCGATG
0656	MVH-1	GCCGGCCATGGCCGAGGTRMAGCTTCAGGAGTCAGGAC
0657	MVH-2	GCCGGCCATGGCCGAGGTSAGCTKCAGCAGTCAGGAC
0658	MVH-3	GCCGGCCATGGCCCAGGTGCAGCTGAAGSASTCAGG
0659	MVH-4	GCCGGCCATGGCCGAGGTGCAGCTTCAGGAGTCSGGAC
0660	MVH-5	GCCGGCCATGGCCGARGTCCAGCTGCAACAGTCYGGAC
0661	MVH-6	GCCGGCCATGGCCCAGGTCCAGCTKCAGCAATCTGG
0662	MVH-7	GCCGGCCATGGCCCAGSTBCAGCTGCAGCAGTCTGG
0663	MVH-8	GCCGGCCATGGCCCAGGTYCAGCTGCAGCAGTCTGGRC
0664	MVH-9	GCCGGCCATGGCCCAGGTYCAGCTYCACGACGTCTGG
0665	MVH-10	GCCGGCCATGGCCGAGGTCCARCTGCAACAATCTGGACC
0666	MVH-11	GCCGGCCATGGCCCAGGTCCACGTGAAGCAGTCTGGG
0667	MVH-12	GCCGGCCATGGCCGAGGTGAASSSTGGTGGAATCTG
0668	MVH-13	GCCGGCCATGGCCGAVGTGAAGYTGGTGAGTCTG
0669	MVH-14	GCCGGCCATGGCCGAGGTGCAGSKGGTGAGTCTGGGG
0670	MVH-15	GCCGGCCATGGCCGAKGTGCAMCTGGTGAGTCTGGG
0671	MVH-16	GCCGGCCATGGCCGAGGTGAAGCTGATGGARTCTGG
0672	MVH-17	GCCGGCCATGGCCGAGGTGCARCTTGTGAGTCTGGTG
0673	MVH-18	GCCGGCCATGGCCGARGTRAAGCTTCTCGAGTCTGGA
0674	MVH-19	GCCGGCCATGGCCGAAGTGAARSTTGAGGAGTCTGG
0675	MVH-20	GCCGGCCATGGCCGAAGTGATGCTGCTGGTGGAGTCTGGG
0676	MVH-21	GCCGGCCATGGCCCAGGTTACTCTRAAAGWGTSTGGCC
0677	MVH-22	GCCGGCCATGGCCCAGGTCCAAC TVCAGCARCCTGG
0678	MVH-23	GCCGGCCATGGCCCAGGTYCARCTGCAGCAGTCTG
0679	MVH-24	GCCGGCCATGGCCGATGTGAAC TGGAAAGTGTCTGG

0680	MVH-25	GCCGGCCATGGCCGAGGTGAAGGTCATCGAGTCTGG
0681	ExtMVH-1	CAGTCACAGATCCTCGCGAATTGGCCCAGCCGGCCATGGCCSANG
0682	ExtMVH-2	CAGTCACAGATCCTCGCGAATTGGCCCAGCCGGCCATGGCCSANC
0683	MJH-Rev1	GGGGGTGTCGTTTGGCTGAGGAGAC GGTGACC GTGG
0684	MJH-Rev2	GGGGGTGTCGTTTGGCTGAGGAGAC TGTGAGA GTGG
0685	MJH-Rev3	GGGGGTGTCGTTTGGCTGCAGAGAC AGTGACC AGAG
0686	MJH-Rev4	GGGGGTGTCGTTTGGCTGAGGAGAC GGTGACT GAGG
0687	ExtMJH-	GGGGGTGTCGTTTGGCTGAGGAGAC GGTGACC GTGG
0688	ExtMJH-	GGGGGTGTCGTTTGGCTGAGGAGAC GGTGACA GTGG
0690	ExtMJH-	GGGGGTGTCGTTTGGCTGAGGAGAC GGTGACC AGAG
0691	ExtMJH-	GGGGGTGTCGTTTGGCTGAGGAGAC GGTGACC GAGG

Table 2

Phage ELISA signal levels as measured at 450nm. TT-coated plates represent plates that were coated with tetanus toxoid. Thyroglobulin coated plates are used as negative controls. 10/10 and 15/15 indicate the number of wash steps with PBS-Tween during panning procedures. The 10/10 tetanus toxoid and 10/10 thyroglobulin plates and the 15/15 tetanus toxoid and 15/15 thyroglobulin plates are duplicates from each other except for the coating agent. OD values higher than 3 times the background are assumed specific.

TT-coated plate 10/10 washings

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.139	0.093	0.089	0.121	0.117	0.598	0.146	0.115	0.18	0.155	0.543	0.601
B	0.136	0.404	0.159	0.187	0.489	0.134	0.216	0.092	0.222	0.108	0.181	0.484
C	0.197	0.526	0.09	0.213	0.395	0.155	0.108	0.12	0.183	0.136	0.092	0.866
D	0.143	0.258	0.101	0.422	0.088	0.243	0.485	0.251	0.304	0.198	0.478	0.091
E	0.445	0.169	0.526	0.481	0.206	0.285	0.111	0.119	0.128	0.2	0.118	0.098
F	0.237	0.291	0.594	0.139	0.206	0.565	0.543	0.091	0.136	0.227	0.228	0.099
G	0.459	0.102	0.152	0.659	0.203	0.452	0.152	0.133	0.094	0.102	0.375	0.098
H	0.341	0.623	0.745	0.415	0.682	0.527	0.655	0.114	0.258	0.284	0.685	0.113

TT-coated plate 15/15 washings

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.247	0.582	0.421	0.428	0.133	0.082	0.262	0.079	0.343	0.414	0.095	0.292
B	0.065	0.364	0.073	0.042	0.049	0.071	0.046	0.103	0.078	0.057	0.048	0.155
C	0.081	0.044	0.066	0.082	0.225	0.444	0.203	0.362	0.122	0.047	0.052	0.309
D	0.092	0.11	0.59	0.22	0.33	0.544	0.058	0.159	0.047	0.174	0.086	0.05
E	0.469	0.577	0.206	0.304	0.13	0.749	0.431	0.062	0.167	0.049	0.056	0.049
F	0.846	0.07	0.561	0.656	0.882	0.094	0.383	0.13	0.152	0.098	0.134	0.048
G	0.537	0.052	0.49	0.105	0.337	0.193	0.514	0.294	0.068	0.35	0.525	0.05
H	0.061	0.306	0.157	0.853	0.054	0.534	0.102	0.235	0.441	0.412	0.565	0.061

Thyroglobulin-coated plate 10/10 washings

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.051	0.045	0.043	0.051	0.044	0.046	0.042	0.047	0.048	0.049	0.05
B	0.042	0.042	0.042	0.042	0.043	0.041	0.041	0.042	0.043	0.045	0.042	0.046
C	0.044	0.043	0.043	0.044	0.043	0.044	0.043	0.042	0.043	0.041	0.044	0.046
D	0.045	0.044	0.044	0.044	0.045	0.046	0.045	0.056	0.045	0.049	0.048	0.73
E	0.046	0.045	0.046	0.044	0.045	0.044	0.044	0.044	0.047	0.046	0.047	0.926
F	0.048	0.045	0.044	0.046	0.044	0.043	0.044	0.046	0.046	0.046	0.046	0.792
G	0.051	0.048	0.045	0.045	0.044	0.043	0.048	0.045	0.048	0.051	0.045	0.053
H	0.064	0.05	0.049	0.047	0.05	0.051	0.047	0.046	0.047	0.047	0.047	0.056

Thyroglobulin-coated plate 15/15 washings

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.036	0.049	0.045	0.044	0.046	0.047	0.046	0.042	0.042	0.043	0.042	0.041
B	0.045	0.042	0.041	0.043	0.043	0.043	0.045	0.045	0.047	0.048	0.044	0.045
C	0.049	0.047	0.047	0.046	0.046	0.046	0.045	0.047	0.046	0.045	0.045	0.052
D	0.047	0.049	0.048	0.048	0.048	0.048	0.047	0.052	0.048	0.046	0.048	0.456
E	0.049	0.047	0.047	0.047	0.047	0.049	0.047	0.048	0.047	0.046	0.048	0.412
F	0.05	0.047	0.046	0.046	0.046	0.046	0.046	0.046	0.046	0.047	0.048	0.528
G	0.05	0.048	0.045	0.045	0.046	0.049	0.048	0.046	0.053	0.049	0.05	0.057
H	0.057	0.05	0.046	0.045	0.047	0.049	0.047	0.047	0.046	0.047	0.053	0.048

Table 3
Protein sequence analysis of ELISA positive tetanus toxoid binders. CDR3 sequence, CDR3 length, VH family members and specific name, JH origin and DH origin of the clones is indicated.

CDR3	CDR3 length	VH	DH	JH	V Gene family
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAYYTYDEKAWFAY	15	musIGHV192	DSP2.11	JH3 mouse	VH7183
HGAFYTYDEKPFWFAY	15	musIGHV192	IGHD2-14*01	JH3 mouse	VH7183
HISYYRYDEEVSFAY	15	musIGHV192	IGHD2-14*01	JH3 mouse	VH7183
HISYYRYDEEVSFAY	15	musIGHV192	IGHD2-14*01	JH3 mouse	VH7183
GWRAFAY	7	musIGHV131	DSP2.9	JH3 mouse	VH7183
GWRAFAY	7	musIGHV131	DSP2.9	JH3 mouse	VH7183
GWRAFAY	7	musIGHV131	DSP2.9	JH3 mouse	VH7183
DRGNYYGMDY	10	musIGHV178	DSP2.1	JH4 mouse	VH7183
LGDYYVDWFFAV	12	musIGHV165	DFL16.1	JH1 mouse	VH7183

NFPWFAF	8	musIGHV547	DST4.3inv	JH3 mouse	VJH558
NFPWFAY	8	musIGHV547	DSP2.1	JH3 mouse	VJH558
NFPWFBV	8	musIGHV547	DSP2.1	JH3 mouse	VJH558
SFTPVPFYGGYDWWYFDV	17	musIGHV532	DSP2.3	JH1 mouse	VJH558
SFTPVPFYGGYDWWYFDV	17	musIGHV532	DSP2.3	JH1 mouse	VJH558
SDYDWWYFDV	9	musIGHV286	DSP2.2	JH1 mouse	VJH558
SDYDWWYFDV	9	musIGHV286	DSP2.2	JH1 mouse	VJH558
DSKWAYYFDY	10	musIGHV532	DST4.3	JH2 mouse	VJH558
GDYTGYGMDY	10	musIGHV125	DSP2.13	JH4 mouse	VHSM7
GDYTGYGMDY	10	musIGHV125	DSP2.13	JH4 mouse	VHSM7
GGYDGYWFPY	10	musIGHV125	DSP2.9	JH3 mouse	VHSM7

Table 4
Vector combinations that were transfected to HEK293T.

Code	HC vector	LC vector	Combined vector	Prep name	Conc. (µg/ml)
A	x	0817676_pSELECT_ 0815426 (IGKV1-39)	x	PIGKV1-39/ P1	-
B	x	0817678_pSELECT_ 0815427 (IGLV2-14)	x	PIGLV2-14/ P1	-
C	MV1110	0817676_pSELECT_ 0815426 (IGKV1-39)	x	PMV1110/ IGKV1-39/P1	11.0
D	MV1110	0817678_pSELECT_ 0815427 (IGLV2-14)	x	PMV1110/ IGLV2-14/P1	15.4
E	x	x	MG1494	MG1494/P2	16.1

Table 5. HLA allotypes considered in T_H-epitope profiling. The corresponding serotypes are shown, as well as allotype frequencies in the Caucasian population (Klitz et al. Tissue Antigens 2003, 62:296-307; Gjertson and Terasake (eds) in: HLA 1997; Gjertson and Terasake (eds) in: HLA 1998; Castelli et al. J. Immunol. 2002, 169:6928-6934). Frequencies can add up to more than 100% since each individual has 2 alleles for each gene. If all allele frequencies of a single gene were known, they would add up to slightly less than 200% due to homozygous individuals.

HLA type	Serotype	Population %	HLA type	Serotype	Population %
DRB1*0101	DR1	17.4	DRB4*0103	DR53	21
DRB1*0102	DR1	4.9	DRB5*0101	DR51	15.8
DRB1*0301	DR17(3)	21.2	DRB5*0302	DR51	5.7
DRB1*0401	DR4	11.5	DQA1*0101/DQB1*0301	DQ5(1)	30.5
DRB1*0402	DR4	3.1	DQA1*0102/DQB1*0302	DQ5(1)	2.6
DRB1*0404	DR4	5.5	DQA1*0102/DQB1*0602	DQ6(1)	26.5
DRB1*0405	DR4	2.2	DQA1*0102/DQB1*0604	DQ6(1)	6.7
DRB1*0407	DR4	<2	DQA1*0103/DQB1*0503	DQ6(1)	11
DRB1*0701	DR7	25.4	DQA1*0104/DQB1*0503	DQ5(1)	4
DRB1*0801	DR8	3.3	DQA1*0201/DQB1*0202	DQ2	20.9
DRB1*0802	DR8	<2	DQA1*0201/DQB1*0303	DQ2	7.2
DRB1*0901	DR9	<2	DQA1*0301/DQB1*0301	DQ7(3)	12.5
DRB1*1101	DR11(5)	17	DQA1*0301/DQB1*0302	DQ8(3)	18.3
DRB1*1104	DR11(5)	5.7	DQA1*0401/DQB1*0402	DQ4	4.5
DRB1*1201	DR12(5)	3.1	DQA1*0501/DQB1*0201	DQ2	24.6
DRB1*1301	DR13(6)	15.4	DQA1*0501/DQB1*0301	DQ7(3)	20.9
DRB1*1302	DR13(6)	10.8	DPA1*0103/DPB1*0301	DPw2	19.9
DRB1*1401	DR14(6)	4.2	DPA1*0103/DPB1*0401	DPw4	65.1
DRB1*1501	DR15(2)	13.2	DPA1*0103/DPB1*0402	DPw4	24.3
DRB1*1601	DR16(2)	5.5	DPA1*0201/DPB1*0101	DPw1	6.3
DRB3*0101	DR52	24.6	DPA1*0201/DPB1*0301	DPw3	<2
DRB3*0202	DR52	43	DPA1*0201/DPB1*0501	DPw5	<2
DRB3*0301	DR52	10	DPA1*0201/DPB1*0901	-	2.4
DRB4*0101	DR53	25.5			

Table 6. T_H epitope counts for IGKV1-39. Peptides binding to multiple HLAs of the same group (DRB1, DRB3/4/5, DP, DQ) are counted as one. Values between brackets refer to germline-filtered peptides.

	DRB1		DRB3/4/5		DQ		DP	
	Strong	Medium	Strong	Medium	Strong	Medium	Strong	Medium
Minus IGKV1-39	0 (+ 8)	0 (+ 16)	0 (+ 0)	0 (+ 5)	0 (+ 3)	0 (+ 9)	0 (+ 0)	0 (+ 9)

Table 7. Mapping of Epibase® predictions for Merus IGKV1-39 in the classical 15-mer peptide format. This table shows the allotype count of critical epitopes and implicated serotypes for each of the 15-mers spanning the Merus IGKV1-39 sequence.

15mer	Start position	15-mer sequence	Allotype count	Implicated serotypes
1	1	DIQMTQSPSSLSASV	6	DR1, DR4, DR7, DR9
2	4	NTQSPSSLSASVDR	5	DR1, DR4, DR9
3	7	SPSSLSASVGERVTE	0	
4	10	SLASVGERVTITCR	0	
5	13	ASVGERVTITCRASQ	0	
6	16	GERVTITCRASQSI	2	DR11(5), DR7
7	19	VTITCRASQSISSYL	4	DQ2, DR11(5), DR4, DR7
8	22	TCRASQSISSYLNWY	2	DQ2, DR4
9	25	ASQSISSYLNWYQQK	5	DR13(6), DR15(3), DR4
10	28	SISSYLNWYQQKPGK	8	DR12(5), DR13(6), DR15(3), DR16(2), DR4, DR8
11	31	SYLNWYQQKPGKAPK	10	DR1, DR13(5), DR16(2), DR4, DR51, DR8
12	34	NWYQQKPGKAPKLLI	9	DR1, DR15(2), DR4, DR51, DR8
13	37	QQKPGKAPKLLIYAA	7	DQ4, DR1, DR11(5), DR15(2), DR51, DR8
14	40	PKAPKLLIYAASSL	7	DQ4, DR1, DR11(5), DR4, DR8
15	43	APKLLIYAASSLQSG	15	DR1, DR11(2), DR12(5), DR13(6), DR14(6), DR15(2), DR4, DR51, DR8, DR9
16	46	LIYAASSLQSGVPS	15	DR1, DR11(5), DR12(5), DR13(6), DR14(6), DR15(2), DR4, DR51, DR8, DR9
17	49	YAASSLQSGVSPFS	1	DR15(2)
18	52	SLQSGVSPFSRSGSG	1	DR15(2)
19	55	QSGVSPFSRSGSGGT	0	
20	58	VDSRFSGSGSGTDFT	0	
21	61	RPDSGSGSGTDFTLT	0	
22	64	GSQSGTDFTLTISSL	1	DR52
23	67	STQSTLTISSLQPE	4	DR4, DR52, DR7, DR9
24	70	DTLTISSLQPEDFA	4	DQ2, DR4, DR7, DR9
25	73	ELISLQPEDFATYY	1	DQ2
26	76	SLQPEDFATYYQQQ	0	
27	79	QPEDFATYYQQQSY	1	DR4
28	82	DFATYYQQQSYSTPE	5	DR4, DR51, DR7
29	85	TYQQQSYSTPEPTFC	4	DR4, DR51, DR7
30	88	QQSYSTPEPTFCQGT	0	
31	91	SYSTPEPTFCQGTKVE	0	
32	94	TPSTFCQGTKVEIK	0	

Table 8: The V_H gene from PG1433 paired with various light chain genes with differing rates of amino acid mutation were compared for production levels with the original clone containing the IGKV1-39 gene.

IgG name	Light chain gene	Number of amino acid mutations	concentration (µg/ml) ⁵
<i>PG1433</i>	<i>1-39</i>	<i>0</i>	<i>63, 45.5, 38.6</i> <i>(avg = 49)</i>
PG1631	1-12	4	10.5
PG1632	1-27	7	9.3
PG1634	1D-12	10	10.8
PG1635	1D-33	6	10.2
PG1642	1-5	8	7.1
PG1644	1-9	3	7.8
PG1650	1D-39	3	9.1
PG1652	2D-28	3	7.1
PG1653	3-15	14	7
PG1654	3-20	2	5.2
PG1674	1-40	7	8.2
PG1678	2-11	2	8.1
PG1680	2-14	15	10.8
PG1682	3-1	13	9.9
PG1683	6-57	6	13.9

subclone	culture days at start batch	avg pH in previous 14 days	% avg	batch started at population countings	maximum viable cell density $\times 10^6$ cells/ml	IVC maximum lgG concentration (10 ⁶ µg/L)	% avg	qAb (mg/ml)	% avg	maximum lgG concentration (mg/L)	% avg	correlation TF	correlation RH	correlation
B224.1	23	35	5.5	99	3	530	92	5.5	97	122	79	0.99	0.95	0.92
	40	41	1.2	115	3.7	536	89	10.8	107	188	122	1	0.89	0.99
	25	26	0.3	104	3.2	627	599	5.8	95	156	100	0.97	0.89	0.98
	30	38			3.3	375		9.8		155				
B224.4	22	36	1	102	2.2	324	888	18.3	119	183	103	1	0.99	0.97
	40	35	0.3	104	3.8	79	79	12.5	102	186	96	1	1	1
	28	34	0.2	98	1.7	378	98	5.8	81	97	87	0.99	1	0.99
	28	34	0.2	98	1.7	378	98	5.8	81	97	87	0.99	1	0.99
B224.15	35	35			2.5	316		12.2		111				
	21	32	1.8	106	2.5	397	591	7.9	79	97	91	0.89	0.99	0.91
	40	32	0.3	97	3.7	339	114	7.3	91	114	195	1	0.97	0.97
	29	31	0.2	94	2.1	310	85	8.8	112	132	98	0.96	0.95	0.99
B224.30	37	33			2.8	400		2		104				
	23	38	1.2	97	1.8	335	89	18.3	112	110	73	0.99	1	0.99
	40	31	0.3	103	2.7	372	888	13.9	107	306	188	1	0.99	0.99
	29	30	0.7	103	1.6	325	89	18.8	92	116	91	0.98	0.99	0.99
B224.37	33	33			2.0	377		13		130				
	23	34	2.5	100	3.1	307	102	13.8	98	238	81	1	0.98	0.99
	42	37	0.7	109	3.6	375	117	18.1	111	219	124	1	0.94	0.95
	31	34	0.2	109	2.3	353	89	14.6	90	263	95	1	1	0.89
B224.53	34	34			3.0	402		16.2		257				
	23	32	0.4	102	3.5	695	595	22.5	134	387	122	0.99	0.89	0.89
	42	24	0.3	105	3.1	378	91	20	101	307	112	0.99	0.97	0.95
	30	31	0.2	98	3.8	334	100	18.8	95	308	89	1	0.98	0.99
B224.59	31	31			3.6	636		13.8		319				
	25	23	0.3	100	3.8	353	99	20.9	107	371	114	0.99	0.89	0.89
	42	32	0.4	97	3.7	805	100	24.3	121	279	116	0.98	0.88	0.94
	31	33	0.1	100	3	524	94	18.3	77	231	73	0.99	0.89	0.94
B224.59	33	33			3.5	581		10.1		327				
	23	35	0.6	104	4.3	790	107	20.9	106	303	104	0.99	0.88	0.88
	42	34	0.2	99	3.6	739	111	18.8	95	384	119	0.98	0.92	0.96
	31	31	0.3	96	2.5	383	82	15.2	79	226	77	0.97	0.99	0.99
B224.63	35				3.7	704		15.3		299				
	23	34	0.6	105	4.3	890	108	33	109	258	117	0.99	0.98	0.95
	42	32	0.4	98	3	891	108	12.3	103	281	119	0.99	0.99	0.99
	31	31	0.1	95	4	860	93	10.5	89	169	87	0.99	0.98	1
B224.63	31	31			3.1	780		11.9		251				
	25	36	1.7	104	3	436	77	5.8	95	366	81	0.98	0.98	0.99
	42	37	0.7	107	3.2	678	122	6.2	101	368	121	1	0.97	0.97
	31	32	0.2	96	3.9	582	100	8.4	104	292	98	0.98	0.98	0.98
B224.63	35				2.8	350		8.1		79				
	23	32	0.8	102	3.1	300	97	9.1	108	112	95	0.97	0.92	0.95
	42	31	0.4	98	3.4	389	104	5.8	93	137	113	1	0.98	0.98
	31	31	0.4	98	2.5	366	106	8.8	121	114	94	0.97	0.99	1
B224.66	31	31			3.0	588		7.1		121				
	23	33	1	99	3	399	88	9.1	108	112	95	0.97	0.92	0.95
	42	36	0.5	107	3.6	1188	88	5.6	104	124	132	1	0.99	0.97
	31	34	0.3	101	3.6	625	74	0.89	108	8	9	0.97	0.98	1
B224.66	34				3.7	800		5.4		92				
	23	34	2.5	100	3.1	307	102	13.8	98	238	81	1	0.98	0.99
	42	37	0.7	109	3.6	375	117	18.1	111	219	124	1	0.94	0.95
	31	34	0.2	109	2.3	353	89	14.6	90	263	95	1	1	0.89

Table 10 Antibody mixtures used for staining of lymphocyte populations.
BM = bone marrow, PC = peritoneal cavity, PP = Peyer’s patches.

Stainings					Mixtures								
	Facs #	tubes #	Monoclonal	Work dilution		volume	1st step	2nd step	3rd step	Final dilution			
A	1	1-8	CD21 ^{FITC}	640	320		0.50	2.00					
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	640									
			CD23 ^{PE-Cy7}	50									
			DAPI	1:20									
			Ckappa mouse ^{BIO-APC}	100									
			Clambda mouse ^{BIO-APC}	100									
			B220 ^{Alex-700}	160									
			FC block	400									
				1:50							3.20	APC	5000
	1:30	3.20	APC	3000									
Spleen	2	9-16	IgD ^{FITC}	640	640		1.00	4.00					
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	500									
			IgM ^{PE-Cy7}	640									
			DAPI										
			Ckappa mouse ^{BIO-APC}	100									
			Clambda mouse ^{BIO-APC}	100									
			B220 ^{Alex-700}	160									
			FC block	400									
				1:50							6.40	APC	5000
	1:30	6.40	APC	3000									
BM	3	17-24	Ckappa mouse ^{FITC}	400	320		0.80	2.00					
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	500									
			IgM ^{PE-Cy7}	640									
			DAPI										
			Clambda mouse ^{BIO-APC}	100									
			B220 ^{Alex-700}	160									
			FC block	400									
				1:30							1.28		
											1.00		
Spleen	4	25-32	Ckappa mouse ^{FITC}	400	640		1.60	4.00					
			lambda ^{FITC}	600									
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	500									
			IgM ^{PE-Cy7}	640									
			DAPI										
			IgD ^{A647}	1280									
			B220 ^{Alex-700}	160									
			PNA ^{BIO-SAV-APC-Cy7}	300									
			FC block	400									
PP	5	33-40	IgM ^{FITC}	160	320		2.00	0.64					
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	500									
			IgM ^{PE-Cy7}	640									
			DAPI										
			IgD ^{A647}	1280									
			B220 ^{Alex-700}	160									
			PNA ^{BIO-SAV-APC-Cy7}	300									
			FC block	400									
				1:30							0.50		
PC	6	41-48	Ckappa mouse ^{FITC}	400	640		1.60	4.00					
			lambda ^{FITC}	600									
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	500									
			IgM ^{PE-Cy7}	640									
			DAPI										
			IgD ^{A647}	1280									
			B220 ^{Alex-700}	160									
			PNA ^{BIO-SAV-APC-Cy7}	300									
			FC block	400									
Spleen	7	49-56	IgM ^{FITC}	160	320		2.00	0.64					
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	500									
			Ckappa mouse ^{BIO-PE-Cy7}	100									
			Clambda mouse ^{BIO-PE-Cy7}	100									
			DAPI										
			CD5 ^{APC}	320									
			B220 ^{Alex-700}	160									
			FC block	400									
				1:50							3.20	PE-Cy7	5000
	1:30	3.20	PE-Cy7	3000									
BM	8	57-64	IgM ^{FITC}	160	640		4.00	4.00					
			Ckappa rat ^{PE}	160									
			CD19 ^{PerCP-Cy5.5}	500									
			Ckappa mouse ^{BIO-PE-Cy7}	100									
			Clambda mouse ^{BIO-PE-Cy7}	100									
			DAPI										
			CD25 ^{APC}	80									
			B220 ^{Alex-700}	160									
			FC block	400									
				1:50							6.40	PE-Cy7	5000
	1:30	6.40	PE-Cy7	3000									
RAT spleen													
Spleen	9	67-104	Ckappa rat ^{PE}	160	80	0.5	0.5						
			rat B220 ^{FITC}	160									
			cyt CD3 ^{FITC}	320									
			cyt Ckappa rat ^{PE}	80									
			cyt CD11c ^{PE-TexasRED}	75									
			cyt NK1.1 ^{BIO-PE-Cy7}	200									
			cyt CD19 ^{PerCP-Cy5.5}	320									
			cyt CD4 ^{APC}	500									
			cyt CD11b ^{Alex-700}	50									
				1:50							1.6	PE-Cy7	5000
	1:30	1.6	PE-Cy7	3000									

Table 11 Numbers of lymphocytes harvested from the bone marrow and spleen of wild type and transgenic mice

Bone Marrow			
	*10e6/ml	total vol	total cells
	cells	(ml)	*10 ⁶
Wt	18.82	5.05	95.0
Wt	19.24	4.96	95.4
CD19-Cre	23.42	5.08	119.0
CD19-Cre	20.58	4.82	99.2
CD19-Cre	25.77	5.15	132.7
CD19-Cre / HuVk1	17.71	5.06	89.6
CD19-Cre / HuVk1	12.60	5.33	67.2
CD19-Cre / HuVk1	18.13	5.27	95.5

Spleen			
	*10e6/ml	total vol	total cells
	cells	(ml)	*10 ⁶
Wt	41.70	5.36	223.5
Wt	37.85	4.71	178.3
CD19-Cre	60.19	3.77	226.9
CD19-Cre	35.06	3.66	128.3
CD19-Cre	80.69	4.60	371.2
CD19-Cre / HuVk1	51.67	4.48	231.5
CD19-Cre / HuVk1	58.80	6.24	366.9
CD19-Cre / HuVk1	24.37	6.25	152.3

Claims.

1. A transgenic non-human mammal comprising, at least in its B
5 cell lineage, a nucleic acid encoding at least an immunoglobulin light chain
or heavy chain, wherein the heavy- or light chain encoding sequence is
provided with a means that renders it resistant to DNA rearrangements
and/or somatic hypermutations.
2. A transgenic non-human mammal according to claim 1, which
10 is a rodent, preferably a mouse.
3. A transgenic non-human mammal according to any one of
claims 1-2, wherein the light/heavy chain encoding sequence is integrated
in the genome of the non-human mammal.
4. A transgenic non-human mammal according to claim 3,
15 wherein the integration is in a locus that is resistant to silencing.
5. A transgenic non-human mammal according to claim 4,
wherein the integration is in the Rosa-locus.
6. A transgenic non-human mammal according to any one of
claims 1-5, wherein the light chain encoding nucleic acid is provided with a
20 means that allows expression of said nucleic acid essentially limited to cells
of B cell lineage.
7. A transgenic non-human mammal according to claim 6,
wherein the light chain encoding nucleic acid is provided with a means that
allows expression of the light chain encoding nucleic acid predominantly
25 during a certain stage of the development of B cells.
8. A transgenic non-human mammal according to claim 7,
wherein said means comprises a promoter selected from the group of CD19,
CD20, μ HC, VpreB1, VpreB2, VpreB3, λ 5, Ig α , Ig β , κ LC, λ LC, and BSAP
(Pax5).
- 30 9. A transgenic non-human mammal according to claim 7 or 8,
wherein said means comprises a cre-lox system or the like.

10. A transgenic non-human mammal according to any one of claims 1-9, wherein the light chain encoding sequence encodes a light chain capable of pairing with at least two different heavy chains encoded by the non-human mammal.

5 11. A transgenic non-human mammal according to any one of claims 1-10, wherein at least one of the endogenous loci encoding an endogenous light chain is functionally silenced.

12. A transgenic non-human mammal according to anyone of claims 1-11, wherein the endogenous κ light chain locus is functionally
10 silenced.

13. A transgenic non-human mammal according to any one of claims 1-12, wherein the sequence of the light chain encoding sequence is a human $\nu\kappa$ sequence.

14. A transgenic non-human mammal according to claim 13
15 wherein the light chain encoding sequence is a germline-like sequence.

15. A transgenic non-human mammal according to claim 14, wherein the light chain encoding sequence is a germline sequence.

16. A transgenic non-human mammal according to claim 15, wherein the germline sequence is based on O12.

20 17. A transgenic non-human mammal according to claim 1 wherein the light chain encoding nucleic acid comprises in 5'-3' direction: a $\nu\kappa$ promoter, a human leader, a human V gene, optionally a MoEki enhancer, a rat constant region (κ) and optionally a (truncated) MoEk3' enhancer.

25 18. A transgenic non-human animal which has been provided with an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B cells, said cassette comprising:
means for preventing silencing of expression of the desired proteinaceous
30 molecule after introduction into a host cell, and
means for timing expression of the desired proteinaceous molecule with the desired developmental stage of the host cell.

19. A transgenic non-human animal which has been provided with an expression cassette according to claim 18, wherein said means for timing expression is a promoter of which the activity is essentially limited to the certain stage of development.

5 20. A transgenic non-human animal which has been provided with an expression cassette according to claim 18 or 19 wherein said certain stage starts at a stage immediately preceding or coinciding with the onset of the expression of light chain molecules by said cells at a certain stage of development into a mature B cell.

10 21. A transgenic non-human animal which has been provided with an expression cassette according to claim 19 or 20, wherein said promoter is selected from the group consisting of the CD19 promoter, the Ig- α promoter, the Ig- β promoter, the μ hc promoter, the vk promoter, or analogues and/or homologues thereof.

15 22. A transgenic non-human animal which has been provided with an expression cassette according to any one of claims 19-21, wherein said promoter drives the expression of a cre-like protein.

23. A transgenic non-human animal which has been provided with an expression cassette according to claim 22 whereby the sequence encoding
20 the desired proteinaceous molecule is activated by the action of said cre-like protein.

24. A transgenic non-human animal which has been provided with a set of nucleic acids that are expression cassettes, wherein one nucleic acid comprises an expression cassette according to claim 22 and the second
25 nucleic acid comprises a sequence encoding a desired proteinaceous molecule under control of a constitutive promoter which can be activated by the action of a cre-like protein.

25. A transgenic non-human animal which has been provided with an expression cassette according to claim 23 or 24, wherein said activation
30 is achieved by removal of a "stop".

26. A transgenic non-human animal which has been provided with an expression cassette according to anyone of claims 18-25, wherein said

desired proteinaceous molecule is a polypeptide chain of an immunoglobulin.

27. A transgenic non-human animal which has been provided with an expression cassette according to claim 26, wherein said polypeptide
5 chain is a light chain.

28. A transgenic non-human animal which has been provided with an expression cassette according to claim 27, wherein the sequence encoding said polypeptide chain is rendered essentially incapable of rearrangement.

10 29. A transgenic non-human animal which has been provided with an expression cassette according to claim 27 or 28, wherein said light chain is a germ line like light chain.

30. A transgenic non-human animal which has been provided with an expression cassette according to claim 29, wherein said germ line like
15 light chain is O12.

31. A transgenic non-human animal which has been provided with an expression cassette according to claim 28-30, wherein said rearrangement is prevented by the absence of elements at least partially responsible for somatic hypermutation such as the MoEki enhancer.

20 32. A transgenic non-human animal which has been provided with an expression cassette according to anyone of claims 18-31, wherein said means for prevention of silencing are means for insertion into a locus in the genome of the host cell that is resistant to silencing.

33. A transgenic non-human animal which has been provided with
25 an expression cassette according to claim 32, wherein said means for insertion are means for homologous recombination into said site resistant to silencing.

34. A transgenic non-human animal which has been provided with an expression cassette according to claim 32 or 33 wherein said locus is the
30 rosa-locus.

35. A transgenic non-human animal which has been provided with an expression cassette according to any one of claims 1-16, which comprises in 5'-3' direction: a $\nu\kappa$ promoter, a human leader, a human V gene, optionally a MoE κ i enhancer, a rat constant region (κ) and optionally a (truncated) MoE κ 3' enhancer.

36. A method for producing a desired antibody comprising exposing a non-human mammal according to any one of claims 1-35 to an antigen such that an antibody response is induced and isolating the antibodies specific for the antigen

37. A method for producing a desired antibody comprising exposing a non-human mammal according to any one of claims 1-36 to an antigen such that an antibody response is induced and isolating cells producing such antibodies, culturing and optionally immortalizing said cells and harvesting said antibodies.

38. A method for producing a desired antibody comprising exposing a non-human mammal according to any one of claims 1-37 to an antigen such that an antibody response is induced and isolating a nucleic acid encoding at least part of such an antibody, inserting said nucleic acid or a copy or a derivative thereof in an expression cassette and expressing said antibody in a host cell.

39. Progeny of a transgenic non-human animal according to any one of claims 1-17, the progeny comprising, at least in its B-cell lineage, a heavy- or light chain encoding sequence together with a means that renders the sequence resistant to DNA rearrangements and/or somatic hypermutations.

40. Progeny of a transgenic non-human animal according to any one of claims 18-35, the progeny comprising an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B cells.

41. A cell that is isolated from a transgenic non-human animal according to any one of claims 1-17, the cell comprising a heavy- or light chain encoding sequence together with a means that renders the sequence resistant to DNA rearrangements and/or somatic hypermutations.

5 42. A cell that is isolated from a transgenic non-human animal according to any one of claims 18-35, the cell comprising an expression cassette for the expression of a desired proteinaceous molecule in cells during a certain stage of development in cells developing into mature B cells.

Figure 1

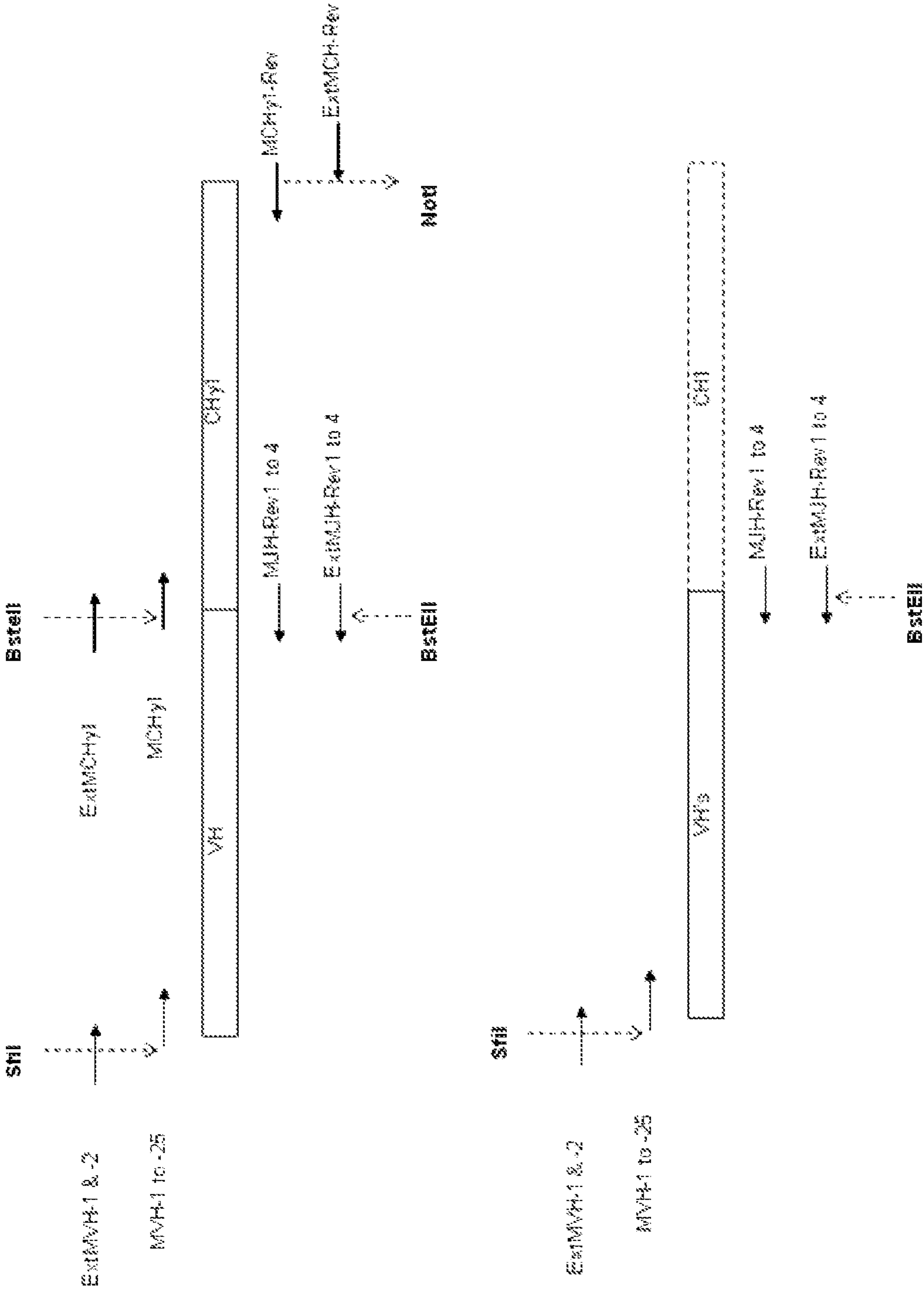


Figure 2

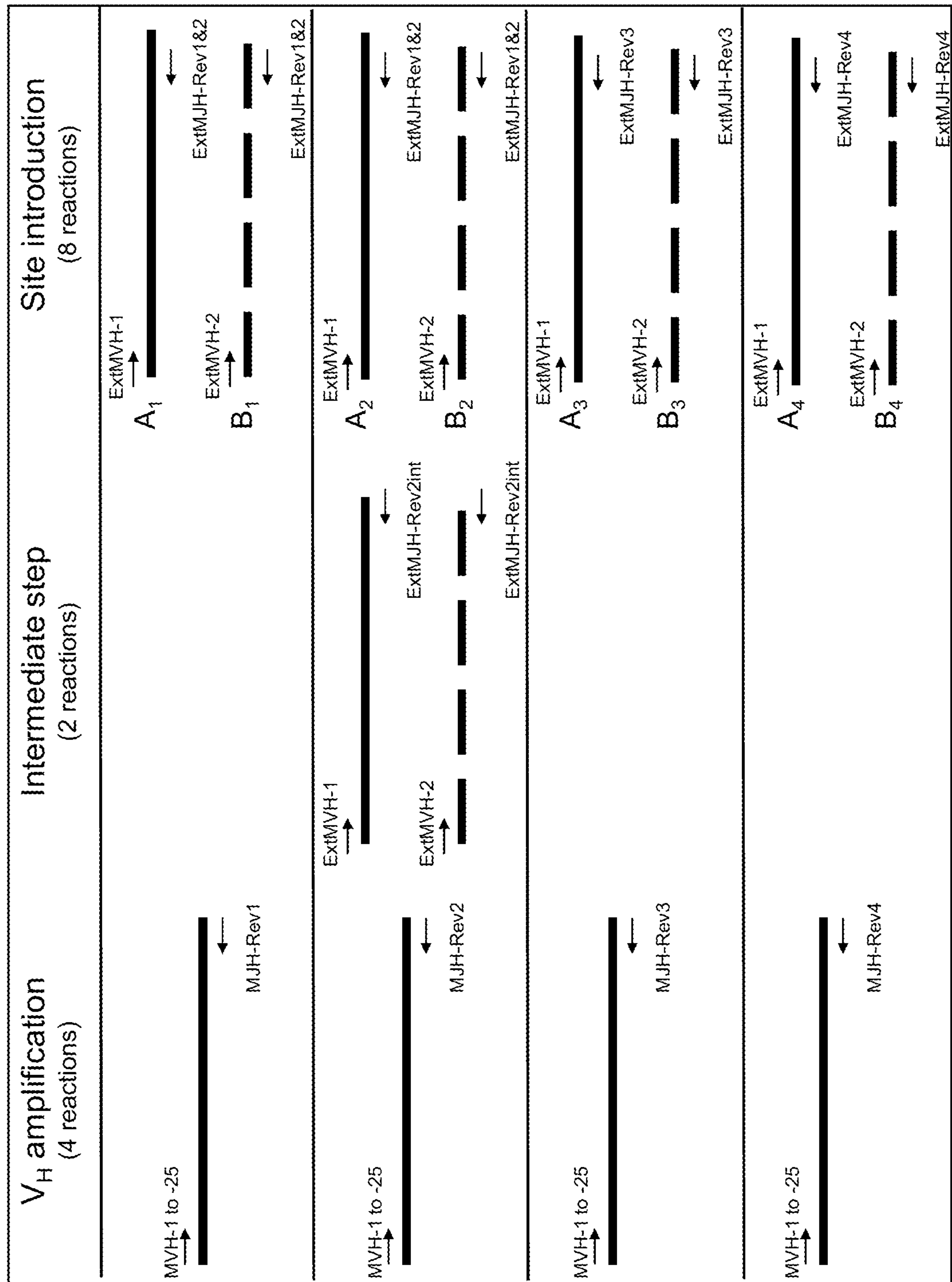


Figure 3

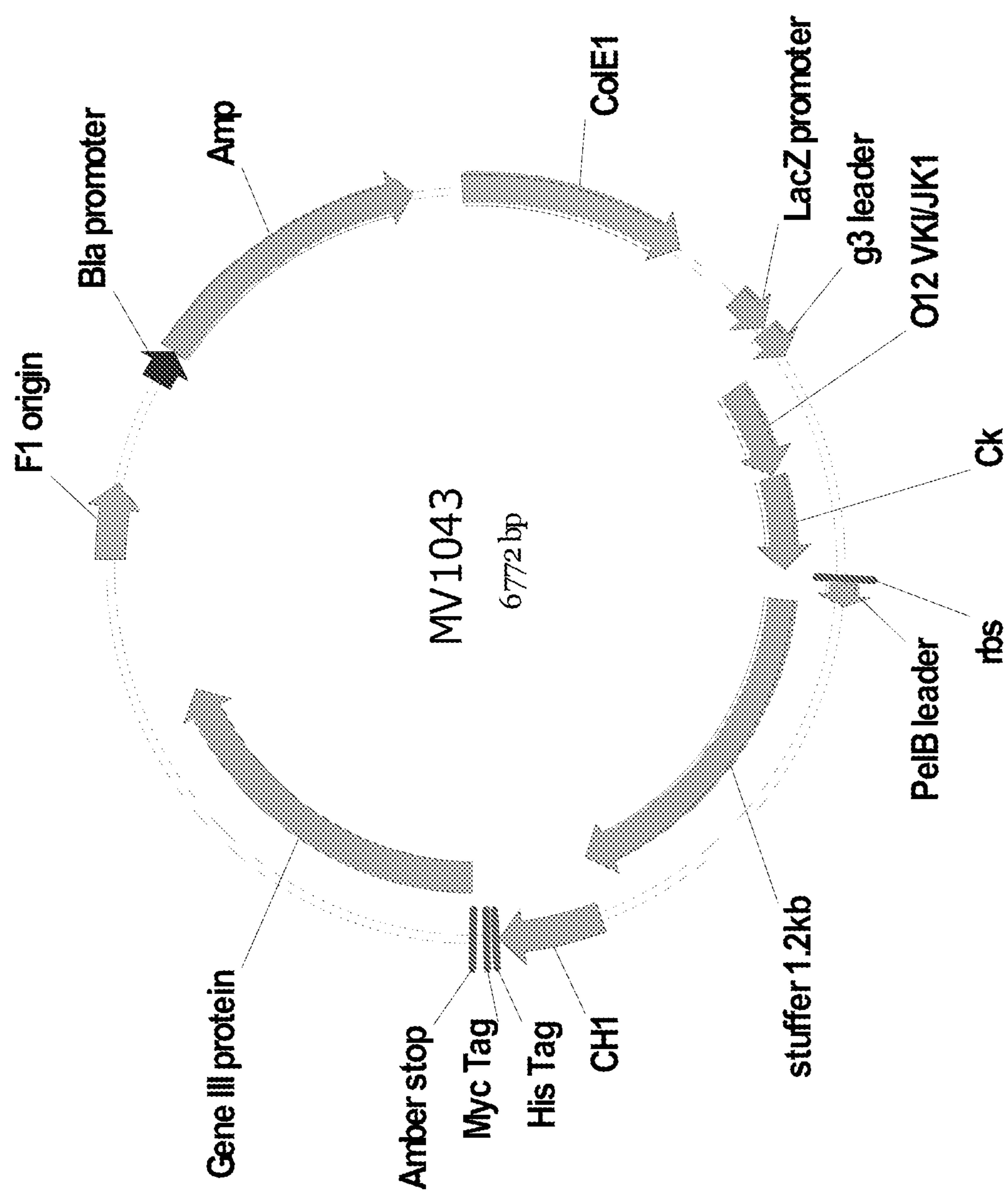


Figure 4

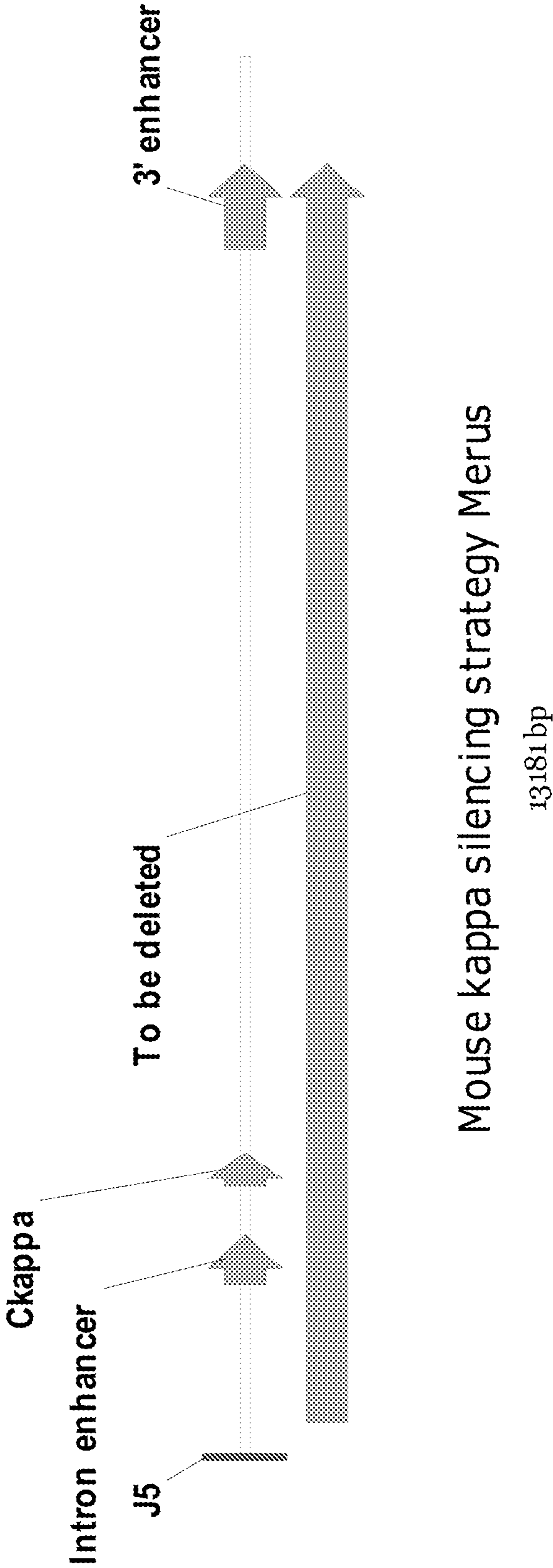


Figure 5

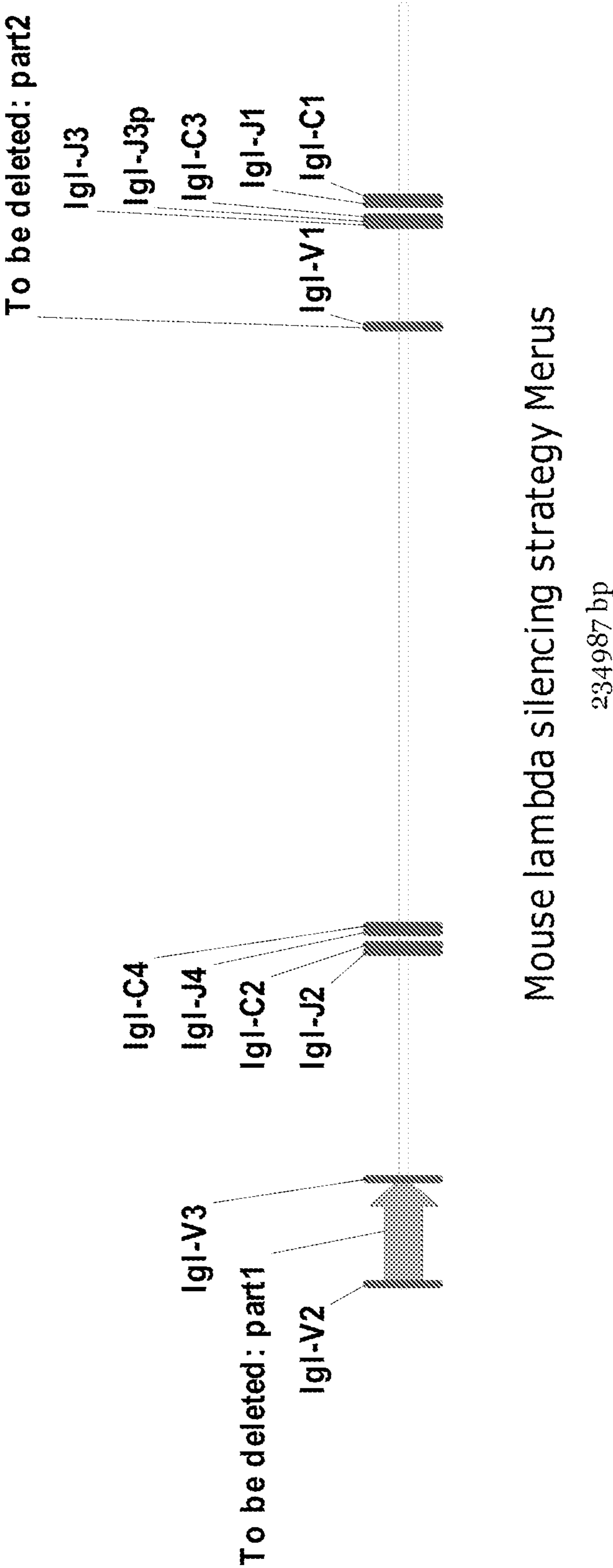


Figure 6

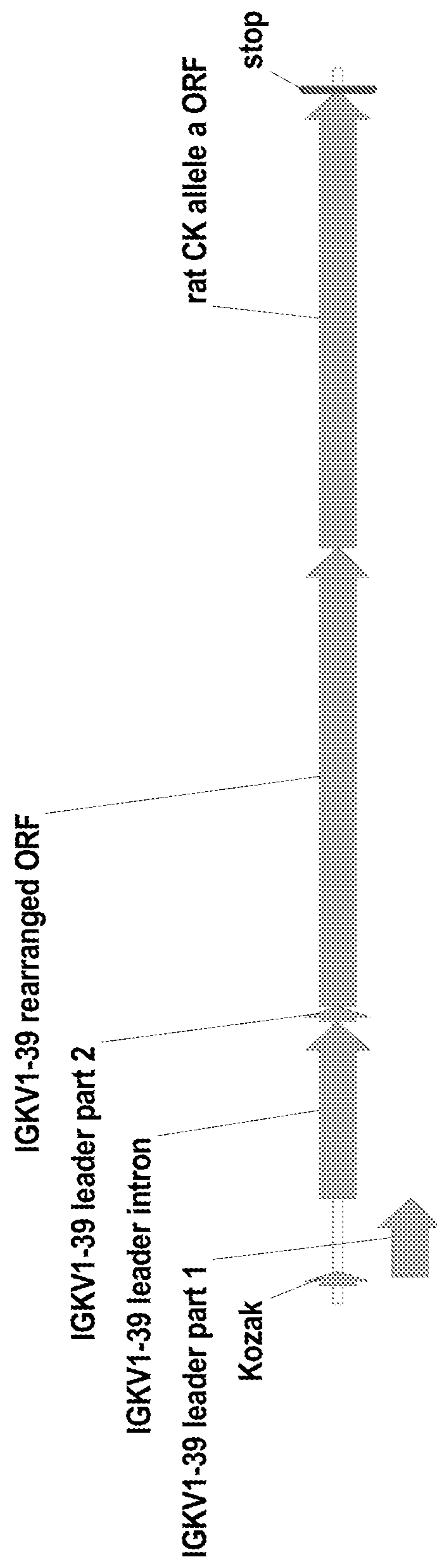


Figure 7

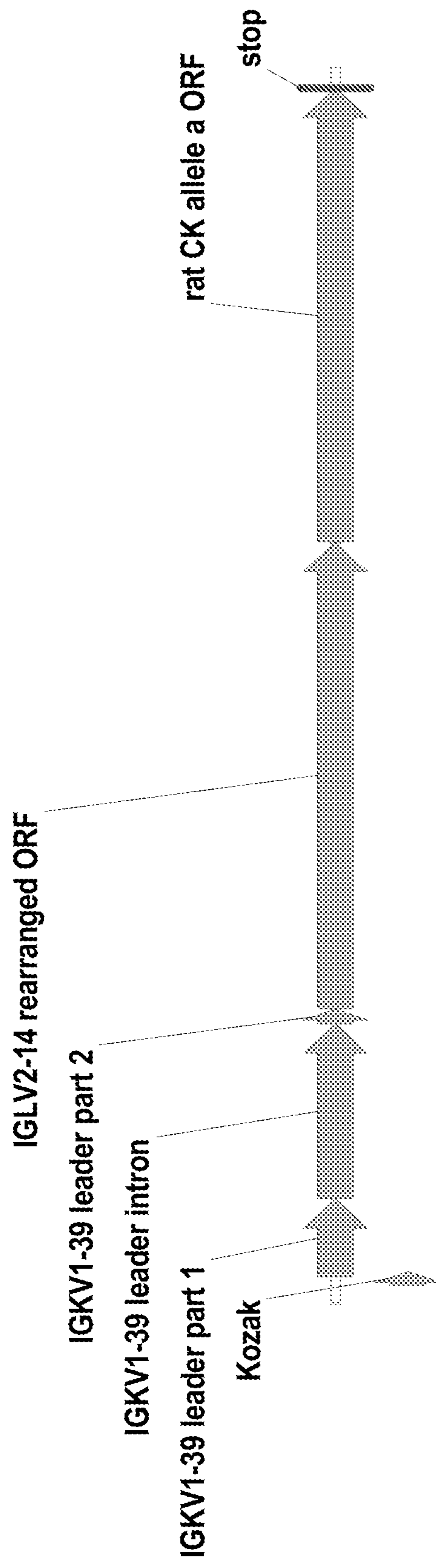


Figure 8

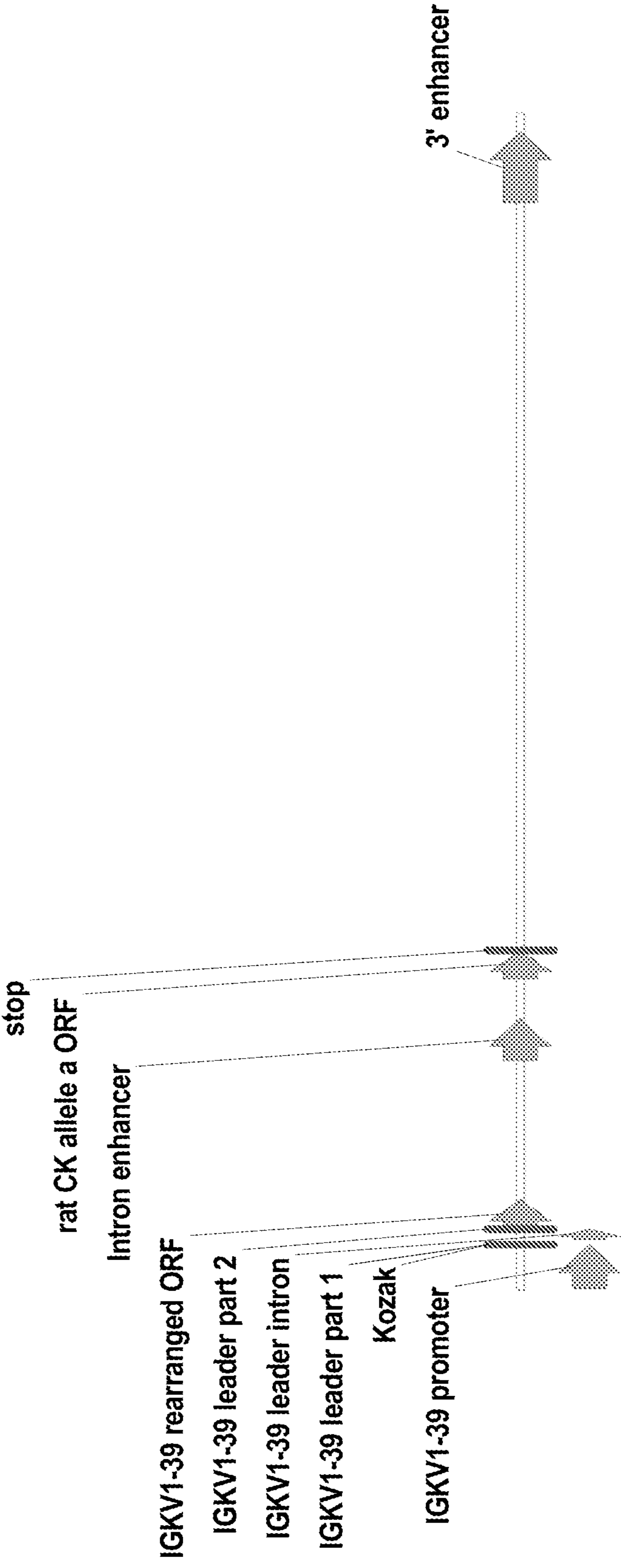


Figure 9

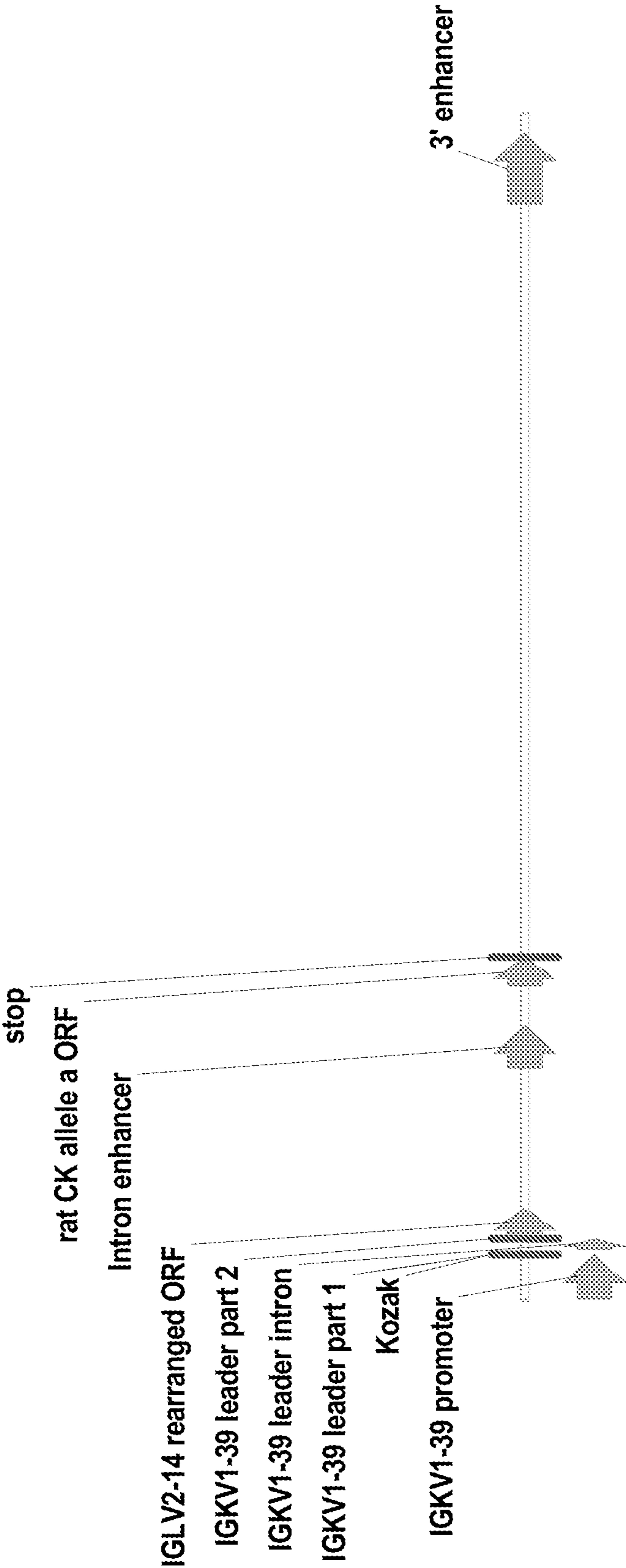


Figure 10

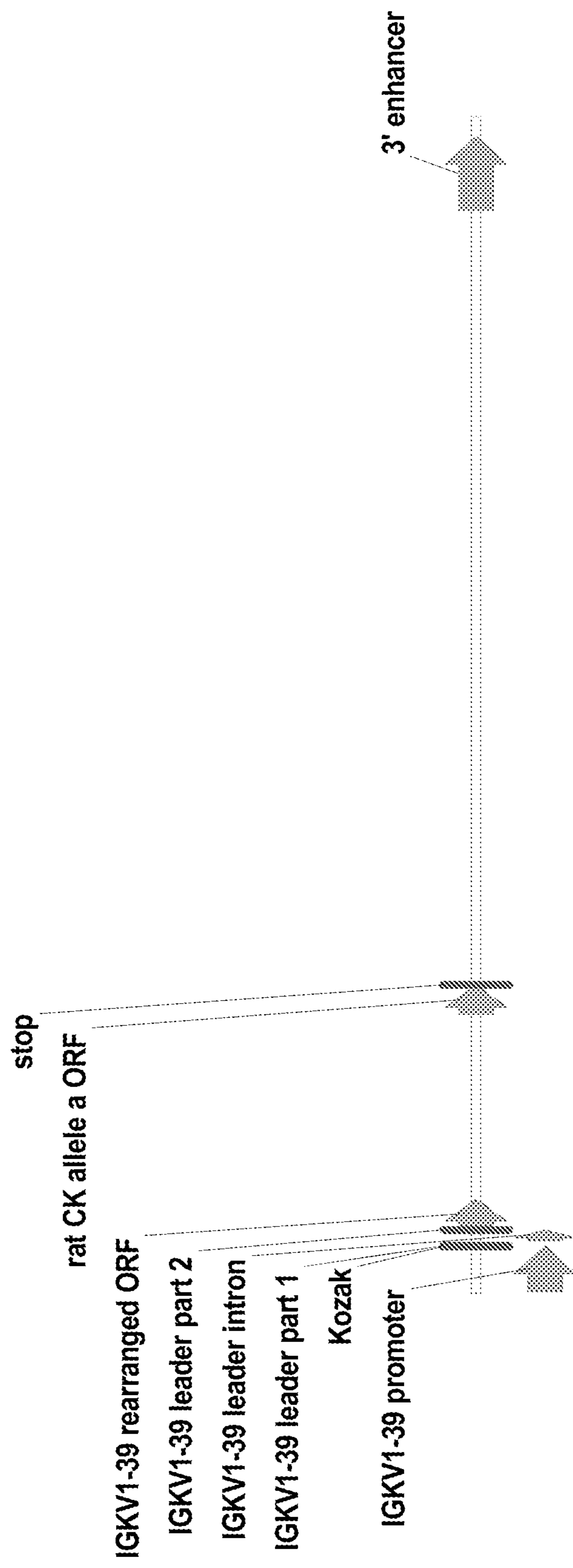


Figure 11

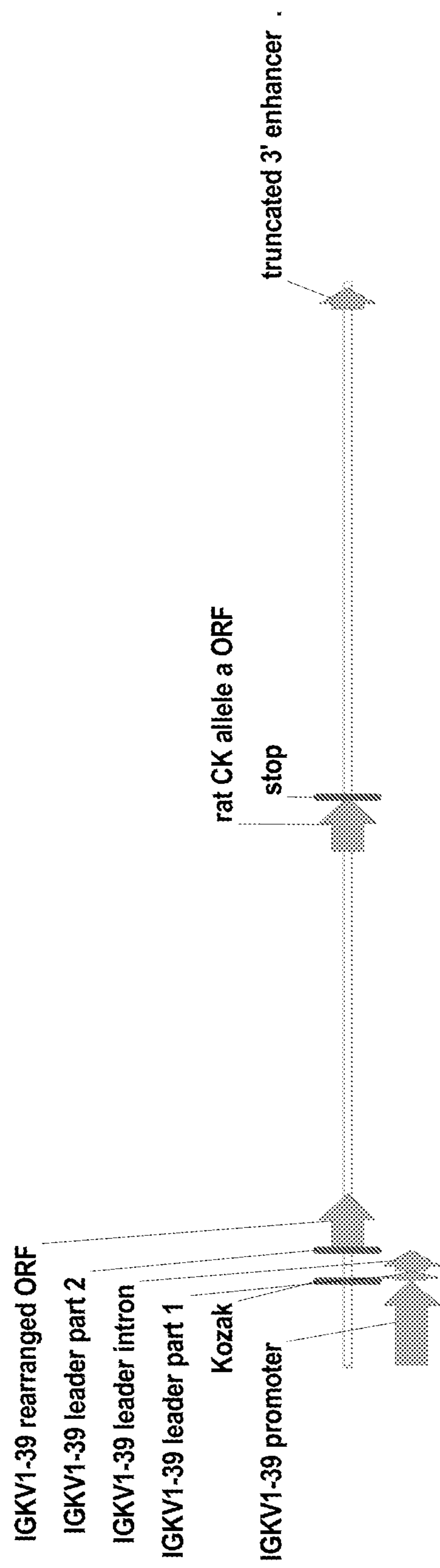


Figure 12

human germline IGKV1-39/J DNA	
1	GAC ATC CAG ATG ACC CAG AGC CCC AGC AGC CTG AGC GCC AGC GTG GGC GAC AGA GTG ACC ATC ACC TGC AGA GCC AGC
79	CAG AGC ATC AGC AGC TAC CTG AAC TGG TAT CAG CAG AAG CCC AAG GGC AAG GGC CCC AAG CTG ATC TAC GCC GCC AGC
157	TCC CTG CAG AGC GGC GTG CCC AGC AGA TTC AGC GGC AGC GGC TCC GGC ACC GAC TTC ACC CTG ACC ATC AGC AGC CTG
235	CAG CCC GAG GAC TTC GCC ACC TAC TAC TGC CAG CAG AGC TAC AGC ACC CCC CCC ACC TTC GGC CAG GGC ACC AAG GTG
313	GAG ATC AAG
human germline IGLV1-39/J Protein	
1	DIQMTQSPSS LSASVGDRVT ITCRASQSI SYLNWYQQKP GKAPKLLIYA
51	ASSLQSGVPS RFGSGSGTD FTLTISLQF EDFATYYCQQ SYSTPPTFGQ
101	GTKVEIK
human germline IGLV2-14/J DNA	
1	CAG TCT GCC CTG ACC CAG CCC GCC TCT GTG TCT GGC AGC CCT GGC CAG AGC ATC ACC ATC AGC TGC ACC GGC ACC AGC
79	AGC GAC GTG GGC TAC AAC TAC GTG TCC TGG TAT CAG CAG CAC CCC GGC AAG GCC AAG CTG ATG ATC TAC GAG
157	GTG TCC AAC AGA CCC AGC GGC GTG AGC AAC AGA TTC AGC GGC AGC AAG AGC GGC AAC ACC GCC AGC CTG ACC ATC AGC
235	GGC CTC CAG GCT GAG GAC GAG GCC GAC TAC TGC AGC AGC TAC ACC AGC AGC TCC ACC CTG GTG TTT GGC GGC GGA
313	ACA AAG CTG ACC GTG CTG
human germline IGLV2-14/J Protein	
1	QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLM I
51	YEVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC SSYTSSSTIV
101	FGGGTKLTVL
Rat IGCK allele a DNA	
1	AGA GCC GAC GCC GCT CCC ACC GTG TCC ATC TTC CCC CCC AGC ATG GAA CAG CTG ACC TCT GGC GGA GCC ACC GTG GTG
79	TGC TTC GTG AAC AAC TTC TAC CCC AGA GAC ATC AGC GTG AAG TGG AAG ATC GAC GGC AGC GAG CAG AGG GAC GGC GTG
157	CTG GAC AGC GTG ACC GAC CAG GAC AGC AAG GAC TCC ACC TAC AGC ATG AGC ACC CTG ACC AAG GTG GAG
235	TAC GAG AGG CAC AAC CTG TAC ACC TGC GAG GTG GTG CAC AAG ACC AGC TCC AGC GTC AAG TCC TTC AAC CGG
313	AAC GAG TGT
Rat IGCK allele a protein	
1	RADAAPTVSI FPPSMEQLTS GGATVVCFVN NFYPRDISVK WKIDGSEQRD
51	GVLDSEVTDQD SKDSTYSMSS TLSLTKVEYE RHNLYTCEVV HKTSSSPVVK
101	SFNRNEC

Figure 12 contd

IGKV1-39/J-Ck	
1	GGT ACC GCG GCC GCG ACC ATG GAC ATG AGA GTG AGA GTG CCC GCC CAG CTC CTG GGG CTC CTG CTC CTA CTC TGG CTC CGA GGT AAG
79	GAT GGA GAA CAC TAG GAA TTT ACT CAG CCA GTG TGC TCA GTA CTG ACT GGA ACT TCA GGG AAG TTC TCT GAT AAC ATG
157	ATT AAT AGT AAG AAT ATT TGT TTT TAT TCC AAT CTC AGG TGC CAG ATG TGA CAT CCA GAT GAC CCA GAG CCC CAG
235	CAG CCT GAG GCG CAG CGT GGT GAC CAT CAC CTG CAG AGC CAG CCA GAG CAT CAG CCA GAG CCA CCA GAA CTG
313	GTA TCA GCA GAA GCC CGG CAA GGC CGC CAG CCA GGT GAT CTA CGC CAG CCA GAG CCA GAG CCA GAG CCA GAG
391	ATT CAG CGG CAG CTA CAG CAC CCC CAG CCA GGT GAC CAT CAG CCA GGT GCA GGT GCA GGT GCA GGT GCA GGT GCA GGT
469	CTG CCA GCA GAG CTA CAG CAC CCC CAG CCA GGT GAC CAT CAG CCA GGT GCA GGT GCA GGT GCA GGT GCA GGT
547	CAC CGT GTC CAT CTT CCC CAG CCA GGT GAC CTA GGT GCA GGT GCA GGT GCA GGT GCA GGT GCA GGT GCA GGT
625	CTA CCC CAG AGA CAT CAG CGT GAA GTG GAA GAT CAG CGG CAG CCA GGT GCA GGT GCA GGT GCA GGT GCA GGT
703	CCA GGA CAG CAA GGA CTC CAG CCA GGT GCA GGT GCA GGT GCA GGT GCA GGT GCA GGT GCA GGT GCA GGT
781	GTA CAC CTG CGA GGT GGT GCA CAA GAC CAG CTC CAG CCC CAG CCA GGT GCA GGT GCA GGT GCA GGT GCA GGT
859	CGA GCT C
IGLV2-14/J-Ck	
1	GGT ACC GCG GCC GCG CAC TAG GAA TTT ACT GAC ATG AGA GTG AGA GTG CCC GCC CAG CTC CTG GGG CTC CTG CTC CTA CTC TGG CTC CGA GGT AAG
79	GAT GGA GAA CAC TAG GAA TTT ACT CAG CCA GTG TGC TCA GTA CTG ACT GGA ACT TCA GGG AAG TTC TCT GAT AAC ATG
157	ATT AAT AGT AAG AAT ATT TGT TTT TAT TCC AAT CTC AGG TGC CAG ATG TGA CAT CCA GAT GAC CCA GAG CCC CAG
235	TGT GTC TGG CAG CCC TGG CCA GAG CAT CAC CAT CAG CTG CAC CGG CAC CAG CCA GGT GAT GAT CTA CGA GGT GCA GGT GCA GGT GCA GGT
313	GTC CTG GTA TCA GCA GCA CCC CGG CAA GGC CCC CAA GCT GAT GAT CTA CGA GGT GTC CAA CAG ACC CAG CGG CGT GAG
391	CAA CAG ATT CAG CGG CAG CAA GAG CGG CAA CAC CAC CCA GGT GAT GAT CTA CGA GGT GTC CAA CAG ACC CAG CGG CGT GAG
469	CTA CTA CTG CAG CAG CTA CAC CAG CAG CCA GGT GAT GAT GAT CTA CGA GGT GTC CAA CAG ACC CAG CGG CGT GAG
547	CGC CGC TCC CAC CGT GTC CAT CTT CCC CAG CAG CCA GGT GAT GAT GAT CTA CGA GGT GTC CAA CAG ACC CAG CGG CGT GAG
625	GAA CAA CTT CTA CCC CAG AGA CAT CTT CCC CAG CAG CCA GGT GAT GAT GAT CTA CGA GGT GTC CAA CAG ACC CAG CGG CGT GAG
703	CGT GAC CGA CCA GGA CAG CAA GGA CTC CAC CTA CAG CAT GAG CAG CCA GGT GAT GAT GAT CTA CGA GGT GTC CAA CAG ACC CAG CGG CGT GAG
781	GCA CAA CCT GTA CAC CTG CGA GGT GGT GCA CAA GAC CAG CTC CAG CCC CAG CCA GGT GAT GAT GAT CTA CGA GGT
859	TTG AGC TAG CGA GCT C

VKP-IGKV1-39/J-Ck	
1	GGC CGG CCC ACA TGA AAC AAT GGG AAC CAT GTG ACA ATC ACA GAG GTG TTG TTA CTA TAG CAA AAG GGA TTG TTA CTC
79	TCC ACA TCC CTT TAA GTA ACT TGA AGG CCT GAT AGA CCC ACC CTC TAA GAC TTC ATT AGA CAT TCC CTA CGA ATG GTT
157	ATA CTC TCC TGT ATA CTC CCA ATA CAA CTC TAA CAG CCG CTC TAG AGA GAA ATA CAT TCC CTC CAG CCA AAT CTA TGC TGC
235	TTT TTT CCT TCA AAA TAT CTC TTG TCA CAA CAG CCG CCT GTG CAG AGC ACA GGT TAC TAA GAT GAT GAT GAT GAT GAT GAT
313	GCT GGT CTG ACC ATG CCC TGG GAC CCA GAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
391	GCC TGC CCC ATG CCC TGG GAC CCA GAG TGA GTC AGG AGC CAC CAT GGA ATT TAC TCA GGT GGT GGT GGT GGT GGT
469	TCG CAC CCA GTG CAG GAG TCA GTC TGA TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
547	GCT ACT CTG GCT CCG AGG TAA CAT GAT TAA TAG TAA GAA TAT TTG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
625	AGG GAA GTT CTC TGA TAA CCA GCA GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT
703	TCC AGA TGA CCC AGA GCG TGA GCG CCA GCG TGG GCG ACA GAG TGA CCA TCA TCA TCA TCA TCA TCA TCA TCA TCA
781	GCA TCA GCA GCT ACC TGA ACT GGT ATC AGC AGA AGC CCG GCA AGG CCC CCA AGC CCG CCA AGC TCA TCA TCA TCA
859	TGC AGA GCG GCG TGC CCA GCA GAT TCA GCG GCA GCG GCT CCG GCA CCG ACT TCA CCA CCA CCA CCA CCA CCA CCA
937	COG AGG ACT TCG CCA CCT ACT ACT GCT GCT GCT GCT GCT GCT GCT GCT GCT GCT GCT GCT GCT GCT GCT GCT
1015	TCA AAC GTA AGT ACA CTT TTC TCA TCT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
1093	GAA AAT CTT GAG AAA ATG GAG AGG GCT CAT TAT CAG TTG ACG TGG CAT ACA GTG TCA GAT TTT CTG TTT AIC AAG CTA

11171	GTG	AGA	TTA	GGG	GCA	AAA	AGA	GGC	TTT	AGT	TGA	GAG	GAA	AGT	AAT	TAA	TAC	TAT	GGT	CAG	CAT	CCA	AGA	GAT	TGG	ATC	
11249	GGG	GAA	TAA	GCA	TGA	GTA	GTT	ATT	GAG	ATC	TGG	GTC	TGA	CTG	CAG	GTA	GCG	TGG	TCT	TCT	AGA	CGT	TTA	AGT	GGG	AGA	
11327	TTT	GGG	GGG	GAT	GAG	GAA	TGA	AGG	AAC	TTT	AGG	ATA	GAA	AAG	GGC	TGA	AGT	CAA	GTT	CAG	CTC	CTA	AAA	TGG	ATG	TGG	
11405	GAG	CAG	ACT	TTG	AAG	ATA	AAC	TGA	ATG	ACC	CAG	AGG	ATG	AAA	CAG	CGC	AGA	TCA	AAG	AGG	GGC	CTG	GAG	CTC	TGA	GAA	
11483	GAG	AAG	GAG	AGT	CAT	CCG	TGT	TGA	GTT	TCC	ACA	AGT	ACT	GTC	TTG	AGT	TTT	GCA	ATA	AAA	GTG	GGA	TAG	CAG	AGT	TGA	
11561	GTG	AGC	CGT	AGG	CTG	AGT	TCT	CTC	TTT	TGT	CTC	CTA	AGT	TTT	TAT	GAC	TAC	AAA	AAT	CAG	TAG	TAT	GTC	CTG	AAA	TAA	
11639	TCA	TTA	AGC	TGT	TTG	AAA	GTA	TGA	CTG	CTT	GCC	ATG	TAG	ATA	CCA	TGG	CTT	GCT	GAA	TAA	TCA	GAA	GAG	GTG	TGA	CTC	
11717	TTA	TTC	TAA	AAT	TTG	TCA	CAA	AAT	GTC	AAA	ATG	AGA	GAC	TCT	GTA	GGA	ACG	AGT	CCT	TGA	CAG	ACA	GCT	CAA	GGG	GTT	
11795	TTT	TTC	CTT	TGT	CTC	ATT	TCT	ACA	TGA	AAG	GTT	TTG	TAA	GAC	AAT	GAT	CTT	TTT	TAT	TAT	AAG	AGT	AGA	AAT	ACA	GTT	GGG
11873	TTT	GAA	CTA	TAT	GTT	TTA	ATG	GCC	ACG	GTT	TTG	TAA	GAC	AAT	TGG	TCC	TTT	GTT	TTT	CTC	CGC	GTT	ACT	CGA	TTG	TAA	
11951	TTT	TAT	ATC	GCC	AGC	AAT	GGA	CTG	AAA	CGG	TCC	GCA	ACC	TCT	TCT	TTA	CAA	CTG	GGT	GAC	CTC	CGC	GCT	GTG	CCA	GCC	
12029	ATT	TGG	CGT	TCA	CCC	TGC	CGC	TAA	GGG	CCA	TGT	GAA	CCC	CCG	CGG	TAG	CAT	CCC	TTG	CTC	CGC	GTG	GAC	CAC	TTT	CCT	
12107	GAG	GCA	CAG	TGA	TAG	GAA	CAG	AGC	CAC	TAA	TCT	GAA	GAG	AAC	AGA	GAT	GTG	ACA	GAC	TAC	ACT	AAT	GTG	AGA	AAA	ACA	
12185	AGG	AAA	GGG	TGA	CTT	ATT	GGA	GAT	TTT	AGA	AAT	AAA	ATG	CAT	TTA	TTA	TAT	TCC	CTT	ATT	TTA	ATT	TTT	TAT	TAT	TAG	
12263	GGA	ATT	AGA	AAG	GGC	ATA	AAC	TGC	TTT	AIC	CAG	TGT	TAT	AAT	AAA	AGC	TTA	ATG	TAT	ATA	AIC	TTT	TAG	AGG	TAA	AAT	
12341	CTA	CAG	CCA	GCA	AAA	GTC	ATG	GTA	AAT	ATT	CTT	TGA	CTG	AAC	TCT	CAC	TAA	ACT	CCT	CTA	AAT	TAT	ATG	TCA	TAT	TAA	
12419	CTG	GTT	AAA	TTA	ATA	TAA	ATT	TGT	GAC	ATG	ACC	TTA	ACT	GGT	TAG	GTA	GGA	TAT	TTT	TCT	TCA	TGC	AAA	AAT	ATG	ATC	
12497	AAT	GAAT	AAA	TTA	GCA	CAA	AAA	TAT	TTT	CCA	ATA	CTT	TAA	TTT	TGT	TAG	AGA	AAA	ATG	TTT	AAC	TCA	GCT	ACT	ATA	ACT	
12575	CCA	TAA	TTT	TGA	AAA	CTA	TTT	ATT	AGC	TTT	TGT	GTT	TGA	CCC	TTT	AGC	CAA	AGG	CAA	CTA	TTT	AAG	GAC	CCT	TTA		
12653	AAA	CTC	TTG	AAA	CTA	CTT	TAG	AGT	CAT	TAA	GTT	ATT	TAA	CCA	CTT	TTA	ATT	ACT	TTA	AAA	TGA	TGT	CAA	TTT	CCT	TTT	
12731	AAC	TAT	TAA	TTT	ATT	TTA	AGG	GGG	GAA	AGG	CTG	CTC	ATA	ATT	CTA	TTG	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TTT	TCG	
12809	TTT	TTA	CTA	CCT	CTG	TCA	CCC	AAG	AGT	TGG	CAT	CTC	AAC	AGA	GGG	GAC	TTT	CCG	AGA	GGC	CAT	CTG	GCA	GTT	GCT	TAA	
12887	GAT	CAG	AAG	TGA	AGT	CTG	CCA	GTT	CCT	CCC	AGG	CAG	GTG	GCC	CAG	AGT	ACA	GTT	GAC	CTG	TTT						

4915	TTT	AAA	ATT	AAG	AGA	GAC	ATA	AGT	TTT	GAG	GTA	GCA	AGA	TGG	AAA	CTC	TGG	GGC	TTG	GGG	AAT	GTT	CTG	TCT	CTC	TGT	
4933	ATG	GGA	TGT	GAA	AGT	TAC	TAT	TGT	GGA	ATT	GGG	ATC	TAT	GTT	CTT	CTT	GTA	TAT	ATT	GTA	TAC	TTC	ATA	ATA	ACT	TCA	
5071	CCT	AAA	GAA	ATA	TCT	AAT	ACC	CAG	TGC	ATA	CAT	AAA	AGA	GGA	TAC	AAG	TGA	ATC	ATA	CGT	CAA	GGC	CAG	AAA	GAC		
5149	AAT	AAA	GTA	GGG	GAT	CCA	GGA	TCA	AAI	CTC	CCA	CAA	CCT	TGA	GCC	TTC	TAC	TAT	TCT	GCC	TTC	CAG	AGC	TCA	AAG	TAC	
5227	AAA	ACA	CAT	AAT	TCA	AAC	ACA	TGA	TCC	CTC	CTT	GGG	GTC	TCT	TCC	TTC	ATG	CAT	CGA	ATT	AGA	AAT	AGC	CAT	GTA	TAA	
5305	AAT	GAG	ATA	GAA	GAG	ACC	TTC	ATC	AAC	AGG	TCA	AAG	AAT	ATA	GGT	AAT	TTT	GTC	TGG	GTA	TGA	AGA	GCC	CAC	GTA	TCA	
5383	AAG	GTT	ACA	TTA	GGG	AAG	GAA	GAG	GAC	ACT	AAC	AGT	GAC	TTT	CAT	TCT	CCC	CCT	CTT	GGA	GGC	CCC	TGC	ATT	TAG		
5461	TCC	CTC	GTG	GGC	TCA	TCC	ACT	CAG	CAC	ACA	TTT	ACT	AAG	CAT	CTT	CTC	AGC	CTA	CAC	TCT	GAA	GGC	AGT	GCA	GAA	TAA	
5539	TGTT	TAG	TGT	CCC	TTC	CCC	CAG	TTA	ATA	TGC	AGT	CCA	GTT	TCC	CTT	CTC	CTT	CCC	TTT	CTC	AGT	CCA	CAT	AAG	GAT	GAT	
5617	GGG	AAA	GGA	GAG	TCA	CCA	AAI	AGG	AGA	GGG	CAA	CCC	TTT	GCC	TTC	CTA	CTT	GAG	AAT	GTA	CAT	CCA	TTT	TAT	CCA	CTT	
5695	TTT	GAA	ACT	TCT	TTT	AAT	TGC	TTT	TTT	TTA	ATT	TGT	CTT	TTC	AAA	TAG	CAT	AAC	CCT	GTT	CAT	CCA	TTT	CTG	GGA	ACC	
5773	AAA	TTT	ATC	AAT	CAA	CAG	TGC	CTC	TAA	TCT	GGC	GAA	AGA	ATA	TAC	AAA	AAT	GCC	TCC	TCA	AAA	TAT	ATA	TGT	TCG	AGT	CTT
5851	ATC	TAA	AAC	AGA	ACC	CAC	AAT	AAA	AAA	TGT	CAA	ATA	AGT	ATA	AGC	ATT	TAT	ATA	ATT	CTG	AGC	AAC	CCT	GTG	CTT	GTG	CTT
5929	TGT	GAA	AAA	AAT	ATA	ATC	TAA	TGT	CAC	ATG	CTG	TAT	TCT	TTT	TAT	TTA	ACA	CTG	GTG	AAA	TTA	TAC	CAT	TAG	AGA	GAA	
6007	AGA	GGA	CAG	ATC	ACT	GAT	CCT	AGG	AIC	TAG	GGA	TGT	TAC	AGA	TAA	GAA	AAC	AAA	TGT	GAC	AAA	GAG	CTG	TCA	CAA	GGA	
6085	GGA	TCT	TCA	AGG	TCA	CAG	AAT	CAC	TGT	CTT	GAT	TTC	AGT	GGT	GGT	TAC	ATA	CAT	TTA	AAT	ATG	TGA	TAA	AAT	GTT	GTT	
6163	GAA	CTA	TAT	TCA	TAT	ATT	GTA	CCA	ATG	TCA	AAT	GCT	TAA	TTT	TGG	CTC	TAT	AGT	ATA	ATT	ATG	CAC	TAA	ATA	ACT	ATT	
6241	TGG	ACA	AAG	AAA	ATG	ATG	TTT	ACA	TCA	AAG	GTG	AGG	CCA	TAT	TTG	TTA	GGA	ACA	TAA	CTT	AAA	AAC	CAT	TTT	GGA	TAA	
6319	CTA	ATG	AAA	AGC	CAT	TTT	GTG	TGC	CTT	GGC	ATA	TCA	TGC	CTA	AGC	TGT	CAC	CAG	ATA	GAT	CTA	ATA	AGA	CCT	AAG	CCT	
6397	CAG	AAG	CAA	GCC	CCT	GCC	CAG	CAA	GCA	GGC	AGC	ACA	GAT	AAG	ACC	GAG	TAA	ACC	CAG	GAC	AGG	CCA	TGA	TAT	GCT	AAT	GAA
6475	CTA	CCT	TCA	AGG	TGT	TGC	TGA	CCT	AGT	GAA	CCA	GCC	CCA	AGC	TGT	GAG	CCC	CAG	CAA	TAG	CAC	AAA	GCT	ACT	GCC	CAA	
6553	AGA	AAT	TAT	ACA	AAA	ATT	GGG	ACT	TTG	GGG	ATG	GTG	TGC	AGG	ATC	GCT	CTG	TAT	GCC	TGG	AAC	ACA	GCT	TCT	TCT	CTA	
6631	TGT	TTT	GTA	TTG	ATA	CCA	GTG	TAG	AAG	CTT	CCA	AAA	CTT	TCT	CAC	TGA	AGA	CTG	AGA	TTT	CCC	ATG	TGG	GAC	CCC	TAC	CTA
6709	CTC	TTT	TGC	CCA																							

8659	TCT	GCC	TCT	GCC	TCT	GCC	TCT	GCC	TCT	GCC	TCT	GCC	TCT	GCC	TCT	GCC	TCT	GCC	TCT	GCC
8737	TCT	GCC	TCT	GCC	TCT	GCC	TAG	TGC	TGG	AAT	TAA	AGG	TTT	GCG	CCA	CAC	CGC	CCG	GTG	AAA
8815	AAC	TTT	ATA	TAT	GTC	TCA	TTT	IAT	TTT	TAI	TTT	TTC	TAT	CAG	ATA	GGA	CTG	TGC	TAA	ACT
8893	AGT	AAI	CGC	AAG	TTG	IAT	TGT	TGT	TGT	TGT	TTT	GCT	TTT	GCT	TTT	GCT	TTT	TTG	CTT	TTT
8971	ITG	CTT	TGC	TTT	GCT	TTT	TGC	CTT	TGC	TTT	GCT	TTT	GCT	TTT	GCT	TTT	TTG	GGT	TTT	TTT
9049	GGG	GAG	GGA	GGG	TGG	AGA	AAG	AAT	CTT	ACT	ATG	AAG	CTC	TGA	CTG	TGG	GAA	CAA	CTC	ACT
9127	TCA	ACT	CAT	AGA	GAT	CTG	CCT	TCT	TCT	GCC	TCC	CAA	GTG	CSA	TGG	ACA	TAA	AAG	GCA	TAC
9205	TCC	CAA	GTT	TTA	GCA	AAA	GTT	TCT	TCT	AGA	CTT	AGA	CTT	GAC	ATT	AAT	AAT	CCA	GTG	CCA
9283	GAG	TTI	ACC	TGA	ATC	ATA	CTC	TAT	ACT	TTA	TCA	GAG	ATT	AAA	TTA	ACA	ITT	AAT	CCA	GTG
9361	CAT	TCA	ATG	GCA	GTG	TTT	GCC	ATC	ATG	CAT	AGG	CTT	AGT	CTT	CAG	TGC	TGA	AAG	GCA	TTG
9439	ATA	CAG	ATG	AGA	AAC	TGG	GAA	AGA	CTT	GCC	TCA	GAT	TCT	CTA	CTG	AAA	GCC	TGA	GTT	TGT
9517	TAC	TTT	CAA	TAT	TTTT	TAA	TGT	ATA	ATT	TTT	TTA	TTT	CCA	CTG	ATT	TTA	TTT	ATT	AAC	ATT
9595	ATG	CAA	TAA	ACC	AAA	TAC	ATG	GAC	AAA	ATA	CAA	GAA	TCA	TAT	GAT	CAC	CTC	AAT	GGA	AGG
9673	AAA	AGT	CTT	TGA	TAA	GAT	TCA	ACA	JTC	APT	CTT	TTT	JTA	TAT	GAT	ATT	TTT	CTC	AAA	AAA
9751	AAA	GCC	CCC	TAT	ACC	TTT	CAA	AAA	ACC	AAG	GCC	CTC	CCC	CAA	CCC	ACC	CAC	TCC	TGC	TTT
9829	GAG	GCA	TAT	GAT	CTT	CAA	AAA	ACC	AAG	GCC	CTC	TCC	TCT	CAT	TGG	TGG	CCG	ACT	ATT	AGG
9907	GCA	ACT	AGA	GAC	ACA	GCT	CTG	GGG	GTT	ACT	GGT	TAG	TTT	ATA	TTG	TTA	GTC	CTC	OTA	TAG
9985	CCT	CTT	GGA	TAC	TTT	CTC	TAG	TTT	CTT	CCT	CTT	GAG	CCC	TGT	GTC	CCA	TCC	AAT	AGA	TGA
10063	GTA	TTT	GCC	AGG	CAC	TGG	CAT	AGC	CTC	ACG	AGA	AAG	AGA	GAG	CTA	TGT	TGT	CCA	GAT	OCT
10141	TAT	GCA	ATA	GTA	TCT	GGT	TTT	GGT	GGT	TGT	ATA	TGG	GAT	GGA	TCC	CAE	AGT	GGA	GCA	GTC
10219	CCA	TCT	CAG	CTC	CAA	ACT	TTG	TCT	CTA	TAA	CTC	CTT	CCA	TGG	GTA	TTT	TGT	TCC	CCA	TTT
10297	CCA	CAC	TTT	GGT	CTT	CCT	TCT	TCT	TGA	GTT	TCA	TAT	GTT	GCA	TCT	TGG	ATA	TTT	TCT	TCT
10375	TAT	CAG	TGA	GTG	CAT	ATC	ATG	OGT	GTT	ATT	TTG	TGA	TTA	GTT	TAC	CTC	ACT	CAG	GAT	GAT
10453	TTT	GCC	TAA	GAA	TTT	CAT	TAA	TTT	ACT	GTT	TTT	AAI	TGC	TGA	ATA	GTA	CTC	CAT	TGT	GTA
10531	TAT	CCA	TTT	CTC	TGT	TGA	GGG	GCA	TCT	GGG	TTT	CCA	GCT	TCT	GGC	TAT	TAT	AAA	TAA	TGG
10609	GGA	GCA	TGT	GTC	CTT	ATC	AAG	TTG	GAA	CAT	CTT	CTA	GGT	ATA	TGC	CCA	GGA	GAG	GAA	TTG
10687	CCA	TCA	ACA	TGC	ATT	CTT	AAT	AAA	AGC	CCT	AGA	ACA	AGG	AGG	ACT	GTA	GGA	AAC	ATA	TTT
10765	GTA	TGA	CAA	ACT	CAT	GAC	CAA	TAT	CAT	CCT	AAA	TGA	ATG	AAA	CCA	TTA	ATA	AGC	TCC	ATT
10843	ACT	ATC	CCT	ACT	TCT	CAT	CCA	TAA	TGA	GAT	TGA	AGC	ATT	AGC	TGG	AGC	AAT	AAG	GCA	AGA
10921	AAA	TAT	TAA	GTC	AAA	TTG	TTT	TCA												

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1	GGC	CGG	CCC	ACA	TGA	AAC	AAI	GGG	AAC	CAT	GTG	ACA	ATC	ACA	GAG	GTG	TTG	TTA	CTA	TAG	CAA	AAG	GGA	TTG	TTA	CTT	CTA
79	TCC	ACA	TCC	CTT	TAA	GTA	ACT	TGA	AGG	CCT	GAT	AGA	CCC	ACC	CTC	TAA	GAC	TTT	ATT	AGA	CAT	TCC	CTA	CGA	ATG	ATT	GTT
157	ATA	CTC	TCC	TGT	ATA	CTC	CCA	ATA	CAA	CTC	TAA	CAAT	ATA	TTA	TTT	CAT	ATA	GTC	CTT	AGG	TTT	GTA	TTA	AAG	TTT	GAC	
235	TTT	TTT	CCT	TCA	AAA	TAT	CTC	TTG	TCA	CAA	CAG	CGG	CTC	TAG	AGA	GAA	ATA	CAT	TCC	CTC	CAG	GCA	AAT	CTA	TGC	TGC	
313	GCT	GGT	CTG	ACC	TGG	GAC	CCT	GGG	GAC	ATT	GCC	CCT	GTG	CTG	AGT	TAC	TAA	GAT	GAG	CCA	GCC	CTG	CAG	CTG	TGC	TCA	
391	GCC	TGC	CCC	ATG	CCC	TGC	TGA	TTG	ATT	TGC	ATG	TTT	CAG	AGC	ACA	GCC	CCC	TGC	CCT	GAA	GAC	TTT	TTT	ATG	GGC	TGG	
469	TGG	CAC	CCT	GTG	CAG	GAG	TCA	GTC	TCA	GTC	AGG	AGC	CAC	CAT	GGA	CAT	GAG	AGT	GCC	CGC	CCA	GCT	CCT	GGG	GCT	CCT	
547	GCT	ACT	CTG	GCT	CCG	AGG	TAA	GGA	TGG	AGA	ACA	CTA	GGA	ATT	TAC	TCA	GCC	AGT	GTG	CTC	AGT	ACT	GAC	TGG	AAC	TTT	
625	AGG	GAA	GTT	CTC	TGA	TAA	CAT	GAT	TAA	TAG	TAA	GAA	TAT	TTG	TTT	TTA	TGT	TTT	CAA	TCT	CAG	GTG	CCA	GAT	GTG	ACA	
703	TCC	AGA	TGA	CCC	AGA	GCC	CCA	GCA	GCC	TGA	GCG	CCA	GCG	TGG	GCG	ACA	GAG	TGA	CCA	TCA	CCT	GCA	GAG	CCA	GCC	AGA	
781	GCA	TCA	GCA	GCT	ACC	TGA	ACT	GGT	ATC	AGC	AGA	AGC	CCG	GCA	AGG	CCC	CCA	AGC	TGC	TGA	TCT	ACG	CCG	CCA	GCT	CCC	
859	TGC	AGA	GCG	GCG	TGC	CCA	GCA	GAT	TCA	GCG	GCA	GCG	GCT	CCG	GCA	CCG	ACT	TCA	CCC	TGA	CCA	TCA	GCA	GCC	TGC	AGC	
937	CCG	AGG	ACT	TGG	CCA	CCT	ACT	ACT	GCC	AGC	AGA	GCT	ACA	GCT	ACA	CCC	CCC	CCT	TGG	GCC	AGG	GCA	CCA	AGG	TGG	AGA	
1015	TCA	AAC	GTA	AGT	ACA	CTT	TTT	TTC	TCA	TCT	TTT	ATG	TGT	AAG	ACA	CAG	GTT	TTT	ATG	TTA	GGA	GTT	AAA	GTC	AGT	TCA	
1093	GAA	AAT	CTT	GAG	AAA	ATG	GAG	AGG	GCT	CAT	TAT	CAG	TTG	ACG	TGG	CAT	ACA	GTG	TCA	GAT	TTT	CTG	TTT	ATC	AAG	CTA	
1171	GTG	AGA	TTA	GGG	GCA	AAA	AGA	GGC	TTT	AGT	TGA	GAG	GAA	AGT	AAT	TAA	TAC	TAT	GGT	CAC	CAT	CCA	AGA	GAT	TGG	ATC	
1249	GGA	GAA	TAA	GCA	TGA	GTA	GTT	ATT	GAG	ATC	TGG	GTC	TGA	CTG	CAG	GTA	GCG	TGG	TCT	TCT	AGA	CGT	TTA	AGT	GGG	AGA	
1327	TTT	GGA	GGG	GAT	GAG	GAA	TGA	AGG	AAC	TTT	AGG	ATA	GAA	AAG	GGC	TGA	AGT	CAA	GTT	CAG	CTC	CTA	AAA	TGG	ATG	TGG	
1405	GAG	CAA	ACT	TTG	AAG	ATA	AAC	TGA	ATG	ACC	CAG	AGG	ATG	AAA	CAG	CGC	AGA	TCA	AAG	AGG	GGC	CTG	GAG	CTC	TGA	GAA	
1483	GAG	AAG	GAG	ACT	CAT	CCG	TGT	TGA	GTT	TCC	ACA	AGT	ACT	GTC	TTG	AGT	TTT	GCA	ATA	AAA	GTG	GGA	TAG	CAG	AGT	TGA	
1561	GTG	AGC	CGT	AGG	CTG	AGT	TCT	CTC	TTT	TGT	CTC	CTA	AGT	TTT	TAT	GAC	TAC	AAA	AAT	CAG	TAG	TAT	GTG	CTG	AAA	TAA	
1639	TCA	TTA	AGC	TGT	TTG	AAA	GTA	TGA	CTG	CTT	GCC	ATG	TAG	ATA	CCA	TGG	CTT	GCT	GAA	TAA	TCA	GAA	GAG	GTG	TGA	CTC	
1717	TTA	TTC	TAA	AAI	TTG	TCA	CAA	AAI	GTG	AAA	ATG	AGA	GAC	TCT	GTA	GGA	ACG	AGT	CCG	TGA	CAG	ACA	GCT	CAA	GGG	GTT	
1795	TTT	TTT</																									

2497	AAT	AAT	AAT	AAT	TTA	GCA	CAA	AAA	TAT	TTC	CCA	ATA	CTTT	TAA	TTT	TGT	GAT	AGA	AAA	ATG	TTT	AAC	TCA	GCT	ACT	ATA	ATC	
2575	CCA	TAA	TTT	TGA	AAA	CTA	TTT	ATT	TGG	CTA	CAC	CAA	AGG	AAG	CCA	TAC	AGA	GGC	TAA	TAT	CAG	AGT	ATT	CTT	GGA	AGA		
2653	GAC	AGG	AGA	AAA	TGA	AAG	TTCT	CTG	TCT	GAA	GCA	TGG	AAC	TGA	AAA	GAA	TGT	AGT	TTT	AGG	GAA	AGG	CAA	TAG	AAG	GAA		
2731	ATA	AAC	TGG	TCT	GAA	TTCT	CTG	TCT	GAA	GCA	TGG	AAC	TTA	CTT	TCT	AAA	GAA	GTA	GCT	AGG	AAC	TAG	GGA	ATA	ACT	TAG	AAA	
2809	GCC	TGA	GAA	TAT	CTT	CAA	AGG	GTC	AGA	CTC	AAT	TTA	CTT	CTT	CTT	AAA	GAA	GTA	GGA	TAA	GGG	TGC	TTT	TTT	GTG	TGT	CTG	
2887	CAA	CAA	GAT	TGT	ATA	TAT	CTG	CAA	CTG	ACA	CAT	AAT	ACA	CTG	AAA	TGG	AGC	CCCT	TCC	TTG	TTA	CTT	CAT	ACC	ATC	CTC	TGT	TCC
2955	TAT	ATA	ACA	TAA	CTG	TTT	CTG	CAA	CTG	ACA	CAT	AAT	ACA	CTG	AAA	TGG	AGC	CCCT	TCC	CTG	TTA	CTT	CAT	ACC	ATC	CTC	TGT	TCC
3043	TTT	CTC	AGG	GGC	CGA	CGC	CGA	CGC	TCC	CAC	CGT	GTC	CAT	CTT	CCC	CCC	CAG	CAT	GGA	ACA	GCT	GAC	CTC	TGG	CGG	AGC	CAC	
3121	CGT	GGT	CTG	CTT	GGT	GAA	CAA	CTT	CTA	CCC	CAG	AGA	CAI	CAG	CGT	GAA	GTG	GAA	GAT	CGA	CGG	CAG	CGA	GCA	GCA	GAG	GGA	
3199	CGG	CGT	GCT	GGA	CAG	CGT	GAC	CGA	CCA	GGA	CGA	CTG	CAA	GGA	CTC	CAC	CAI	GAG	CAG	CAC	CCCT	GAG	CGT	GAC	CCT	GAC	CAA	
3277	GGT	GGA	GTA	CGA	GAG	GCA	CAA	CCT	GTA	CAC	CTG	CGA	GGT	GGT	GCA	CAA	GAC	CAG	CTC	CAG	CCC	CGT	GGT	CAA	GTC	CTT		
3355	CAA	CCG	GAA	CGA	GTG	TTG	AAG	ACA	AAG	GTC	CTG	AGA	CGC	CAC	CAG	CTC	CAG	CTC	CCC	AGC	TCC	ATC	CTA	TCT	TCC	CTT	CTA	
3433	AGG	TCT	TGG	AGG	CTT	CCC	CAC	AAG	CGA	CCT	ACC	ACT	GTT	GGG	GTG	CTC	CAA	ACC	TCC	TCC	CCA	CCT	CCT	TCT	CCT	CCT	CCT	
3511	CCT	CCC	TTT	CCT	TGG	CTT	TTA	TCA	TGC	TAA	TAT	TTG	CAG	AAA	ATA	TTT	TGC	AGT	AAA	GTG	AGT	CTT	TGC	ACT	TGA	GAT	CTC	
3589	TGT	CTT	TCT	TAC	TAA	AUG	GTA	ATA	AAT	GTA	AAT	TGT	TTT	TCC	AGT	TAC	CTG	TTT	TCT	AAA	GAA	GTT	AAA	TGT	TTA	TGT	TTA	
3667	GTT	GCC	CTG	AAA	TCC	ACC	ACA	CTT	AAA	GGA	TAA	ATA	AAA	CCC	CTC	ACT	TGC	CCT	GGT	TGG	CTG	TCC	ACT	ACA	TGG	CAG		
3745	TCC	TTT	CTA	AGG	TTT	ACG	AGT	ACT	ATT	CAT	GGC	TTA	TTT	CTC	TGG	GCC	ATG	GTA	GGT	TTG	AGG	AGG	CAT	ACT	TCC	TAG		
3823	TTT	TCT	TCC	CCT	AAG	TCG	TCA	AAG	TCC	TGA	AGG	GGG	ACA	GTC	TTT	ACA	AGC	ACA	TGT	TCT	TGT	ATC	TGA	TTT	AAC	CTA		
3901	CCC	AGT	AAA	CTT	GGC	GAA	GCA	AAG	TAG	AAT	CAT	TAT	CAC	AGG	CAA	AGG	CAA	CCT	AAA	TGT	GCA	AGC	AAT	AGG	AAA	AAA		
3979	ATG	TGG	AAG	CCC	ATC	ATA	GTA	CTT	GGA	CTT	CAT	CTG	CTT	TTG	TGC	CTT	CAC	TAA	GTT	TTT	AAA	CAI	GAG	CTG	GCT	CTT		
4057	ATC	TGC	CAT	TGG	CAA	GGC	TGG	GCA	CTA	CCC	ACA	ACC	TAC	TTT	AAG	GAC	CTC	TAT	ACC	GTG	AGA	TTA	CAC	ACA	TAC	ACT		
4135	AAA	ATT	TGG	GAA	AAG	TTT	TAC	CAA	GCT	GAG	AGC	TGA	TCA	CCC	CAC	TCT	TAG	GTG	CTT	ATC	TCT	GTA	CAC	CAG	AAA	CCT		
4213	TAA	GAA	GCA	ACC	AGT	ATT	TAG	AGA	CTC	ATT	TAT	GAA	AGT															

Figure 12 contd

6241 ACA ACT GCT TCC CTC CTC GTG TGA TAT CTG TTT TAT GTT AGC ATA ATA TTA TAA GGA ATG TTT GTG TGA ATA
6319 AAC CAA ACA TAT TTT AAA AGC AAA TAT TGT ATG CAC ATC CTA ATT GCT AAA AAG TTT ACA GCT AAT AGT CCC ATG CTC
6397 TCC ACA ATA CTG GAT CCA AAT AAG TCC TAA TGT TGG GCA TCT TTA CAG AGA GAA AGA CAT TAA AAA TGA AGA
6475 GAC ATG CAG AGA GTG CAC CAT GAT GCC ATC GTG GAG ACA GAC TGA AGT GAC ACA ACT GTT AGT CAA AGA GGA TTA AGG ACT
6553 TCC AGA AGC CAC CAA AGG AAG GAG GTA TGA AGT GGT TTC TCC CTC AGA GHA TCC AGA GGA GAC TAA ACC AAC CAA CAC
6631 CTT TTT GCT TAA GAC TTC GTG CCT TCA GGA CTG TGA GAA GGT AGC TTC CTA CTG ATA GCT GAG JTT TCC AGG CCC GTA TGT GGC ATT
6709 TTG TTA AGG TAG AGT CAA GAA ACC AAT AAA ATG CAG ACA GAC AAT GGT TTT GGT TAT GTT TTG TTT GGG GAG TTT TTT
6787 ATT TTT GGT TTT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT
6865 GGG GTT TTT TTG GGT TTT GTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
6943 TTT TTG TTT TTT TGA GAC AGT GTT TCT CTG TAT AGC CCT GGC TGT TTT GGA GTT CCT TCT ATC TCT AAT GTC TAC ATC
7021 TCA GAG GGG ATC CTC TAA TTT CAA ATG AGC AGT AGC TCT CCA TTT TTA GCT CTT ATT TAT TCA TTT ATT TAC TTA CTT
7099 ACT TAT TGT CTG TAG ATG AAA GAA TTT TGG AGT GGG AAA GGG TTC ATG AGC CCC CAG CAA CTA ATG AGG AGC TAC AGA
7177 CAA TTG ATG TTT CTG GGG AAT GGA GAC TCA GTT TCT AGA GTA TAG CTT CTG ATG GGT CAA CCA TGT TCC TGT GGC
7255 TGA TGT CAC ACC CAG GAG TAT GCA GAG AAT AAA TAC AAT TAA ATG AAG TGA GTT GGT TTA AAA ATA AAG GGC ATG AAG
7333 CTT GGG ATA GAA ATT AAG GAT AAA TGA GAT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT
7411 AAA AAA AAT CCA GAA TTA CCT ATG ATG CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT
7489 AAA AGG TAG AGA GGA GAA TAG TGG AAG AGA GAG ATA AGG AGA CTT CAA AAG GAG TGG AGG GAG ATA GAG GAG GAG AAA
7567 GCA GAA GCA ATG GCT GAT AGA CAC AGG ATA AGA GGG AAC AGA AAG GAG AAA GAG GAA GCC AGG ATG AGT GGT ATT TCT TTG
7645 CCT ATC TGT GAC TTG CAC ATG ACC AGG CCC CCA GTA TGC TCA TGT ATG TAT CAG GAT GTG CTA CAA CTT TTT AAT ACA AAA TTT TGA
7723 GAG TCT TTC TTT CTT ATA ACC AGG CCC CCA GTA TGC TCA TGT ATG TAT CAG GAT GTG CTA CAA CTT TTT AAT ACA AAA TTT TGA
7801 GTT TAT AAC TGG GTA ACT TTG TGA AGG GAA GGA AGT GCA CAC TGA GAT GTG CTA CAA CTT TTT AAT ACA AAA TTT TGA
7879 AGA GTT TGT ACA ATG TAT GTA TAA TTA ATA ATT AAT ATT ATG CAC TTT AGA TTT TGA TTT CAA CTC AAG ATA CTA ATT
7957 CTA TAT ATA TGG GTT AAA TCA ATA TAT TAA GTT TAA TTT CAC ATG CTT ATT TTT ATT GTG GTT TTC GAG ACA GGG
8035 TTT CTC TGT ATA GCC CTG GCT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT CTT
8113 CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT
8191 CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT
8269 CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT CTG CCT
8347 TCT CAT TCT ATT TCT ATC AGA TAG GAC TGT GCT AAA CTA ATA AAT GTG CCC TCA AAC TTT TAA ACT TTA TAT ATG
8425 TGT ATT GTT GTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG
8503 CTT TGC TTT GCT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG CTT TTG
8581 GGA GAA AGA ATC TTA CTA TGA AGC TCT GAC TGT CCT GAG TAA TAT AGA TCA GGC TCA GGC TTT TTT CCG GGG AGG GAG GGT
8659 ATC TGC CTT CTT CTG CCT CCC AAG TGC TGG GAA TAA AGG CAT ACA CCT CCA TGC CCA GAT AGT GAT CCC AAG TTT TAG
8737 CAA AAG TTT CTA GAC TTG ACA TTA ATC GAT GGA GAT AGA CAT GAA TTA CAC AAA GAA CTA ATG TGG AGT TTA CCT GAA
8815 TCA TAC TCT ATA CTT TAT CAG AGA TTA ATC CAT TTA ATA ATC CAG TGC CAG TGC CAG GGC CAA TAT TAC TCA TTA TAC AGA TGA GAA
8893 TGT TTG CCA TCA TGC ATA GGC TTA GTC TTT CAG ATT CTC TAC TGA AAG GCT GAG TTT GTG GCT TCT AGA AAA TCT TTT ACT TTC AAT ATT
8971 ACT GGG AAA GAC TTG CCT CAG ATT TTC TAT TTT TTA ACA TTT ATA AGA AAT AAA TGC AAT AAA CCA
9049 TTT AAT GTA TAA TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
9127 AAT ACA TGG ACA AAA TAC AAG AAT CAT ATG ATC ACC TCA ATG GAA GGA AAA AAG AAA GAA AAA GTC TTT GAT
9205 AAG ATT CAA CAT TCA TTC TTT TAT TAG ATA TTT TCT TCA TTT ACA TTT CAA ATG CTA TCC CCA AAG CCC CCT ATA
9283 CCT TCC CCT GCC CTG CTC CAC CCC ACT CCT GCT TTT TGG CCC TGG CAT TCC TCT GTA CTG AGG CAT ATG ACA CTA GAG ACA
9351 TTC AAA AAA CCA AGG GCC TCT CCT CTC ATT GGT GGC CGA CTA TTA GGC CAT CTT TTG CTA CAT ATG CAA CTA GAG ACA
9439 CAG CTC TGG GGG TTA CTG GTT AGT TCA TAT TGT TAG TCC TCC TAT AGA GTT GCA GAC CCC TTT AGC TCC TTG GAT ACT
9517 TTC TCT AGT TCC TTC ATT AGG GGC CCT GTC CCA ATA GAT GAC TGT GAG CAT CCA CTT TTG TAT TTG CCA GGC
9595 ACT GGC ATA GCC TCA CGA GAA AGA GAG AGC TAT GTC AGG ATC CTG TCA GTA AAA TCT TTC TGG CAT ATG CAA TAG TAT
9673 CTG GGT TTG GTG GTT GTA TAT GGG ATG GAT CCC CAA GTG GAG CAT TCT CTG AAT GGT CCT TTC TCC CAT CTC AGC TCC
9751 AAA CTT TGT CTC TAT AAC TCC TTC CAT ATG TGG TGA AGA ATC CAC ACT TTT GTC
9829 TTC CTT CTT GAG TTT CAT ATG TTG CAT CTT GGA TAT TCT AAG TTT CTG GGT TAA TAT CCA CGT ATC AGT GAG TGC
9907 ATA TCA TGC GTG TTA TTT TGT GAT TAG TAT ACC TCA CTC AGG ATG ATA TCC TCC AGA TGC ATC CAT TTG CCT AAG AAT

9985	TTC	ATT	AAT	TCA	CTG	TTT	TTA	ATT	GCT	GAA	TAG	TAC	TCC	ATT	GTG	TAA	ATG	TAC	CAC	AAT	TTC	TGT	ATC	CAT	TCC	TCT	
10063	GTT	GAG	GGG	CAT	CTG	GGT	TCT	TTC	CAG	CTT	CTG	GCT	ATT	ATA	AAT	AAG	GCT	GCT	ATG	AGC	ATA	GCg	GAG	CAT	GTG	TCC	
10141	TTA	TCA	AGT	TGG	AAC	ATC	TTT	TAG	GTA	TAT	GCC	CAG	GAG	AGG	AAT	TGC	TGG	ATC	TTC	CGG	TAG	TAC	CAT	CAA	CAI	GCA	
10219	TTT	TTA	ATA	AAA	GCC	CTA	GAA	CAA	GGA	GGA	CTG	TAG	GAA	ACA	TAT	TCC	AAC	ATA	ATA	AAG	GTT	ATG	TAT	GAC	AAA	CTC	
10297	ATG	ACC	AAT	ATC	ATC	CTA	AAT	GAA	TGA	AAC	CAT	TAA	TAA	GCT	CCA	TTA	AAA	TCA	GAG	GAC	TGC	CCA	CTA	TCC	CTA	CTT	
10375	CTC	ATC	CAT	AAT	GAG	ATT	GAA	GCA	TTA	GCT	GGA	GCA	ATA	AGG	CAA	GAG	AAG	GGA	TAC	AAA	TGG	GAA	AAT	ATT	AAG	TCA	
10453	AAT	TGT	TTT	CAA	TTG	AAG	ATT	ATA	TTA	TCT	TAT	ACC	CAA	TGA	CCT	CAA	ATT	TTG	ACT	AGA	AAA	ATT	GTA	GAA	ATT	ATC	
10531	AAT	AAT	TTC	AGC	AAA	GTG	TTA	TGA	TGC	ACC	ACA	TCC	TTA	TTT	TTC	TCC	CCA	GCT	TCT	TGT	TTC	TCT	CTT	CTT	GCT	GCT	
10609	CTT	CAT	CCT	TTC	TGT	CCT	TCC	ATC	TGC	CTG	CAC	TCT	TGT	CTC	AAG	ACT	GAG	TGC	AGC	GTG	TAA	CTC	TCC	TGT	GAC	TGA	
10687	GTA	TCT	CAC	AAA	ACG	TTT	TAC	TAC	CTG	CCA	AAC	CTG	GAT	GAG	CCC	TTT	GTG	TTT	CTG	AAG	CTA	TGA	GGC	TCT	CTA	CAT	AGA
10765	CTC	AAG	AAG	GAA	ATG	ACA	GGG	AGG	AGG	TAA	TAA	TGA	AGT	GGG	GAA	GGC	TGA	CAT	TAG	CAT	TGC	TCC	TGT	GTG	GCT	CCT	
10843	TAA	TTT	CTC	ATA	CTT	CAC	ACT	GAG	ATG	TTA	TTA	ACT	GTG	ACT	CAT	AGG	TGA	AGA	AGC	CAG	AGC	TAA	GGT	TCT	CAT	ATT	
10921	TGA	GTG	TTA	TAG	AAT	GAG	TAG	AGC	AGT	AGT	TCT	CAA	ACT	ATG	GGT	CAT	GAC	TCC	TTT	ATG	GGT	CAA	ACT	ACC	CTT	TCA	
10999	CAC	AGG	TTG	CAT	ATC	AGA	TAT	CCT	AAT	TTT	AJA	TAC	ATA	TAT	ATA	TGC	ATA	TGT	ATA	TAT	ATA	TAT	TTC	ACA	ACA	GTA	
11077	GGA	AAA	TTA	TTT	AGT	AAT	CAT	TTT	ATA	GTT	GTG	GGT	CAT	GGC	AAC	ATG	AGG	AAC	TGT	ATT	AAA	GGG	TTG	CAG	CAT	TAG	
11155	GAA	TGT	TGA	GAC	CCA	CTG	TAA	TAG	ACA	ATG	AGG	CTT	AAG	GCA	GGG	CTA	TAA	AGC	CCA	ATG	GAC	CAT	GTG	CCT	TTT	CCA	
11233	ACA	TTT	GCC	ACA	TGG	TAA	GCT	CTG	TAT	AGA	CTT	TTT	AAA	GAA	CAT	TGG	TTT	GTA	ATT	TTA	AAT	GGA	TAA	GGG	TCT	TCA	
11311	CTG	TCT	ATC	ACC	CAT	CTA	TAT	AAT	AAA	TAC	ATA	AGT	TTT	GAT	TCC	ACC	ATG	GAT	TCA	AAT	GCA	AAA	ATC	CTC	AAC	CTA	
11389	AGA	TCT	ATC	AGT	GAA	ACA	TTA	ATG	ACC	AAA	TAG	GAA	ATC	CAT	GTA	GAG	ACC	TTT	TAT	CTT	CTG	ATG	GCT	CCA	CAG	GCA	
11467	CCA	TCT	TGC	AAC	AGA	GTT	CTA	CTT	TGC	TAC	TGA	TAA	TGA	ATA	CAG	TGT	CTC	AAC	TCC	TGC	CAT	TGA	ATC	TTC	AGG	AAG	
11545	CCC	CTG	AAA	TGA	CTT	GTA	CTA	CAC	CAT	TTC	TTA	AAG	ACA	GAA	AAG	CTA	AGA	CTT	AGA	GGG	AAT	AAA	TGT	CAT	GCC	TGA	
11623	GAT	CAT	GCA	ACC	AAT	TAA	GTC	CAA	CTT	GGC	CTG	ATC	AAG	AGG	CAC	AAT	TCA	AAA	GCA	ATG	TTG	TTT	CAT	CAC	TAG	CTC	
11701	TTG	TGT	ATG	GTT	GCT	GAT	TCC	GGA	AGC	AAA	GTA	TCA	CTG	AAT	ATC	CCT	AGT	GGG	AAA	AGA	CTT	GGG	AAT	CAA	ATG	TCT</	

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1	GGC	CGG	CCC	ACA	TGA	AAC	AAT	GGG	AAC	CAT	GTG	ACA	ATC	ATC	ACA	GAG	GTG	TTG	TTA	CTA	TAG	CAA	AAG	GGA	TTG	TTA	CTC
79	TCC	ACA	TCC	CTT	TAA	GTA	ACT	TGA	AGG	CCT	GAT	AGA	CCC	ACC	CTC	TAA	GAC	TTT	ATT	AGA	CAT	TCC	CTA	CGA	ATG	GTT	
157	ATA	CTC	TCC	TGT	ATA	CTC	CCA	ATA	CAA	CTC	TAA	AAT	ATA	TTA	TTT	CAT	ATA	GTG	CTT	AGG	TTT	GTA	TTA	AAG	TTT	GAC	
235	TTT	TTT	CCT	TCA	AAA	TAT	CTC	TTG	TCA	CAA	CAG	CGG	CTC	TAG	AGA	GAA	ATA	CAT	TCC	CTC	CAG	GCA	AAT	CTA	TGC	TGC	
313	GCT	GGT	CTG	ACC	TGG	GAC	CCT	GGG	GAC	ATT	GCC	CTT	GTG	CTG	AGT	TAC	TAA	GAT	GAG	CCA	GCC	CTG	CAG	CTG	TGC	TCA	
391	GCC	TGC	CCC	ATG	CCC	TGC	TGA	TTG	ATT	TGC	ATG	TTT	CAG	AGC	ACA	GCC	CCC	TGC	CCT	GAA	GAC	TTT	TTT	ATG	GGC	TGG	
469	TCG	CAC	CCT	GTG	CAG	GAG	TCA	GTC	TCA	GTC	AGG	AGC	CAC	CAT	GGA	CAT	GAG	AGT	GCC	CGC	CCA	GCT	CCT	GGG	GCT	CCT	
547	GCT	ACT	CTG	GCT	CCG	AGG	TAA	GGA	TGG	AGA	ACA	CTA	GGA	ATT	TAC	TCA	GCC	AGT	GTG	CTC	AGT	ACT	GAC	TGG	AAC	TTT	
625	AGG	GAA	GTT	CTC	TGA	TAA	CAT	GAT	TAA	TAG	TAA	GAA	TAT	TTG	TTT	TTA	TGT	TTT	CAA	TCT	CAG	GTG	CCA	GAT	GTG	ACA	
703	TCC	AGA	TGA	CCC	AGA	GCC	CCA	GCA	GCC	TGA	GCG	CCA	GCG	TGG	GCG	ACA	GAG	TGA	CCA	TCA	CCT	GCA	GAG	CCA	GCC	AGA	
781	GCA	TCA	GCA	GCT	ACC	TGA	ACT	GGT	ATC	AGC	AGA	AGC	CCG	GCA	AGG	CCC	CCA	AGC	TGC	TGA	TCT	ACG	CCG	CCA	GCT	CCC	
859	TGC	AGA	GCG	GCG	TGC	CCA	GCA	GAT	TCA	GCG	GCA	GCG	GCT	CCG	GCA	AGG	CCA	TCA	CCC	TGA	TCA	TCT	AGC	CCA	GCC	TGC	AGA
937	CCG	AGG	ACT	TCG	CCA	CCT	ACT	ACT	GCC	AGC	AGA	GCT	ACA	GCA	CCC	CCC	CCA	CCT	TCG	GCC	AGG	GCA	CCA	AGG	TGG	AGA	
1015	TCA	AAC	GTA	AGT	ACA	CTT	TTT	TCA	TCT	TTT	TTT	ATG	TGT	AAG	ACA	CAG	GTT	TTT	CTC	ATG	TTA	GGA	GTT	AAA	GTG	TCA	
11093	GAA	AAT	CTT	GAG	AAA	ATG	GAG	AGG	GCT	CAT	TAT	CAG	TTG	ACG	TGG	CAT	ACA	GTG	TCA	GAT	TTT	CTG	TTT	ATC	AAG	CTA	
11171	GTG	AGA	TTA	GGG	GCA	AAA	AGA	GGC	TTT	AGT	TGA	GAG	GAA	AGT	AAT	TAA	TAC	TAT	GGT	CAC	CAT	CCA	AGA	GAT	TGG	ATC	
1249	GGA	GAA	TAA	GCA	TGA	GTA	GTT	ATT	GAG	ATC	TGG	GTC	TGA	CTG	CAG	GTA	GCG	TGG	TCT	TCT	AGA	CGT	TTA	AGT	GGG	AGA	
1327	TTT	GGA	GGG	GAT	GAG	GAA	TGA	AGG	AAC	TTT	AGG	ATA	GAA	AAG	GCG	TGA	AGT	CAA	GTT	CAG	CTC	CTA	AAA	TGG	ATG	TGG	
1405	GAG	CAA	ACT	TTG	AAG	ATA	AAC	TGA	ATG	ACC	CAG	AGG	ATA	AAG	AAA	CAG	CGC	AGA	TCA	AAG	AGG	GGC	CTG	GAG	CTC	TGA	GAA
1483	GAG	AAG	GAG	ACT	CAT	CCG	TGT	TGA	GTT	TCC	ACA	AGT	ACT	GTG	TTG	AGT	TTT	GCA	ATA	AAA	GCG	GGA	TAG	CAG	AGT	TGA	
1561	GTG	AGC	CGT	AGG	CTG	AGT	TCT	CTC	CTA	AGT	TTT	TAT	GAC	TAC	AAA	AAT	CAG	TAG	TAT	GTG	TAT	GTG	CTG	CTG	AAA	TAA	
1639	TCA	TTA	AGC	TGT	TTG	AAA	GTA	TGA	CTG	CTT	GCC	ATG	TAG	ATA	CCA	TGG	CTT	GCT	GAA	TAA	TCA	GAA	GAG	GTG	TGA	CTC	
1717	TTA	TTC	TAA	AAT	TTG	TCA	CAA	AAT	GTC	AAA	ATG	AGA	GAC	TCT	GTA	GGA	ACG	AGT	CCT	TGA	CAG	ACA	GCT	CAA	GGG	GTT	
1795	TTT																										

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625	AGG	GAA	GTT	CTC	TGA	TAA	CAT	GAT	TAA	TAG	TAA	GAA	TAT	TTG	TTT	TTA	TGT	TTC	CAA	TCT	CAG	GTG	CCA	GAT	GTC	AGT
703	CTG	CCC	TGA	CCC	AGC	AGC	CGT	CTG	TGT	TGT	GCA	GCC	CTG	GCC	AGA	GCA	TCA	CCA	CCG	GCA	CCG	GCA	CCA	GCA	CGC	
781	ACG	TGG	GCG	GCT	ACA	ACT	ACG	TGT	CCT	GGT	ATC	AGC	AGC	ACC	CCG	GCA	AGG	CCC	CCA	AGC	TGA	TCT	ACG	AGG	TGT	
859	CCA	ACA	GAC	CCA	GCG	TGA	GCA	ACA	GAT	TCA	GCG	GCA	GCA	AGC	ACA	CCG	GCA	CCA	CCG	TGA	CCA	TCA	TCA	GCG	GCC	
937	TCC	AGG	CTG	AGG	ACG	AGG	CCG	ACT	ACT	ACT	GCA	GCT	ACA	CCG	GCA	GCT	CCA	CCC	TGG	TGT	TTG	GCG	GCG	GAA	CAA	
1015	AGC	TGA	CCG	TGC	TGC	GTA	AGT	ACA	CTT	TTC	TCA	TCT	TTT	TTT	ATG	TGT	AAG	ACA	CAG	GTT	TTT	ATG	TTA	GGA	GTT	AAA
11033	GTC	AGT	TCA	GAA	AAT	CTT	GAG	AAA	ATG	GAG	AGG	GCT	CAT	TAT	CAG	TTG	ACG	TGG	CAT	ACA	GTG	TCA	GAT	TTTT	CTG	TTT
11171	ATC	AAG	CTA	GTG	AGA	TTA	GGG	GCA	AAA	AGA	GGC	TTT	AGT	TGA	GAG	GAA	AGT	AAT	TAA	TAC	TAT	GGT	CAC	CAT	CCA	AGA
11249	GAT	TGG	ATC	GGA	GAA	TAA	GCA	TGA	GTA	GTT	ATT	GAG	ATC	TGG	GTC	TGA	CTG	CAG	GTA	GCG	TGT	TCT	TCT	AGA	CGT	TTA
11327	AGT	GGG	AGA	TTT	GGA	GGG	GAT	GAG	GAA	TGA	AGG	AAC	TTT	AGG	ATA	GAA	AAG	GGC	TGA	AGT	CAA	GTI	CAG	CTC	CTA	AAA
11405	TGG	ATG	TGG	GAG	CAA	ACT	TTG	AAG	ATA	AAC	TGA	ATG	ACC	CAG	AGG	ATG	AAA	CAG	CGC	AGA	TCA	AAG	AGG	GGC	CTG	GAG
11483	CTC	TGA	GAA	GAG	AAG	GAG	ACT	CAT	CCG	TGT	TGA	GTT	TCC	ACA	AGT	ACT	GTG	AAA	CAG	CTG	AGT	TTT	GCA	ATA	AAA	TAG
11561	CAG	AGT	TGA	GTG	AGC	CGT	AGG	CTG	AGT	TCT	CTC	TTT	TGT	CTC	CTA	AGT	TTT	TAT	GAC	TAC	AAA	AAT	CAG	TAG	TAT	GTC
11639	CTG	AAA	TAA	TCA	TTA	AGC	TGT	TTG	AAA	GTA	TGA	CTG	CTT	GCC	AJG	TAG	ATA	CCA	TGG	CTT	GCT	GAA	TAA	TCA	GAA	GAG
11717	GTG	TGA	CTC	TTA	TTC	TAA	AAI	TTG	TCA	CAA	AAI	GTC	AAA	ATG	AGA	GAC	TCT	GTA	GGA	ACG	AGT	CCT	TGA	CAG	ACA	GCT
11795	CAA	GGG	GTT	TTT	TTC	CTT	TGT	CTC	ATT	TCT	ACA	TGA	AAG	TAA	ATT	TGA	AAT	GAT	CTT	TTT	TAT	TAT	AAG	AGT	AGA	AAT
11873	ACA	GTT	GGG	TTT	GAA	CTA	TAT	GTT	TTA	ATG	GCC	ACG	GTT	TTG	TAA	GAC	AAT	TGG	TCC	TTT	GTT	TTT	CCA	GTT	ATT	ACT
11951	CGA	TTG	TAA	TTT	TAT	ATC	GCC	AGC	AAT	GGA	CTG	AAA	CGG	TCC	GCA	ACC	TCT	TTA	CAA	CTG	GGT	GAC	CTC	GCG	GCT	
2029	GTG	CCA	GCC	ATT	TGG	CGT	TCA	CCC	TGC	GCG	TAA	GGG	CCA	TGT	GAA	CCC	CCG	TAG	CAT	CCC	TTG	CTC	GCG	GTG	GAC	
2107	CAC	TTT	CCT	GAG	GCA	CAG	TGA	TAG	GAA	CGC	AGC	CAC	TAA	TCT	GAA	GAG	AAC	AGA	GAT	GTG	ACA	GAC	TAC	ACT	AAT	GTC
21195	AGA	AAA	ACA	AGG	AAA	GGG	TGA	CTT	ATT	GGA	GAT	TTT	AGA	AAT	AAA	ATG	CAT	TTA	TTA	TAT	TCC	TTT	ATT	TTA	ATT	
22263	TTT	TAT	TAG	GGA	ATT	AGA	AAG	GGC	ATA	AAC	TGC	TTT	ATC	CAG	TGT	TAT	ATT	AAA	AGC	TTA	ATG	TAT	ATA	ATC	TTT	TAG
2341	AGG	TAA	AAT	CTA	CAG	CCA	GCA	AAA	GTC	ATG	ATG	ATT	ATT	ACT	CAA	TCT	CAC	TAA	ACT	CCT	CTA	AAT	TAT	TAT	ATG	
2419	TCA	TAT	TAA	CTG	GTT	AAA	TTA																			

4369	TTC	TGT	AAT	CTG	AAT	CCT	ACC	CAG	TAA	ACT	TGG	CGA	AGC	AAA	GTA	GAA	TCA	TTA	TCA	CAG	GAA	GCA	AAG	GCA	ACC		
4447	TAA	ATG	TGC	AAG	CAA	TAG	GAA	AAT	GTG	GAA	GCC	CAT	CAT	AGT	ACT	TGG	ACT	TCA	TCT	GCT	TTT	GTG	CCT	TCA	CTA	AGT	
4525	TTT	TAA	ACA	TGA	GCT	GGC	TCC	TAT	CTG	CCA	TTG	GCA	AGG	CTG	GGC	ACT	ACC	CAC	AAC	CTA	CTT	CAA	GGA	CCT	CTA	TAC	
4603	CGT	GAG	ATT	ACA	CAC	ATA	CAT	CAA	AAT	TTG	GGA	AAA	GTT	CTA	CCA	AGC	TGA	GAG	CTG	ATC	ACC	CCA	CTC	TTA	GGT	GCT	
4681	TAT	CTC	TGT	ACA	CCA	GAA	ACC	TTA	AGA	AGC	AAC	CAG	TAT	TGA	GAG	ACT	CAT	TTA	TGA	AAG	TCT	AAA	ACT	GGA	TAC	AAC	
4759	CAA	AAT	GTC	CAC	CAA	CAG	TTA	AAT	TAT	GAC	ATG	TTT	ACA	ATT	GAG	CTA	TTA	CTT	AAT	AAG	GAG	AAT	TAA	TAA	AAT	AAA	
4837	ACT	TAA	GAG	CAT	AGT	TTA	ATC	TCA	TAA	ACA	AGA	TAA	GCA	AAA	CAA	AAC	AAT	TTT	TCA	TCC	ATG	TAA	GTT	TAA	AAG		
4915	CAG	GTA	AAA	TTT	AAA	ATT	AAG	AGA	GAC	ATA	AGT	TTT	GAG	GTA	GCA	AGA	TGG	AAA	CTC	TGG	GGC	TTG	GGG	AAT	GTT	CTG	
4993	TCT	CTC	TGT	ATG	GGA	TGT	GAA	AGT	TAC	TAT	TGT	GGA	ATT	GGG	ATC	TAT	GTT	CTT	CCT	GTA	TAT	ATT	GTA	TAC	TTT	ATA	
5071	ATA	ACT	TCA	CCT	AAA	GAA	ATA	TCT	AAT	ACC	CAG	TGC	AAT	CAT	AAA	AGA	GGA	TAC	AAG	GAA	TGA	ATC	ATA	CGT	CAA	GGC	
5149	CAG	AAA	GAC	AAT	AAA	GTA	GGG	GAT	CCA	GGA	TCA	AAT	CTC	CCA	CAA	CCT	TGA	GCC	TTT	TAC	TAT	TCT	GCC	TTT	CAG	AGC	
5227	TCA	AAG	TAC	AAA	ACA	CAT	AAT	TCA	AAC	ACA	TGA	TCC	CTC	CTT	GGG	GTC	TCT	TCC	TTT	ATG	CAT	CGA	ATT	AGA	AAT	AGC	
5305	CAT	GTA	TAA	AAT	GAG	ATA	GAA	GAG	ACC	TTT	ATC	AAC	AGG	TCA	AAG	AAT	ATA	GGT	AAT	TTT	GTG	TGG	GTA	TGA	AGA	GCC	
5383	CAC	GTA	TCA	AAG	GTT	ACA	TTA	GGG	AAG	GAA	GAG	GAC	ACT	AAC	AGT	GAC	TTT	CAT	TCT	CCC	CCT	CTT	GGA	GGC	CCC		
5461	TGC	AAT	TAG	TCC	CTC	GTG	GGC	TCA	TCC	ACT	CAG	CAC	ACA	TAT	ACT	AAG	CAT	CTT	CTC	AGC	CTA	CAC	TCT	GAA	GGC	AGT	
5539	GCA	GAA	TAA	TGT	TAG	TGT	CCC	TTT	CTC	CAG	TTA	ATA	TGC	AGT	CCA	GTT	TCC	CTG	CTC	CTT	CTC	AGT	CCA	CAT			
5617	AAG	GAT	GAT	GGG	AAA	GGA	CAG	TCA	CCA	AAT	AGG	AGA	GGG	CAA	CCC	TTT	GCC	TTT	CTA	CCT	CTT	GAG	AAT	GTA	CAT	TAT	
5695	TAT	CCA	CTT	TTT	GAA	ACT	TCT	TTT	AAT	TGC	TTT	TTT	TTT	TTA	ATT	TGT	CTT	TTT	AAA	TAG	CAT	AAC	CTT	GTT	CAT	CCA	TTT
5773	CTG	GGA	ACC	AAA	TTT	ATC	AAT	CAA	CAG	TGC	CTC	TAA	TCT	GGC	TAT	TAA	TAC	AAA	AAT	GCC	TCC	TCA	AAA	TAT	ATA	TGT	
5851	TCG	AGT	CTT	ATC	TAA	AAC	AGA	AAC	CAC	AAT	AAA	GAA	GAA	ATA	CAT	ATA	AGC	ATT	TAT	ATA	ATT	CTG	AGC	AAC			
5929	CTT	GTG	CTT	TGT	GAA	AAA	AAT	ATA	ATC	TAA	TGT	CAC	ATG	CTG	TAT	TCT	TTT	TAT	TTA	ACA	CTG	GTG	AAA	TTA	TAC	CAT	
6007	TAG	AGA	GAA	AGA	GGA	CAG	ATC	ACT	AGT	AGG	ATC	TAG	GGA	TGT	TAC	AGA	TAA	GAA	AAC	AAA	TGT	GAC	AAA	GAG	CAG	CTG	
6085	TCA	CAA	GGA	GGA	TCT	TCA	AGG	TCA	CAG	AAT	CAC	TGT	CTT	GAT	TTT	TAC	AGT	GGT	TAC	ATA	CAT	TTA	AAT	ATG	TGA	TAA	
6163	AAT	GTT	GTT	GAA	CTA	TAT	TCA	TAT	ATT	GTA	CCA	ATG															

Figure 12 contd

8113 AGC CAG GAT GGG TAT TTC TTT TTT GCC TAT GTC TAT CTG TGA CTT GCA CAT GGT CTT GGC AAT TAT TGA TGA TGA GGT CAA GGC TTA ATT
8131 CTT CAC TTG TGC CAA CTC CAC TAG CAA ATT TTG AAG AGT TGT TTA TAT TAT GCA CTG AGA TGT TCA GGT CCT
8263 CTT ATC TCC TTA TAG CAA TCC TGT TTA TAA CTG GGT AAC TTT GTG AAG GGA AGG AAT TAA TAT TAT TTA ATT TCA CAT GCT TAT TTG
8347 ACT TTT TAA TAC AAA ATT TTG AAG AGT TGT TTA TAA ATC AAT ATA TTA ATA TTA TTA ATT TCA CAT GCT TAT TTT
8425 ATT TCA ACT CAA GAT ACT AAT TTT CGA GAC CCA CTT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT
8503 TAT TGT GGT TTT CAA GAT ACT AAT TTT CGA GAC CCA CTT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT
8581 AAA CTC AGA AAC CTA CTT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT
8653 GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT
8737 GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT GGC TCT
8815 AAA TTT TTA AAC TTT ATA TAT GTC TCA TTC TAT TAT CAG ATA GGA CTG TGT AGA CTG TGC TAA ACT AAT AAA TGT
8893 GCC CTC AAA AGT AAT CGC AAG TTG TAT TGT TGT TAT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
8971 TGC TTT GCT TTG CTT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
9043 TTT TTT CCG GGG GAG GGA CTT GAT AGA GAT CTG CTT TCT GGC TCC CAA GTG CTG GGA ATA AAG GCA TAC ACC TCC ATG ATC
9127 AGG CTT GAT TCA ACT CAT GAT AGA GAT CTG CTT TCT GGC TCC CAA GTG CTG GGA ATA AAG GCA TAC ACC TCC ATG ATC
9205 AGA TAG TGA TCC CAA GTT TTA GCA AAA GTT TCT AGA CTT GAC AAT AAT CGA TGG AGA TAG ACA TGA AAT ACA CAA AGA
9283 ACT AAT GTG GAG TTT ACC TGA ATC ATA CTC TAT ACT TTA TCA GAG AAT AAA TTA ACA TTT AAT CCA GTG CCA GGC
9361 TAG AGG CAC CAT TCA ATG GCA GTG TTT GCC ATC ATG CAT AGG CTT AGT CTT CTA CTG TGC TGA AAG GCA TTG GGG GCA ATA
9439 TTA CTC ATT ATA CAG ATG AGA AAC TGG GAA AGA CTT GCC TCA GAT TCT CTA CTG AAA GGC TGA GTT TGT GGC TTC TAG
9517 AAA ATC TTT TAC TTT CAA TAT TTT TAA TGT ATA ATT TTT TTA TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
9595 AAG AAA TAA ATG CAA TAA ACC AAA TAC ATG GAC AAA AAA ATA CAA GAA TCA TAT GAT CAC CTC AAT GGA AGG AAA AAA
9673 AAA GAA AGA AAA AGT CTT TGA TAA GAT TCA ACA TTC ATT CTT TTA TTA GAT AAT TTC TTC TAC ATT TCA AAT
9751 GCT ATC CCC AAA GCC CTT TGA TAA GAT TCA ACA TTC CTT CCA CCC ACC CAC TCC TGC TTT CTG GCC CTG GCA TTC
9829 CTC TGT ACT GAG GCA TAT GAT CTT CAA AAA ACC AAG GGC CTC TCC TCT CAT TGG TGG CCG ACT ATT AGG CCA TCT TTT
9907 GCT ACA TAT GCA ACT AGA GAC ACA GCT CTG GGG GTT ACT GGT TAG TTC ATA TTG TTA GTC CTC CTA TAG AGT TGC AGA
9985 CCC CTT TAG CTC CTT GGA TAC TTT AGG CAC TGG CAT AGC CTC ACC AGA AAG AGA GAG CTA TGT CAG GAT CCT GTC AGT AAA ATC
10063 TCC ACT TCT GTA TTT GCC AGG CAC TGG CAT AGC CTC ACC AGA AAG AGA GAG CTA TGT CAG GAT CCT GTC AGT AAA ATC
10141 TTT CTG GCA TAT GCA ATA GTA TCT GGT TTT GGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
10219 TCC TTC CTT CCA TCT CAG CTC CAA ACT TGT CCA TAA TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT
10297 GTG AAG AAT CCA CAC TTT GGT CTT CCA ACT TGT TGA TAT TGT TGA TTA TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
10375 ATA TCC ACG TAT CAG TGA GTG CAT AAT TTT CCA TAA TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT
10453 ATG CAT CCA TTT GCC TAA GAA TTT CAT TTA TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT TCA TAT
10531 CAT TTT CTG TAT CCA TTC CTC TGT TGA GGG GCA TCT GGT TTC TTT CCA GCT TGT TGT TGT TGT TGT TGT TGT TGT TGT
10609 GAG CAT AGC GGA GCA TGT GTC CTT ATC AAG TTG GAA CAT CTT CTA GGT ATA TGC CCA GGA GAG GAA TTA TTA TTA TTA TTA
10687 CCG GTA GTA CCA TCA ACA TGC AAT CTT AAT AAA AGC CCT AGA ACA AGG AGG ACT GTA GGA AAC ATA TTC CAA CAT AAT
10765 AAA GGT TAT GTA TGA CAA ACT CAT GAC CAA TAT CAT CCT AAA TGA ATG AAA CCA TTA ATA AGC TCC AAT AAA ATC AGA
10843 GGA CTG CCC ACT ATC CCA TAA TGA GAT TGA AGC AAT AGC TGG AGC AAT AAG GCA AGA GAA GGA ATA
10921 CAA ATG GGA AAA TAT TAA GTC AAA TTG TTT TCA ATT GAA GAT TAT AAT ATC TTA TAC CCA ATG ACC TCA AAT TTT GAC
10999 TAG AAA AAT TGT AGA AAT TAT CAA TAA TTT CAG CAA AGT GTT ATG ATG CAC CAC ATC CTT ATT ATT CTC CCC AGC TTC
11077 TGC TTG CTT CTC TCT TCT TGC TCT TCA TCC TTT CTG TCC TTC CAT CTG CCA CTC TTG TCT CAA GAC TGA GTG CAG
11155 CGT GTA ACT CTC CTG TGA CTG AGT ATC TCA CAA AAC GTT CTA CCT GCC AAA CCT GGA TGA GCC CTT TGT CTT TCT GAA
11233 GCT ATG AGG CTC TCT ACA TAG ACT CAA GAA AAT GAC AGG GAG GTA ATA ATG AAG TGG GGA AGG CTG ACA TTA
11311 GCA TTG CTC CTG TGT GGC TCC TTA AAT TCT CAT ACT TCA CAC TGA GAT GTT TTT TCA AAC TAT GGG TCA TGA CTC CTT
11389 CCA GAG CTA AGG TTC TCA TAT TTG AGT GTT ATA GAA TGA GTA CAG TAG TTT TCA AAC TAT GGG TCA TGA CTC CTT
11467 TAT GGG TCA AAC TAC CCT TTC ACA CAG GTT GCA TAT CAG ATA TCC TAA TTT TAT TGT GGG TCA TGG CAA CAT GAG GAA CTG
11545 ATA TAT ATA TTT CAC AAC AGT AGG AAA ATT ATT TAG TAA TCA TTT TAT AGT TGT GGG TCA TGG CAA CAT GAG GAA CTG
11623 TAT TAA AGG GTT GCA GCA TTA GGA ATG TTG AGA CCC ACT GTA ATA GAG AAT GAG GCT TAA GGC AGG GCT ATA AAG CCC
11701 AAT GGA CCA TGT GCC TTT TCC AAC AAT TGC CAC ATG GTA AGC TCT GTA TAG ACT TTT TAA AGA ACA TTG GTT TGT AAT
11779 TTT AAA TGG ATA AGG GTC TAT CAC CCA TCT ATA TAA TAA TAG TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT

Figure 12 contd

11857 AAA TGC AAA AAT CCT CAA GGC ACC AAG ACA TAG CAG TGA AAC BAC ATT GAT GAC CAA ATA GGA AAT CCA TGT AGA GAC CTT CTA
11935 TCT TCT GAT GGC TCC ACA GAG AGT TGT ACT TGT ACT ACA CCA TTT CTA CCA GTA ATG AAT ACA GTG TCT CAA CTC
12013 CTG CCA TTG AAT CTT CAG GAA GCC CCT GAA ATG ACT TGA TTA AGT CCA ACT TGG CCA AAG GAT CAA GAG GCA CAA TTC AAA AGC
12091 AGG GAA TAA AAT CCA TGC CTG AGA TCA TGC TAT GGT TGA TTA GGA GAT GAA ACG CCA AAG CCA TAT CCC TAG TGG GAA
12169 AAT GTT GTT CCT TCA CTA AAT GTC TCA TTT AAC AGA TTA GGA GAT GAA ACG CCA GCT TAG TCT GTG TAG TTTG TAC ACC CCT GTG
12247 AAG ACT TGG AAA TCA AAT GTC TCA TTT AAC AGA TTA GGA GAT GAA ACG CCA GCT TAG TCT GTG TAG TTTG TAC ACC TAT CTA AAA
12325 ATC CCA TCG CTA GGA AGA CTG AGG CAG GAA GTC CTC GAG ATC TAA TGA TCA GCT TAG TCT GTG TAG TTTG TAC ACC TAT CTA AAA
12403 AAT AAT TAC TAA CTA CTT AAT AGG AGA TTG GAT GTT AAG ATC TAA TGA TCA GCT TAG TCT GTG TAG TTTG TAC ACC TAT CTA AAA
12481 TAT TTT CTA CCT GGT ATG TTT TAA ATT GCA GTA AGG ATC TAA TGA TCA GCT TAG TCT GTG TAG TTTG TAC ACC TAT CTA AAA
12559 GCA ACA ACA GAG AGT GTT AGA TTT GTT TGG AAA AAT TTA TCA TCA GCT TAG TCT GTG TAG TTTG TAC ACC TAT CTA AAA
12637 AGT ACC CAC CCA TAT CTC CCT ACC CAC TAC CAG ACT GGT TAT TGA TTT TCA TGG TGA CTG GCC TGA GAA GAT
12715 TAA AAA AAG TAA TGC TAC CTT ATT GGT AGT GTC CCA TGG ACC AAG ATA GCA ACT GTG CTA ACC GTC ACA CTG CTT
12793 TGA TCA AGA AGA CCA TTT TGG GAA CCA TGG CCA TGG CCA TGG CCA TGG CCA TGG CCA TGG CCA TGG CCA TGG CCA TGG CCA
12871 AGC CTG GGT GGT GCA CTC CTC CAC ACC CTT TCA AGT TTT TCA AGT TTT TCA AGT TTT TCA AGT TTT TCA AGT TTT TCA AGT TTT
12949 GGC TGT ATC CAG CAC TGG GAT GAA AAT AAT ACC CCA CCT CCA TCT TGT TTTG ATA TTA CTC TAT CTC AAG CCC CAG GTT
13027 AGT CCC CAG TCC CAA TGC TTT TGC ACA GTC AAA ACT CAA CTT GGA ATA ATC AGT ATT GAA GAG TTT TGA TAT GGT
13105 CAC TGG GCC CAT ATA CCA TGT AAG ACA TGT GGA AAA GAT GTT TCA TGG GGC CCA GAG TTT TGA TAT GGT
13183 GTG GCA AAA AAT AGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
13261 AGC ATT GCA GGA TAG TTG TCA GTA AAC ACC CCA CAA CCC ATA ACA GAA GTA TTT TCT TCT TCT TCT TCT TCT TCT TCT TCT
13339 TCC ATG TAG ATG GCT GTC TTC ATA TTT GTT CTA GAC GGC CCG CC

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1 GCG GCC GCA ATA AAA TAT CTT TAT TTT CAT TAC ATC TGT GTG TTG GTT TTT TGT GTG AAT CGT AAC TAA CAT ACG CTC
79 TCC ATC AAA ACA AAA CGA AAT CCA TAG CAA AAT AAG CTG TCC CCA GTG CAG GTG CCA CAG TCC CCG AGA AGT TGG
157 TCT ATC GAA GGA TCT GCG ATC GCT CCG GTG CCT AGA GAA GGT GGC CAG TAG TCG CCG TGA ACG TTC TTT TTT GCA ACG GGT
235 GGG GAG GGG TCG GCA ATT GAA CCG GTG GAG AAC CCG GTG CAG TGT CTT CAC GCG CCC GCG CTA CCT GAG GCC GCG
313 TCC GCC TTT TTC CCG AGG GTC GTC CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
391 TTG CCG CCA GAA CAC AGC TGA GCG TTC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
469 ATC CAC GCC GGT TGA GTC GCG AAC TCT ACG TCG AGC CCA CCA TGG ACA TGA GAG TGC CCG CCG CCG CCG CCG CCG CCG CCG
547 TAA AGC TCA CCC TGC TTG CTC AAC GGC ATG AGG ATA TTA CTC CAG TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
625 GCG TGA CCC TGC TTG CTC AAC GGC ATG AGG ATA TTA CTC CAG TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
703 CCG CTA CCT GAG ATC ACC GGC ATG AGG ATA TTA CTC CAG TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
781 TCT GGC TCC GAG GTA ACA TGA TTA ATA GTC AAT TTA CTC CAG TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
859 AGT TCT CTG ATA ACA TGA TTA ATA GTC AAT TTA CTC CAG TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
937 ATG ACC CAG AGC CCC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC
1015 AGC AGC TAC CTG AAC TGG TAT CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
1093 AGC GGC GTG CCC AGC AGA TTC AGC GGC TCC GGC ACC CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
1171 GAC TTC GCC ACC TAC TAC TGC CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG
1249 AGA GCC GAC GCC GCT CCC ACC CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
1327 TGC TTC GTG AAC AAC TTC TAC CCC AGA GAC ATC AGC GTG AAG TGG AAG ATC GAC GGC AGC AGC AGC AGC AGC AGC AGC AGC
1405 CTG GAC AGC GTG ACC GAC CAG GAC AGC AAG GAC TCC ACC TAC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC AGC
1483 TAC GAG AGG CAC AAC CTG TAC ACC TGC GAG GTG CAC AAG ACC AAG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
1561 AAC GAG TGT TGA GCT AGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC
1639 AAA AAA TGC TTT ATT TGT GAA ATT TGT GAT TTA TTT GTA ACC ATT ATA AGC TGC AAT AAA CAA GTT AAC
1717 AAC AAC AAT TGC ATT CAT TTT ATG TTT CAG GTT CAG GTT TTA TTT TTA TTT TTA TTT TTA TTT TTA TTT TTA TTT TTA
1795 AAA TGT GGT ATG GAA TTC TAA AAT AAT ACA TAG CAA AAC TTT AAC CTC CAA ATC AAG CCT CTA CTT GAA TCC TTT TCT
1873 GAG GGA TGA ATA AGG CAT AGG CAT CAG GGC CTG TTG CCA ATG TGC ATT AGC TGT TTG CAG CCT CAC CTT TCA TGG

Figure 12 contd

1951	AGT	TTA	AGA	TAT	AGT	GTA	TTT	TCC	CAA	GGT	TTG	AAC	TAG	CTC	TTC	ATT	TCT	TTA	TGT	TTT	AAA	TGC	ACT	GAC	CTC	CCA		
2029	CAT	TCC	CTT	TTT	AGT	AAA	ATA	GCC	CCT	TCA	TAA	AAA	TAG	ACA	TCA	TTG	TTG	CAA	TGA	AAA	TAA	ATG	TTT	ATT	AGG	CAG		
2107	AAT	CCA	GAT	GCT	CAA	AGA	AAG	CGA	CCT	TCA	TAA	TAT	CCC	CCA	GTT	TAG	TTG	GAC	TTA	GGG	AAC	AAA	GGA	ACC	TTT	AAT	AGA	
2185	AAT	TGG	ACA	GCA	AGA	AAG	CGA	GCT	TCT	AGC	GAA	TTC	TCG	ACT	CAT	TCC	TTT	GCC	CTC	GGA	CGA	GTG	CTG	GGG	CGT	CGG		
2263	TTT	CCA	CTA	TCG	GCG	AGT	ACT	ACT	TCT	ACA	CAG	CCA	TCG	GTC	CAG	ACG	GCC	CGT	CTT	CTG	CGG	CGG	ATT	TGT	GTA	CGC	CCG	
2341	ACA	GTC	CCG	GCT	CCG	GAT	TGG	TCA	AGA	CCA	ATG	CGG	AGC	ATA	TAC	GCC	CGG	CAA	GCT	GCA	TCA	TCG	AAA	TTG	CCG	TCA	ACC	
2419	AAG	CTC	TGA	TAG	AGT	TGG	TCG	CGC	GTC	TGC	TCC	ATA	CAA	GCC	AAC	CAC	GGC	CTC	CAG	AAG	ATG	TTG	GCG	ACC	TCG	TAT	TGC	
2497	CTC	CGC	TCG	AAG	TAG	CGC	ATC	GCC	TGC	TCG	CTC	CAG	TCA	ATG	ACC	GCT	ATT	CGG	CCA	TTG	TCC	GTC	AGG	ACA	TTG	TTG	GAG	
2575	TGG	GAA	TCC	CCG	AAC	TGC	ATC	AGC	AGG	TGC	ACG	ACT	TCG	GGG	CAG	TCC	TGC	GGC	ACA	ATC	AGC	TCA	TCG	AGA	GCC	TGC	GCG	
2653	CCG	AAA	TCC	GGG	TGC	ACG	ATC	AGC	AGG	TGC	TCG	TCC	ATC	ACA	GTT	TGC	CAG	TGA	TCA	GGG	ACA	ATC	GGG	CAT	ATG	AAA	TCA	
2731	ACG	GAC	GCA	CTG	ACG	TAT	TGA	GTG	TAT	CGG	ATT	CCT	TGC	GGT	CCG	AAT	GGG	CGC	ATC	AGC	ATC	GGG	CAT	ATG	AAA	TCA		
2809	CGC	CAT	GTA	GTG	TAT	GTA	GTG	TAT	CGG	ATT	CCT	TGC	GGT	CCG	AAT	GGG	CGC	ATC	AGC	ATC	GGG	CAT	ATG	AAA	TCA			
2887	ATC	GCA	TCC	ATG	AGC	TCC	GGG	ACG	AGT	CGT	TGC	AGA	ACA	GGG	AGT	GGG	CGC	ATC	AGC	ATC	GGG	CAT	ATG	AAA	TCA			
2965	TGT	GCA	CGG	CGG	GAG	ATG	CAA	TAG	CTC	TCC	GGT	AAT	TCC	CGC	ATC	GGG	CGC	ATC	AGC	ATC	GGG	CAT	ATG	AAA	TCA			
3043	GCC	GAT	GCA	AAG	TGC	CGA	TAA	ACA	TAA	CGA	TCT	TTG	TAG	AAA	CCA	TCC	GGG	CGC	ATC	AGC	ATC	GGG	CAT	ATG	AAA	TCA		
3121	CGC	CCT	CCT	ACA	TCG	AAG	TTC	TCG	AAA	GCA	GAT	TCT	TCG	CCC	TCC	GAG	AGC	ATC	AGC	ATC	GGG	CAT	ATG	AAA	TCA			
3199	TTT	TCG	ATC	AGA	AAC	TTC	TCG	ACA	GAC	GTC	GGG	ATG	TCA	GGC	TTC	TTT	TTT	ATG	ATG	ATG	GGC	CTC	CTA	TAG	TGA	GTC	GTA	
3277	TTA	TAC	TAT	GCC	GAT	ATA	CTA	TCG	CGA	TGA	TTA	ATT	GTC	AAA	ACA	GCG	TGG	ATG	ATG	GGG	TCT	CCA	GCT	TAT	CTG	ACG	GTT	
3355	CAC	TAA	ACG	AGC	TCT	GCT	TAT	ATA	GAC	CTC	CCA	CCG	TAC	ACG	ACA	ACT	ACC	GCC	CAT	TTG	CGT	CAA	TGG	GGC	GGA	GTT	GTT	
3433	ACG	ACA	TTT	TGG	AAA	GTC	CCG	CTA	TCC	ACG	CCC	ATT	TAC	TAG	TCA	AAA	CAA	ACT	CCC	ATT	GGT	AAT	GGG	GTG	GAG	ACT	TGG	AAA
3511	TCC	CCG	TGA	GTC	AAA	CCG	CTA	TCC	ACG	CCC	ATT	GAT	GTA	CTG	CCA	AAA	CCG	CAT	CAA	GGT	AAT	GGT	AAT	AGC	GAT	GAC	TAA	
3589	TAC	GTA	GAT	GTA	CTG	CCA	AGT	AGG	AAA	GTC	CCA	TAA	GGT	CAT	GTA	CTG	CCA	GGC	ATA	ATG	CCA	GGC	CCA	TTT	ACC	GTC	TAA	
3667	ATT	GAC	GTC	AAT	AGG	GGG	CGT	ACT	TGG	CAT	ATG	ATA	CAC	TTG	ATG	TAC	TGC	CAA	GTG	GGC	AGT	TTA	CCG	TAA	ATA	CTC	TAA	
3745	CAC	CCA	TTG	ACG	TCA	ATG	GAA	AGT	CCC	TAT	TGG	CGT	TAC	TAT	GGG	AAC	ATA	CGT	CAT	TAT	TGA	CGT	CAA	TGG	GCG	GGG	TAA	
3823	GTC	GTT	GGG	CGG	TCA	GCC	AGG	CGG	GCC	ATT	TAC	CGT	AAG	TTA	TGT	AAC	GCC	TGC	AGG	TTA	ATT	AAG	AAC	ATG	TGA	GCA	TAA	
3901	AAA	GGC	CAG	CAA	AAG	GCC	AGG	AGG	AAC	CGT	AAA	AAG	GCC	GGG	TTG	CTG	GCG	TTT	TTT	CTT	CTT	AGG	CTC	CGC	CCC	CTT	GAC	GAG
3979	CAT	CAC	AAA	AAT	CGA	CGC	TCA	AGT	CAG	AGG	TGG	CGA	AAC	CCG	ACA	GGA	CTA	TAA	AGA	TAC	CAG	GCG	TTT	CCC	CCT	GGA	TTC	
4057	AGC	TCC	CTC	GTG	CGC	TCT	CCT	GTT	CCG	ACC	CTG	CCG	CTT	ACC	GGA	TAC	GTG	TCC	GCC	TTT	CTC	CCT	TCG	GGG	AGC	GTG	TTC	
4135	GCG	CTT	TCT	CAT	AGC	TCA	CGC	TGT	AGG	TAT	CTC	AGT	TCG	GTG	TAG	GTC	GTT	CGC	TCC	AAG	CTG	GGC	TGT	GTG	CAC	GAA	TTC	
4213	CCC	CCC	GTT	CAG	CCC	GAC	CGC	TGC	TGC	TTA	TCC	GGT	AAC	TAT	CGT	CTT	GAG	TCC	AAC	CCG	GTA	AGA	CAC	GAC	TTA	TCG	TTC	
4291	CCA	CTG	GCA	GCA	GCC	ACT	GGT	AAC	AGG	ATT	AGC	AGA	GCG	AGG	TAT	GTA	GGC	GGT	GCT	ACA	GAG	TTT	TTC	AAG	TGG	TGG	TTC	
4369	CCT	AAC	TAC	GGC	TAC	ACT	AGA	AGA	ACA	GTA	TTT	GGT	ATC	TGC	GCT	CTG	CTG	AAG	CCA	GTT	ACC	TTT	GGA	AAA	AGA	GTT	TTC	
4447	GGT	AGC	TCT	TGA	TCC	GGC	AAA	CAA	ACC	ACC	GCT	GGT	AGC	GGT	GGT	GGT	TTT	TTT	GTG	TGC	AAG	CAG	CAG	ATT	ACG	CGC	AGA	
4525	AAA	AAA	GGA	TCT	CAA	GAA	GAT	CCT	TTG	ATC	TTT	TCT	ACG	GGG	TCT	GAC	GCT	CAG	TGG	AAC	GAA	AAC	TCA	CGT	TAA	GGG	TTC	
4603	ATT	TTG	GTC	ATG	GCT	AGT	TAA	TTA	ACA	TTT	AAA	TCA															TTC	

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1	GCG	GCC	GCA	ATA	AAA	TAT	CTT	TAT	TTT	CAT	TAC	ATC	TGT	GTG	TTG	GTT	TTT	TGT	GTG	AAT	CGT	AAC	TAA	CAT	ACG	CTC	
79	TCC	ATC	AAA	ACA	AAA	CGA	AAC	ACA	AAC	TAG	CCC	ATC	TAG	CAA	AAT	AGG	CTG	TCC	CCA	GTG	CAG	GTG	CCA	GAA	CAT	TTC	
157	TCT	ATC	GAA	GGA	TCT	CGG	ATC	GCT	CCG	GTG	CCC	GTC	AGT	AGT	GGG	CAG	AGC	GCA	CAT	CGC	CCA	CAG	TCC	CCG	AGA	AGT	TGG
235	GGG	GAG	GGG	TCG	GCA	ATT	GAA	CGG	GTG	CCT	AGA	GAA	GGT	GGC	GCG	GGG	TAA	ACT	GGG	AAA	GTG	ATG	TCG	TGT	ACT	GGC	
313	TCC	GCC	TTT	TTC	CCG	AGG	GTG	GGG	GAG	AAC	CGT	ATA	TAA	GTG	CAG	TAG	TCG	CCG	TGA	ACG	TTT	TTC	TTC	GCA	ACG	GGT	
391	TTG	CCG	CCA	GAA	CAC	AGC	ATC	AGC	TTC	GAG	GGG	CTC	GCA	TCT	CTC	CTT	CAC	GCG	CCC	GCC	CTA	CCT	GAG	GCC	GCC	GCT	
469	ATC	CAC	GCC	GGT	TGA	GTC	CGG	TTC	TGC	CGC	CTC	CGC	CTT	GAA	CTG	GAA	CTG	CAA	CTG	CGT	CCG	CCG	TCT	AGG	TAA	GTT	
547	TAA	AGC	TCA	GGT	CGA	GAC	CGG	GCC	TTT	GTC	CGC	TCC	TCC	CTT	GGA	GCC	TAC	CTA	GAC	TCA	GCC	GGC	TCT	CCA	CGC	TTT	
625	GCC	TGA	CCC	TGC	TTG	CTC	ATC	TCT	ACG	TCT	TTG	TTT	CGT	TTT	CTG	TTC	TGC	GCC	GTT	ACA	GAT	CCA	AGC	TGT	GAC	CGG	
703	CGC	CTA	CCT	GAG	ATC	ACC	GGC	GTG	TCG	ACG	CCA	TGG	ACA	TGA	GAG	TGC	CCG	CCC	AGC	TCC	TGG	GGC	TCC	TGG	TGC	TAC	TTC

Figure 12 contd

781	TCT	GGC	TCC	GAG	GTA	AGG	ATG	GAG	AAC	ACT	AGG	AAT	TTA	CTC	AGC	CAG	TGT	GCT	CAG	TAC	TGA	CTG	GAA	CTT	CAG	GGA		
859	AGT	TCT	CTG	ATA	ACA	TGA	TTA	ATA	GTA	AGA	ATA	TTT	GTT	TTT	ATG	TTT	CCA	ATC	TCA	GGT	GCC	AGA	TGT	CAG	TCT	GCC		
937	CTG	ACC	CAG	CCC	GCC	TCT	GTG	TCT	GGC	AGC	CCT	GGC	CAG	AGC	ATC	ACC	ATC	AGC	TGC	ACC	GGC	ACC	AGC	GAC	GTG			
1015	GGC	GGC	TAC	AAC	TAC	GTG	TCC	TGG	TAT	CAG	CAG	CAC	CCC	GGC	AAG	GCC	AAG	CCC	CTG	ATG	ATC	TAC	GAG	GTG	TCC	AAC		
11093	AGA	CCC	AGC	GGC	GTG	AGC	AAC	AGA	TTC	AGC	GGC	AGC	AAG	AGC	GGC	AAC	ACC	AGC	AGC	CTG	ACC	ATC	AGC	GGC	CTC	CAG		
11171	GCT	GAG	GAC	GAG	GCC	GAC	TAC	TGC	AGC	AGC	TAC	ACC	AGC	AGC	TCC	ACC	CTG	AGC	CTG	GGC	GGC	ACA	AAG	CTG	CAG			
12449	ACC	GTG	CTG	AGA	GCC	GAC	GCC	GCT	CCC	ACC	GTG	TCC	ATC	TTT	CCC	CCC	AGC	ATG	GAA	CAG	CTG	ACC	TCT	GGC	GGA	GCC		
13227	ACC	GTG	GTC	TGC	TTT	GTG	AAC	AAC	TTC	TAC	CCC	AGA	GAC	ATC	AGC	GTG	AAG	TGG	AAG	ATC	GAC	GGC	AGC	GAG	CAG	AGG		
14005	GAC	GGC	GTG	CTG	GAC	AGC	GTG	ACC	GAC	AGC	AAG	GAC	TCC	ACC	TAC	AGC	ATG	AGC	ATG	AGC	AGC	ACC	CTG	AGC	CTG	AGG		
14483	AAG	GTG	GAG	TAC	GAG	AGG	CAC	AAC	CTG	TAC	ACC	TGC	GAG	GTG	GTG	CAC	AAG	ACC	AGC	TCC	AGC	CCC	GTG	GTG	AAG	TCC		
15661	TTT	AAC	CGG	AAC	GAG	TGT	TGA	GCT	AGC	TGG	CCA	GAC	ATG	ATA	AGA	TAC	ATT	GAT	GAG	TTT	GGA	CAA	ACC	ACA	ACT	AGA		
937	CTG	ACT	CAG	CCT	GCC	TCC	GTG	TCT	GGG	TCT	CCT	GGG	CAG	TCG	ATC	ACC	ATC	TCC	TGC	ACT	GGA	ACC	AGC	AGT	GAC	GTT		
10115	GGT	GGT	TAT	AAC	TAT	GTC	TCC	TGG	TAC	CAA	GAG	CAC	CCA	GGC	AAA	GCC	CCC	AAA	CTC	ATG	ATT	TAT	GAG	GTG	AGT	AAT		
11093	CGG	CCC	TCA	GGG	GTT	TCT	AAT	CGC	TTT	TCT	GGC	TCC	AAG	TCT	GGC	AAC	ACG	AGC	TCC	CTG	ACC	ATC	TCT	GGG	CTC	CAG		
11171	GCT	GAG	GAC	GAG	GCT	GAT	TAT	TAC	TGC	AGC	TCA	TAT	ACA	AGC	AGC	ACT	CTC	GTA	TTC	GGC	GGA	GGG	ACC	AAG	CTG			
12449	ACC	GTC	CTA	CGG	GCT	GAT	GCT	GCA	CCA	ACT	GTA	TCC	ATC	TTT	CCA	CCA	TCC	ATG	AGC	CAA	TTC	ACA	TCT	GGA	GGT	GCC		
13227	ACA	GTC	GTG	TGC	TTT	GTG	AAC	AAC	TTC	TAT	CCC	AGA	GAC	ATC	AGT	GTC	AAG	TGG	AAG	ATT	GAT	GGC	AGT	GAA	CAA	CGA		
14005	GAT	GGT	GTC	CTG	GAC	AGT	GTT	ACT	GAT	CAG	GAC	AGC	AAA	GAC	AGC	ACG	TAC	AGC	ATG	AGC	ACC	AGC	ACC	CTC	TGG	TTG	ACC	
14483	AAG	GTT	GAA	TAT	GAA	AGG	CAT	AAC	CTC	TAT	ACC	TGT	GAG	GTT	GTT	CAI	AAG	ACA	TCA	TCC	TCA	CCC	GTC	GTC	AAG	AGC		
15661	TTT	AAC	AGG	AAT	GAG	TGT	TAG	GCT	AGC	TGG	CCA	GAC	ATG	ATA	AGA	TAC	ATT	GAT	GAG	TTT	GGA	CAA	ACC	ACA	ACT	AGA		
16339	ATG	CAG	TGA	AAA	AAA	TGC	TTT	ATT	TGT	GAA	ATT	TGT	GAT	GCT	ATT	GCT	TTA	TTT	GTA	ACC	ATT	ATA	AGC	TGC	AAT	AAA		
17171	CAA	GTT	AAC	AAC	AAC	AAT	TGC	ATT	CAT	TTT	ATG	TTT	CAG	GTT	CAG	GGG	GAG	GTG	TGG	GAG	GTT	TTT	TAA	AGC	AAG	TAA		
17795	AAC	CTC	TAC	AAA	TGT	GGT	ATG	GAA	TTT	TAA	AAT	ACA	GCA	TAG	CAA	AAC	TTT	AAC	CTC	CAA	ATC	AAG	CCT	CTA	CTT	GAA		
18773	TCC	TTT	TCT	GAG	GGA	TGA	ATA	AGG	CAT	AGG	CAT	AGG	CAT	CAG	GGG	CTG	TTG	CCA	ATG	TGC	ATT	AGC	TGT	TTG	CAG	CCT	CAC	CTT
19551	CTT	TCA	TGG	AGT	TTA	AGA	TAT	AGT	GTA	TTT	TCC	CAA	GGT	TTG	AAC	TAG	CTC	TTT	CTT	ATT	TCT	TTA	TGT	TTT	AAA	TGC	ACT	
20229	GAC	CTC	CCA	CAT	TCC	CTT	TTT	AGT	AAA	ATA	TTT	AGA	AAI	AAT	TTA	AAI	ACA	TCA	TTG	CAA	TGA	AAA	TAA	ATG	TTT	TTT	TTT	
2107	ATT	AGG	CAG	AAI	CCA	GAT	GCT	CAA	GGC	CCT	TCA	TAA	TAT	CCC	CCA	GTT	TAG	TAG	TTG	GAC	TTA	GGG	AAC	AAA	GGA	ACC		
2185	TTT	AAT	AGA	AAT	TGG	ACA	GCA	AGT	ACA	AGC	GCA	GCT	TCT	AGC	GAA	TTT	TCG	ACT	CAT	TCC	TTT	GCC	CTC	GGA	CGA	GTG	CTG	
2263	GGG	CGT	CGG	TTT	CCA	CTA	TCG	GCG	AGT	ACT	TCT	ACA	CAG	CCA	TCG	GTC	CAG	ACG	GCC	GCG	CTT	CTG	CGG	GCG	ATT	TGT	TGT	
2341	GTA	CGC	CCG	ACA	GTC	CCG	GCT	CCG	GAT	CGG	AGT	CGG	ACT	CGG	TCG	CAT	CGA	CCC	TGC	GGC	CAA	GCT	GCA	TCA	TCG	AAA	TTG	AAA
2419	CCG	TCA	ACC	AAG	CTC	TGA	TAG	AGT	TGG	TCA	AGA	CCA	ATG	CGG	AGC	ATA	TAC	GCC	CGG	AGC	CGC	GAT	CCT	GCA	AGC	AGC	AGC	
2497	TCC	GGA	TGC	CTC	CGC	TCG	AAG	TAG	CGC	GTG	TCC	ATA	CAA	GCC	AAC	CAC	GGC	CTC	CAG	AAG	AAG	ATG	TTG	GCG	AGC	AGC	AGC	
2575	ACC	TCG	TAT	TGG	GAA	TCC	CCG	AAC	ATC	GCC	TCG	CTC	CAG	TCA	ATG	ACC	GCT	ATT	ATG	CGG	CCA	TTG	TCC	GTG	AGG	ACA	ACA	
2653	TTG	TTG	GAG	CCG	AAA	TCC	GCG	TGC	ACG	AGG	TGC	CGG	ACT	TCG	GGG	CAG	TCC	TCG	GCC	CAA	AGC	ATC	AGC	TCA	TCG	AGA	AGA	
2731	GCC	TGC	GCG	ACG	GAC	GCA	CTG	ACG	GTG	TAT	TGA	CCG	ATT	CCT	TGC	GGT	CCG	AAI	GGG	CCG	AAC	CCG	CTC	GTG	AGA	TCG	TCG	
2809	ATG	AAA	TCA	CGC	CAT	GTA	GTG	TAT	TGA	CCG	ATT	CCT	TGC	GGT	CCG	AAI	GGG	CCG	AAI	GGG	CCG	AAI	GGG	CCG	AAI	GGG	CCG	AAI
2887	GCC	GCA	GCG	ATC	GCA	TCC	ATG	AGC	TCC	GCG	ACG	GGT	TGC	AGA	ACA	GCG	GGC	AGT	TCG	GTT	TCA	GGC	AGG	TCT	TGC	AAC	AAC	
2965	GTG	ACA	CCC	TGT	GCA	CGG	CGG	GAG	ATG	CAA	TAG	GTG	CTC	AGG	CTC	TCG	CTG	AAI	TCC	CCA	ATG	TCA	AGC	ACT	TCC	GGA	ATC	
3043	GGG	AGC	GCG	GCC	GAT	GCA	AGG	TGC	CGA	TAA	ACA	TAA	GCA	CGA	TCT	TTG	TAG	AAA	CCA	TCG	GCG	CAG	CTA	TTT	ACC	CGC	AGG	
3121	ACA	TAT	CCA	CGC	CCT	CCT	ACA	TCG	AAG	CTG	AAA	GCA	CGA	GAT	TCT	TCG	CCC	TCC	TCC	GAG	AGC	TGC	ATC	AGG	TCG	GAG	ACG	
3199	CTG	TCG	AAC	TTT	TCG	ATC	AGA	AAC	TTT	TCG	ACA	GAC	GTG	GCG	GTG	AGT	TCA	GGC	TTT	TTC	ATG	ATG	GCC	CTC	CTA	TAG	TAG	
3277	TGA	GTC	GTA	TTA	TAC	TAT	GCC	GAT	ATA	CTA	TGC	CGA	TGA	TTA	ATT	GTC	AAA	ACA	GCG	TGG	ATG	GCG	TCT	CCA	GCT	TAT	TAT	
3355	CTG	ACG	GTT	CAC	TAA	ACG	AGC	TCT	GCT	TAT	ATA	GAC	CTC	CCA	CCG	TAC	ACG	CCT	ACC	GCC	CAT	TTG	CGT	CAA	TGG	GGC	GGC	
3433	GGA	GTT	GTT	ACG	ACA	TTT	TGG	AAA	GTC	CCG	TTG	ATT	TAC	TAG	TCA	AAA	CAA	ACT	CCC	ATT	GAC	GTG	AAT	GGG	GTG	GAG	GAG	
3511	ACT	TGG	AAA	TCC	CCG	TGA	GTC	AAA	CCG	CTA	TCC	ACG	CCC	ATT	GAT	GTA	CTG	CCA	AAA	CCG	CAT	CAT	CAT	GGT	AAT	AGC	AGC	
3589	GAT	GAC	TAA	TAC	GTA	GAT	GTA	CTG	CCA	AGT	AGG	AAA	GTC	CCA	TAA	GGT	CAT	GTA	CTG	GGC	ATA	ATG	CCA	GGC	AGT	CCA	CCA	
3667	TTT	ACC	GTC	ATT	GAC	GTG	AAI	AGG	GGG	CGT	ACT	TGG	CAT	ATG	ATA	CAC	TTG	ATG	TAC	TGC	CAA	GTG	GGC	AGT	TTA	CCG	CCG	
3745	TAA	ATA	CTC	CAC	CCA	TTG	ACG	TCA	ATG	GAA	AGT	CCC	TAT	TGG	CGT	TAC	TAT	TAT	GGG	AAC	ATA	CGT	CAT	TAT	TGA	CGT	CAA	

[illegible]

2653	GAC	CAT	GAT	TAC	GCC	AAG	CTT	TGG	AGC	CTT	TTT	TTT	GGA	GAT	TTT	CAA	CGT	GAA	AAA	ATT	ATT	CGC	AAT	TCC	TTT
2731	AGT	TGT	TCC	TTT	CTA	TTT	TCA	CAG	TGC	ACA	GAT	CCA	AAT	GAC	CCA	GTC	TCC	ATC	CTC	GTC	TGC	ATC	TGT	AGG	AGA
2809	CAG	AGT	CAC	CAT	CAC	TTG	CCG	GGC	AAG	TCA	GAG	CAT	TAG	CAG	CTA	CTT	AAA	TTG	GTA	TCA	GCA	GAA	ACC	AGG	GAA
2887	CCC	TAA	GCT	CCT	GAT	CTA	TGC	TGC	ATC	CAG	TTTT	GCA	AAG	TGG	GGT	CCC	ATC	AAG	GTT	CAG	TGG	CAG	TGG	ATC	TGG
2965	AGA	TTT	CAC	TCT	CAC	CAT	CAG	CAG	TCT	GCA	ACC	TGA	AGA	TTT	TGC	AAA	TTA	CTA	CTG	TCA	ACA	GAG	TTA	CAG	
3043	TCC	AAC	GTT	CGG	CCA	AGG	GAC	CAA	GCT	CGA	GAT	CAA	ACG	TAC	TGT	GGC	TGC	ACC	ATC	TGT	CTT	CAT	CTT	CCC	
3121	TGA	TGA	GCA	GTT	GAA	ATC	TGG	AAC	TGC	CTC	TGT	TGT	GTG	CTT	GCT	GAA	TAA	CTT	CTA	TCC	CAG	AGA	GGC	CAA	
3199	GTG	GAA	GGT	GGA	TAA	CGC	CCT	CCA	ATC	GGG	TAA	CTC	CCA	GGA	GAG	TGT	CAC	AGA	GCA	GGA	CAA	GGA	CAG	CAC	
3277	CAG	CCT	CAG	CAG	CAC	CCT	GAC	GCT	GAG	CAA	AGC	AGA	CTA	CGA	GAA	ACA	AGT	CTA	CGC	CTG	CGA	AGT	CAC	CCA	
3355	GGG	CCT	GAG	CTC	GCC	CGT	CAC	AAA	GAG	CTT	CAA	CAG	GGG	AGA	GTG	TTA	GTA	AGG	CGC	GCC	AAT	TCT	ATT	TCA	
3433	CAG	TCA	TAA	TGA	ATC	ACC	TAT	TGC	CTA	CGG	CAG	CCG	CTG	GAT	TGT	TAT	TAC	TCG	CGG	CCC	AGC	CGG	CCA	TGG	
3511	CTG	CTT	GCC	GAA	TAT	CAT	GCT	GGA	AAA	TGG	CCG	CTT	TTT	TGA	AGA	GCT	TGG	ATC	CAT	CGA	CTG	TGG	GGC	GGA	
3589	CTA	TCA	GGA	CAT	AGC	GTT	GGC	TAC	CCG	TGA	TAT	TGC	TGA	AGA	GCT	TGG	CGG	CGA	ATG	GGC	TGA	CGT	CGT	GCT	
3667	TTA	CGG	TAT	CGC	CGC	TCC	CGA	TTT	CGA	TTT	CGA	TTT	CGA	TTT	CGA	TTT	TGA	AAG	GTT	GGG	CTT	CGG	AGT	CTG	
3745	GGG	TTT	GGT	GCT	ACG	AGA	TTT	CGA	TTT	CGA	TTT	CGA	TTT	CGA	TTT	CGA	TTT	TGA	AAG	GTT	GGG	CTT	CGG	GGA	
3823	CGG	CTG	GAT	GAT	CCT	CCA	GGC	GGA	TCT	CAT	GCT	GGA	GTT	CTT	CGC	CCA	CCC	CAA	CTT	GTT	TAT	TGC	AGC	TTA	
3901	TGG	TTA	CAA	ATA	AAG	CAA	TAG	CAT	CAC	AAA	TTT	CAC	AAA	TAA	AGC	ATT	TTT	TTT	ACT	GCA	TTT	TAG	TGG	TTT	
3979	CAA	ACT	CAT	CAA	TGT	ATC	TTA	TCA	TGT	CTG	TAT	ACC	GTC	GAC	CTC	TAG	CTA	GAG	CTT	GGC	GTA	ATC	ATG	ATA	
4057	GTT	TCC	TGT	GTG	AAA	TTG	TTA	TCC	GCT	CAC	AAT	TCC	ACA	CAA	CAT	ACG	AGC	CGG	AAG	CAT	AAA	GTG	TAA	AGC	
4135	TGC	CTA	ATG	AGT	GAG	CTA	ACT	CAC	ATT	AAT	TGC	GTG	GGC	CTC	ACT	GCC	CGC	TTT	CCA	GTG	GGG	AAA	CCT	GTG	
4213	GAA	TTG	CAT	GAA	GAA	TCT	GCT	TAG	GGT	TAG	GGT	TTT	TGC	GCT	GCT	CTA	GGT	CAA	TAT	TGG	CCA	TTA	GCC	ATA	
4291	TTA	TTC	ATT	GGT	TAT	ATA	GCA	TAA	ATC	AAT	ATT	GGC	TAT	TGG	CCA	TTG	CTA	TTG	TAT	CCA	TAT	CAAT	AAT	ATG	
4369	ATT	TAT	ATT	GGC	TCA	TGT	CCA	ACA	TTA	CCG	CCA	TGT	TGA	CAT	TGA	TTT	TTG	ACT	AGT	TAT	TAA	TAG	TCA	ATT	
4447	GGG	TCA	TTA	GTT	CAT	AGC	CCA	TTG	ACG	TCA	ATG	ATG	ATG	ATG	ACA	TAA	CTT	ACG	GTA	AAT	GGC	CGT	GGA	CCC	
4525	AAC	GAC	CCC	CGC	CCA	TTG	ACG	TCA	ATA	ATG	ACG														

MV1057

1	TAC	TCT	TCC	TTT	TTT	CAAT	AAT	ATT	ATT	GAA	GCA	TTT	ATC	AGG	GTT	ATT	GTC	TCA	TGA	GCG	GAT	ACA	TAT	TTG	AAT	GTA	TTT
79	AGA	AAA	ATA	AAC	AAA	TAG	GGG	TTT	CGC	GCA	CAT	TTT	CCC	GAA	AAG	TGC	TGC	CAC	CTG	CAC	CTG	ACG	TGC	GAT	CGG	GAG	ATC
157	CGA	TCC	CCT	ATG	GTG	CAC	TCT	CAG	TAC	AAT	CTG	CTC	TGA	TGC	CGC	ATA	GTT	AAG	CCA	GTA	TCT	TCT	GCT	CCC	TGC	TTG	TGT
235	GTT	GGA	GGT	CGC	TGA	GTA	GTG	CGC	GAG	CAA	AAT	TTA	AGC	TAC	AAC	AAG	GCA	AGG	CTT	GAC	CGA	CAA	TTG	CAT	GAA	GAA	
313	TCT	GCT	TAG	GGT	TAG	GCG	TTT	TGC	GCT	GCT	TCG	CTA	GGT	GAT	CAA	TAT	TGG	CCA	TTA	GCC	ATA	TTA	TTT	ATT	GCT	TAT	
391	ATA	GCA	TAA	ATC	AAT	ATT	GGC	TAT	TGG	CCA	TTG	CAT	ACG	TTG	TAT	CCA	TAT	CAT	AAT	ATG	TAC	ATT	TAT	ATT	GCG	TCA	
469	TGT	CCA	ACA	TTA	CCG	CCA	TGT	TGA	CAT	TGA	TTA	TTG	ACT	AGT	TAT	TAA	TAG	TAA	TCA	AAT	ACG	GGG	TCA	GTT	CAT		
547	AGC	CCA	TAT	ATG	GAG	TTT	CGC	GTT	ACA	TAA	CTT	ACG	GTA	AAT	GGC	CCG	CCT	GGC	TGA	CCG	CCC	AAC	GAC	CCC	CGC	CCA	
625	TTG	ACG	TCA	ATA	ATG	ACG	TAT	GTT	CCC	ATA	GTA	ACG	CCA	ATA	GGG	ACT	TTT	CAT	TGA	CGT	CAA	TGG	GTG	GAG	TAT	TTA	
703	CGG	TAA	ACT	GCC	CAC	TTG	GCA	GTA	CAT	CAA	GTG	TAT	CAT	ATG	CCA	AGT	ACG	CCC	CCT	ATT	GAC	GTG	AAT	GAC	GGT	AAA	
781	TGG	CCC	GCC	TGG	CAT	TAT	GCC	CAG	TAC	ATG	ACC	TTA	TGG	GAC	TTT	CCT	ACT	TGG	CAG	TAC	ATC	TAC	GTA	TTA	GTC	AAA	
859	GCT	ATT	ACC	ATG	GTG	ATG	CGG	TTT	TGG	ACG	TAC	ATC	ATC	AAT	GGG	CGT	GGA	TAG	CGG	TTT	GAC	TCA	CGG	GGA	TTT	CCA	
937	CTC	CAT	CCC	ATT	GAC	ATC	AAT	GGG	AGT	TTG	TTT	TGG	CAC	CAA	AAT	CAA	AGC	AGA	GCT	CGT	TTA	TGT	AAC	AAC	TCC	AGT	
1015	GCC	CCA	TTG	ACG	CAA	ATG	GGC	GGT	AGG	CGT	GTA	CGG	TGG	GAG	GTC	TAT	ATA	AGC	AGA	GCT	CGT	TTA	GTG	AAC	CGT	CAG	
1093	ATC	GCC	TGG	AGA	CGC	CAT	CCA	CGC	TGT	TTT	GAC	CTC	CAT	AGA	AGA	CAC	CGG	GAC	CGA	TCC	AGC	CTC	CGC	GGC	CGG	GAA	
1171	COG	TGC	ATT	GGA	AGC	TTG	GTA	CCG	GTG	AAT	TGG	CCG	CGC	CGC	GCT	ATC	CTC	TTT	GTA	CTG	CTG	ACC	GAC	CGG	TTT	GCG	
1249	AGA	GGC	CGC	AAT	TCC	CTA	GCC	ACC	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTT	TTG	GTA	CTG	CTG	GCC	CAG	COG	GCC	GCC	
1327	ATG	GGG	CGG	AGA	ATG	GGC	GGA	ACT	GGG	CGG	AGT	TAG	GGG	CGG	GAT	GGG	CGG	AGT	TAG	GGG	CGG	GAC	TAT	GGT	TGC	TGA	
1405	CTA	ATT	GAG	ATG	CGG	ATC	CGC	TGG	CAC	GAC	AGG	TTT	CCC	GAC	TGG	AAA	CGC	GGC	AGT	GAG	CGC	AAC	GCA	ATT	AAT	GTG	
1483	AGT	TAG	CTC	ACT	CAT	TAG	GCA	CCC	CAG	GCT	TTA	CAC	TTT	ATG	CTT	CCG	GCT	ATG	TTG	TGT	GGA	ATT	GTG	AGC	GGA		
1561	TAA	CAA	TTT	CAC	ACA	GGA	AAC	AGC	TAT	GAC	CAT	GAT	TAC	GCC	AAG	CTT	GGG	CTG	CAG	GTT	TCC	GCC	TCA	GAA	GCC		
1639	ATA	GAG	CCC	ACC	GCA	TCC	CCA	GCA	TGC	CTG	CTA	TTG	TCT	TCC	CAA	TCC	TCC	CCC	TTG	CTG	TCC	CCC	ACC	CCA	CCC		
1717	CCC	AGA	ATA	GAA	TGA	CAC	CTA	CTC	AGA	CAA	TGC	GAT	GCA	ATT	TCC	TCA	TTT	TAT	TAG	GAA	AGG	ACA	GTG	GGA	GTG	G	

3121	GTG	ACA	AAA	CTC	ACA	CAT	GCC	CAC	CAG	CAC	CTG	AAC	TCC	TGG	GGG	GAC	CGT	CAG	TCT	TCC	TCT	TCC	CCC	CAA		
3139	AAC	CCA	AGG	ACA	CCC	TCA	TGA	TCT	CCC	GGA	CCC	CTG	AGG	TCA	CAT	GCG	TGG	TGG	ACG	TGA	GCC	ACG	AAG	ACC	CTG	
3277	AGG	TCA	AGT	TCA	ACT	GGT	ACG	TGG	ACG	GCG	TGG	TGC	ATA	ATG	CCA	AGA	CGC	GGG	AGG	AGC	AGT	ACA	AGT	ACA	ACA	
3355	GCA	CGT	ACC	GTG	TGG	TCA	GCG	TCC	TCA	COG	TCC	TGC	ACC	AGG	ACT	GGC	TGA	ATG	GCA	AGG	AGT	ACA	AGT	GCA	AGG	TCT
3433	CCA	ACA	AAG	CCC	TCC	CAG	CCC	CCA	TCG	AGA	AAA	CCA	TCT	CCA	AAG	CCA	AAG	GGC	AGC	CCC	GAG	AAC	CAC	AGG	TGT	ACA
3511	CCC	TGC	CCC	CAT	CCC	GGG	AGG	AGA	TGA	CCA	AGA	ACC	AGG	TCA	GCC	TGA	CGT	GCC	TGG	TCA	AAG	GCT	TCT	ATC	CCA	CGG
3589	ACA	TCG	CCG	TGG	AGT	GGG	AGA	GCA	ATG	GGC	AGC	CGG	AGA	ACA	ACT	ACA	AGA	CCA	CGC	CTC	COG	TGC	TGG	ACT	COG	ACG
3667	GCT	CCT	TCT	TCC	TCT	ATA	GCA	AGC	TCA	CCG	TGG	ACA	AGA	GCA	GGT	GGC	AGC	AGG	GGA	ACG	TCT	TCT	CAT	GCT	CCG	TGA
3745	TGC	ATG	AGG	CTC	TGC	ACA	ACC	ACT	ACA	CGC	AGA	AGA	GCC	TCT	CCC	TGT	CTC	CGG	GTA	AAT	GAG	TTT	AAC	GGA	TCT	TAA
3823	TTA	ATC	CGA	GCT	CGG	TAC	CAA	GCT	TAA	GTT	TAA	ACC	GCT	GAT	CAG	CCT	CGA	CTG	TGC	CTT	CTA	GTI	GCC	AGC	CAT	CTG
3901	TTG	TTT	GCC	CCT	CCC	CCG	TGC	CTT	CCT	TGA	CCC	TGG	AAG	GTG	CCA	CTC	CCA	CTG	CCG	TGG	GGC	AGG	ATA	ATG	AGG	AAA
3979	TTG	CAT	CGC	ATT	GTC	TGA	GTA	GGT	GTC	ATG	CTA	TTT	TGG	GGG	GTG	GGC	AGG	ACA	GCA	AGG	GGG	AGG	ATT	GGG		
4057	AAG	ACA	ATA	GCA	GGC	ATG	CTG	GGG	ATG	CGG	TGG	GCT	CTA	TGG	CTT	CTG	AGG	CGG	AAA	GAA	CCA	GCT	GGG	GCT	CTA	GGG
4135	GGT	ATC	CCC	ACG	CGC	CCT	GTA	GCG	GCG	CAT	TAA	GCG	CGG	CTG	TGG	TGG	TTA	CGC	GCA	GCG	TGA	CCG	CTA	CAC	TTG	
4213	CCA	GCG	CCT	AGC	GCC	CTC	TCC	TTT	CGC	TTT	CGC	TTT	CTT	CCC	TTT	CTT	CGC	CAC	GGT	CGC	CTT	TCC	COG	TCA	AGC	TCT
4291	AAA	TCG	GGG	GCT	CCC	TTT	AGG	GTT	CCG	ATT	TAG	TGC	TTT	ACG	GCA	CCT	CGA	CCC	CAA	AAA	ACT	TGA	TTA	GGG	TGA	TGG
4369	TTT	ACG	TAG	TGG	GCC	ATC	GCC	CTG	ATA	GAC	GGT	TTT	TCG	CCC	TTT	GAC	GGT	GGA	GTC	CAC	GGT	TAA	TAG	TGG	ACT	
4447	CTT	GTT	CCA	AAC	TGG	AAC	ACT	CAA	CCC	TAT	CTC	GGT	CTA	TTT	TGA	TTT	TGA	ATT	ATA	AGG	GAT	TTT	GCC	GAT	TTT	GCC
4525	CTA	TTG	GTI	AAA	AAA	TGA	GCT	GAT	TTA	ACA	AAA	ATT	TAA	CGC	GAA	TTA	ATT	CTG	TGG	AAT	GTG	TGT	CAG	TTA	GGG	TGT
4603	GGA	AAG	TCC	CCA	GGC	TCC	CCA	GCA	GGC	AGA	AGT	ATG	CAA	AGC	ATG	CAT	CTC	AAT	TAG	TCA	GCA	ACC	AGG	TGT	GGA	AAG
4681	TCC	CCA	GGC	TCC	CCA	GCA	GGC	AGA	AGT	ATG	CAA	AGC	ATG	CAT	CTC	AAT	IAG	TCA	GCA	ACC	ATA	GTG	CCC	CTA	ACT	
4759	CGG	CCC	ATC	CGC	CCC	CTA	ACT	CCG	CCC	AGT	TCC	GCC	CAT	TCT	CCG	CCC	CAT	GGC	TGA	CTA	ATT	TTT	TTT	ATT	TAT	GCA
4837	GAG	GCC	GAG	GCC	GCC	TCT	GCC	TCT	GAG	CTA	TTT	CAG	AAG	TAG	TGA	GGG	GGC	TTT	GCA	GGC	CTA	GGC	TTT	TTT	TGC	AAA
4915	AAG	CTC																								

Figure 12 contd

6865 AGC GGT TTG ACT CAC GGG GAT TTC CAA GTC CAC GGT GAT GGC GAT TTC CAA GTC CAC GGT GAT TTC CAA GTC CAC GGT GAT TTC CAA GTC CAC
6943 GGG ACT TTC CAA AAT GTC GAT GTC GGT GAT GTC GGT GAT GTC GGT GAT GTC GGT GAT GTC GGT GAT GTC GGT GAT GTC GGT GAT GTC GGT GAT GTC GGT
7021 TAA GCA GAG CTC GTT TAG TGA ACC GTC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
7099 GGG ACC GAT CCA GCC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
7177 ATC GAT CCG ACC GAC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
7255 GTG ATC TCC ACC TGT CTT GAA TTC TCC ATG GTC CCG GCA AGT GCT GCA TCC GCT GCA TCC GCT GCA TCC GCT GCA TCC GCT GCA TCC GCT GCA TCC
7333 GGA GAC AGA GTC ACC CTC ACT TTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT
7411 AAA GCC CCT AAG CTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
7489 GGG ACA GAT TTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC ACT CTC
7567 ACC CCT CCA ACG TTC GGC CAA GGG ACC AAG GTG GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
7645 AAA CAT CAA GAT TTT AAA TAC GCT TCT TGG TCT CCT TGC TAT AAT TAT CTG GGA TAA GCA TGC TGT TTT CTG TGT TTT CTG TGT TTT CTG
7723 CCT AAC ATG CCC TGT GAT TAT CCG CAA ACA ACA GCG GAA GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
7801 TCT TTC CTC AGG AAC TGT GGC TGC ACC ATC TGT CTT CAT CTT GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
7879 CTC TGT TGT GTG CCT GCT GAA TAA CTT CTA TCC CAG AGA GGC CAA AGT ACA GTG GAA GGT TGA GCA GGT GAA GGT TGA GCA GGT GAA GGT TGA
7957 GGG TAA CTC CCA GGA GAG TGT CAC AGA GCA GGA CAG CAA GCG CCA TCA CAG CCG CCA TCA CAG CCG CCA TCA CAG CCG CCA TCA CAG CCG CCA
8035 CAA AGC AGA CTA CGA GAA GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
8113 CTT CAA CAG GGG AGA GTG TTA GGT TTA ACG GAT CCG AGC TCG GTA CCA AGC TCA CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG
8191 ACT GTG CCT TCT AGT TGC CAG CCA TCT GTT GTC TCC CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
8269 ACT GTC CTT TCC TAA TAA AAT GAG GAA ATT GCA TCG CAT TGT CTG AGT AGG CAT GCT GGG GAT GCG GTG GCG GTG GCG GTG GCG GTG GCG GTG GCG
8347 GGG CAG GAC AGC AAG AGC TGC ATT AAT GAA TCG GCC AAC CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
8425 GCG GAA AGA ACC AGC AGC TGC ATT AAT GAA TCG GCC AAC CCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG
8503 CCT CGC TCA CTG ACT CCG TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC TGC
8581 TAT CCA CAG AAT CAG GCG ATA ACG CAG GAA AGA ACA TGT GAG CAA AAG GCC AGC AAA ATC GAC GCT CAA GGT CAA GGT CAA GGT CAA GGT CAA
8659 CCG CGT TGC TGG CGT TTT TCC ATA GGC TCC GCC CCC CTG ACG AGC ATC ACA AAA ATC GAC GCT CAA GGT CAA GGT CAA GGT CAA GGT CAA GGT
8737 GAA ACC CGA CAG GAC TAT AAA GAT ACC AGG CGT TTC CCC CTG GAA GCT CGC TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG TCG
8815 CGC TTA CCG GAT ACC TGT CCG CCT TTC TCC CTT CGG GAA GCG TGG CGC TTT CTC ATA GCT CAC GCT GTA GGT ATC TCA TCA TCA TCA TCA TCA
8893 GTT CGG TGT AGG TCG TTC GCT CCA ACC CAG TTA CCG GAT TAT CCG CAC TGG CAG CCA CTG ACA CTA GAA GAA CAG TAT TTG TCA TCA TCA TCA
8971 GTA ACT ATC GTC TTG TAG GCG GTG CTA CAG AGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT
9049 GAG CGA GGT ATG TAG GCG GTG CTA CAG AGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT GGT
9127 GTA TCT GCG CTC TGC TGA AGC CAG TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG
9205 GTA GCG GTG GTT TTT TTG TTT GCA AGC AGC AGA TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG GAT TTA CCG
9283 CTA CGG GGT CTG ACG CTC AGT GGA ACG AAA ACT CAC GGT AAG GGA TTT TGG TCA TGA GAT CTC AAG AAG AAG AAG AAG AAG AAG AAG AAG AAG
9361 CCT AGA TCC TTT TAA ATT AAA AAT GAA GTT TTA AAT CAA TCT AAA GTA TAT ATG AGT AAA CTT GGT TAT CCA AAA GGA TCT TCA TCA TCA TCA
9439 AAT GCT TAA TCA GTG AGG CAC CTA TCT CAG CGA TCT GTC TAT TTC CAT CCA TAG TAC CGC GAG ACC CAC GCT CAC GCT CAC GCT CAC GCT CAC
9517 AGA TAA CTA CGA TAC GGG AGG GCT TAC CAT CTG GCC CCA GTG CCA GAA GTG GTC TTA ATA GTT TGC GCA ACG TTG TGC CCA TTG CCA TTG CCA
9595 CAG ATT TAT CAG CAA TAA ACC AGC CAG CCG GAA GCG AGC GCA GAA GTG GTC TTA ATA GTT TGC GCA ACG TTG TGC CCA TTG CCA TTG CCA TTG
9673 AGT CTA TTA ATT GTT GTC GCG GCG AAG CTA GAG TAA GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC GTC
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9829 GAT CCC CCA TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT TGT
9907 TAT CAC TCA TGG TTA TGG CAG CAC TGC ATA ATT CTC TTA CTG TCA TGC CAT CCG TAA GAT GCT TTT CTG TGA TGA TGA TGA TGA TGA TGA TGA
9985 AGT ACT CAA CCA AGT CAT TCT GAG AAT AGT GTA TGC GGC GAC CGA GTT GCT CTT GCC CGG GAA AAC TCT CAA TAC GGG ATA ATA TAC TAC
10063 CCG CGC CAC ATA GCA GAA CTT TAA AAG TGC TCA TCA TTG GAA AAC GTT CTT CGG GCG GAA AAC TCT CAA TAC GGG ATA ATA TAC TAC TAC TAC
10141 CGC TGT TGA GAT CCA GTT CGA TGT AAC CCA CTC GTG CAC CCA ACT GAT CTT CAG CAT CTT TTA CTT TCA CCA CCG TTT TCA TCA TCA TCA
10219 CTG GGT GAG CAA AAA CAG GAA GGC AAA ATG CCG CAA AAA AGG GAA TAA GGG CGA CAC GGA AAT GTT GAA TAC TAC TCA TCA TCA TCA

Figure 13

A

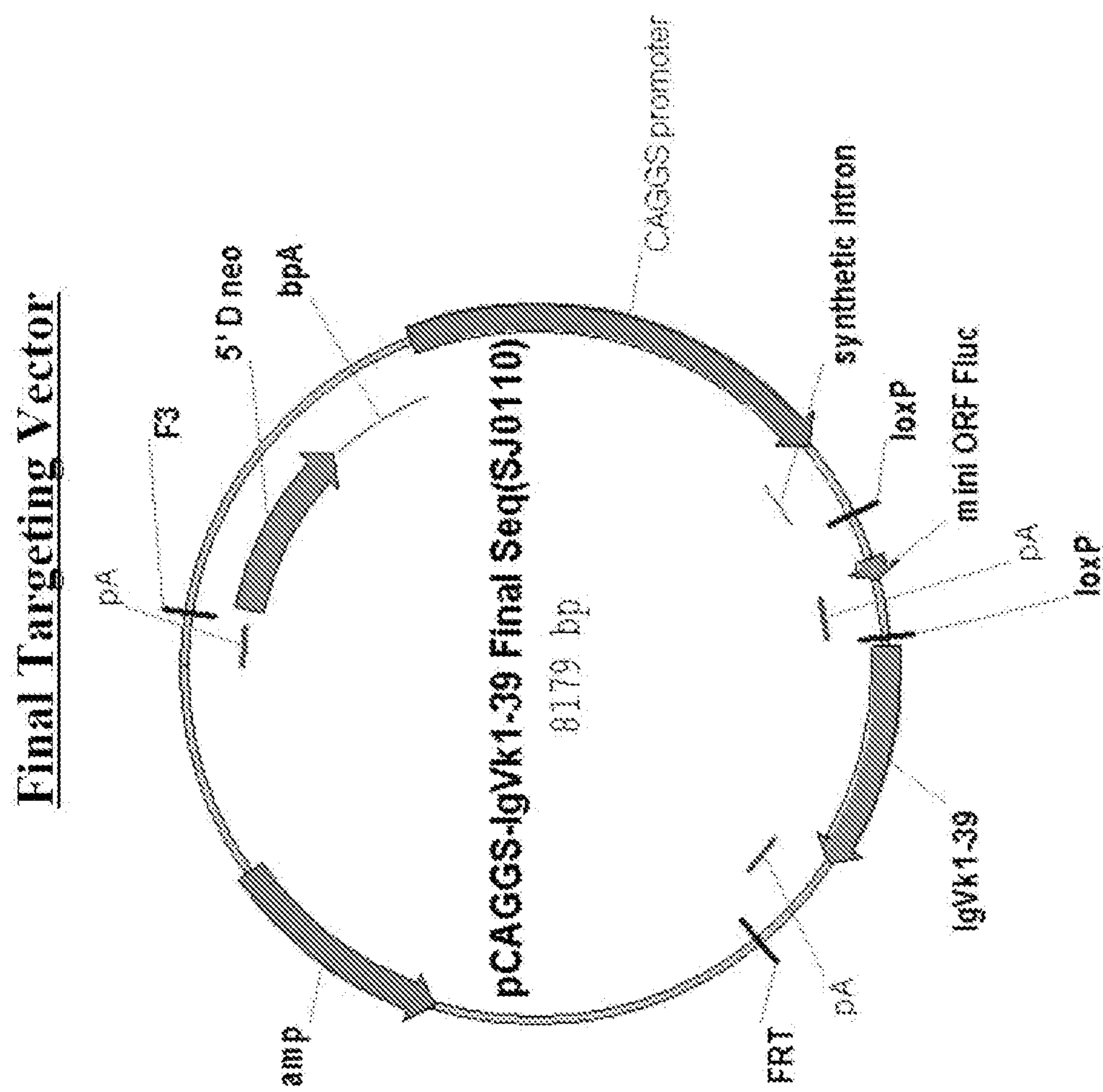


Figure 13 contd**B**

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121 aatgaccttg ggggaggggg aggccagaat gaccttgggg gagggggagg ccagaatgag
181 gcgcggatcc ggagaaagtcc ctattccgaa gttcctattc ttcaaatagt ataggaaactt
241 cgctcgaggg atcgggcatb gaacaagatg gattgcacgc aggttctccg gccgcttggg
301 tggagaggct attcggctat gactgggcac aacagacaaat cggctgctct gatgccgccg
361 tgttccggct gtcagcgag gggcgcccg tcttcttgt caagaccgac ctgtccggtg
421 ccctgaatga actgcaggac gaggcagcgc ggctatcgtg gctggccacg acgggcgttc
481 cttgcgcagc tgtgtcgac gttgtcactg aagcgggaaag ggactggctg ctattggggc
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601 tggctgatgc aatgcggcgg ctgcatacgc ttgatccggc tacctgcccc ttcgaccacc
661 aagcgaataa tcgcatcgag cgagcacgta ctcggatgga agccgggtctt gtcgatacag
721 atgatctgga cgaagagcat caggggctcg cgccagccga actgttcgcc aggctcaagg
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901 accgctatca ggacatagcg ttggctaccc gtgatattgc tgaagagctt ggcggcgaat
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Figure 13 contd

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1561 ttgattattg actagttatt aatagtaatc aattacgggg tcattagttc atagcccata
1621 tatggagttc cgcgttacat aacttacggt aaatggcccg cctggctgac cgcccaacga
1681 cccccgccc ttgacgtcaa taatgacgta tgttcccata gtaacgccc aa tagggacttt
1741 ccattgacgt caatgggtgg agtatttacg gtaaaactgcc cacttggcag tacatcaaagt
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2461 tgcgtgggga gcgcgcgtg cggcccgcg tgcgcggcg ctgtgagcg tcggggcgcg
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2581 gcggtgccgg ggggctgcga ggggaacaaa ggctgcgtgc ggggtgtgtg cgtggggggg
2641 tgagcagggg gtgtgggcgc ggcggtcggg ctgtaacccc ccctgcacc cccctccccg
2701 agttgctgag cacggccccg cttcgggtgc ggggctcgtg gcggggcggt gcgcggggct
2761 cgccgtgccg ggcgggggggt ggcggcaggt gggggtgccc ggcggggcctcg
2821 ggcgggggag ggtcggggg agggcgcgcg cggccccgga gcgcggcg ctgtcgaggc
2881 gcggcgagcc gcagccattg ccttttatgg taatcgtgag agagggcgca gggacttctc
2941 ttgtcccaaa tctgtgcgga gccgaaatct gggaggcgcc gccgacccc ctctagcggg

Figure 13 contd

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Figure 13 contd

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Figure 13 contd

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Figure 13 contd

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Figure 13 contd

C

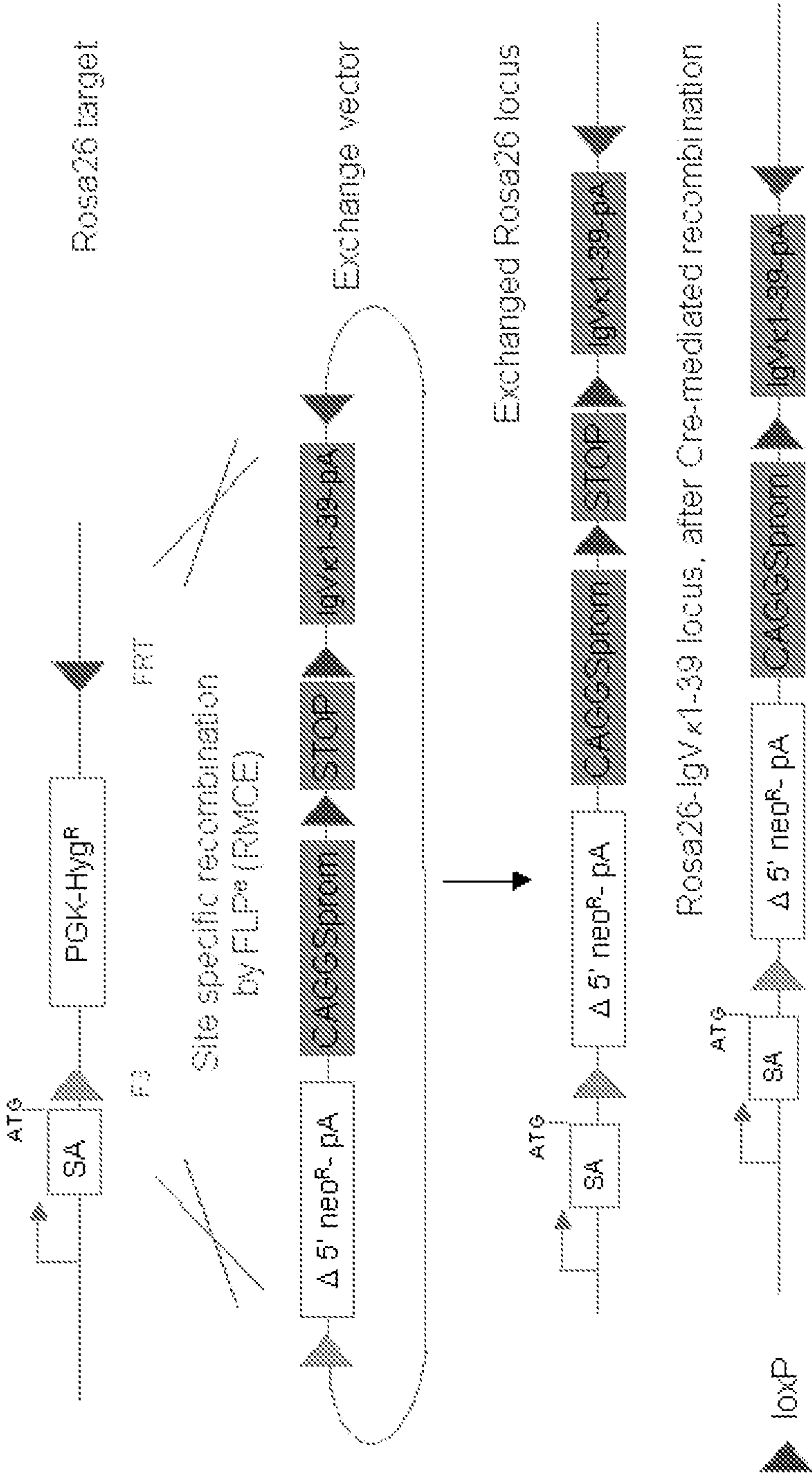
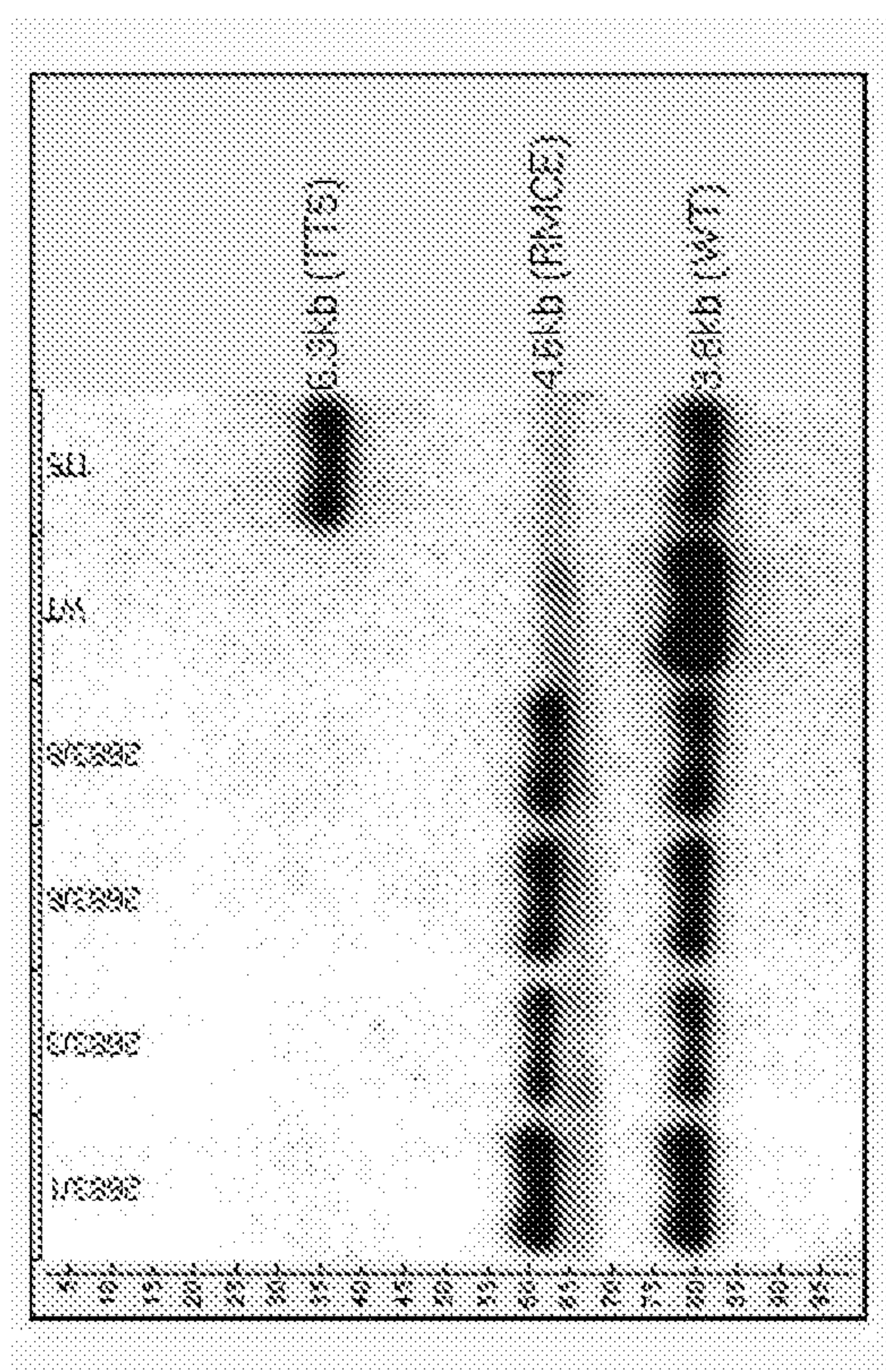


Figure 14

A



B

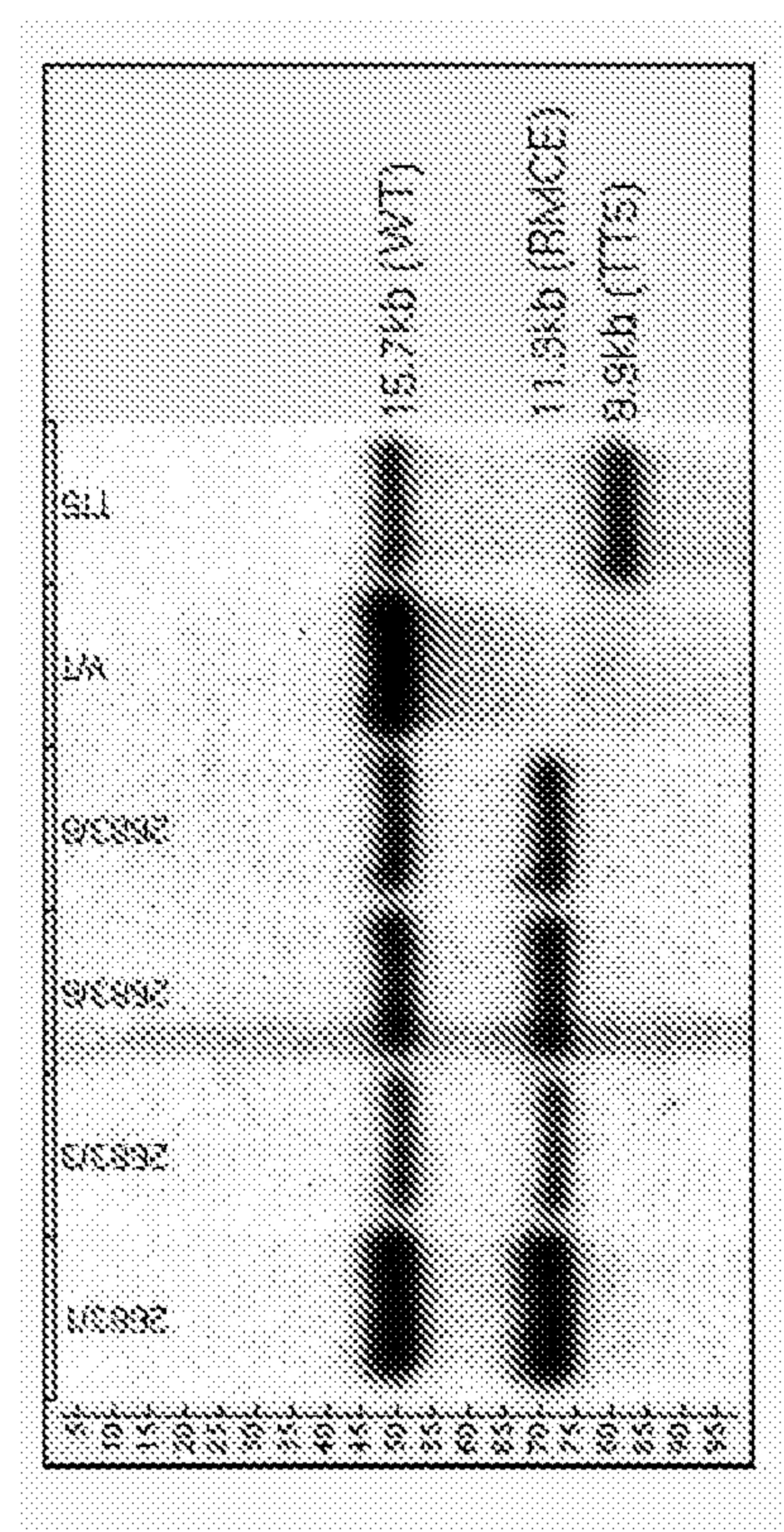


Figure 14 contd

C

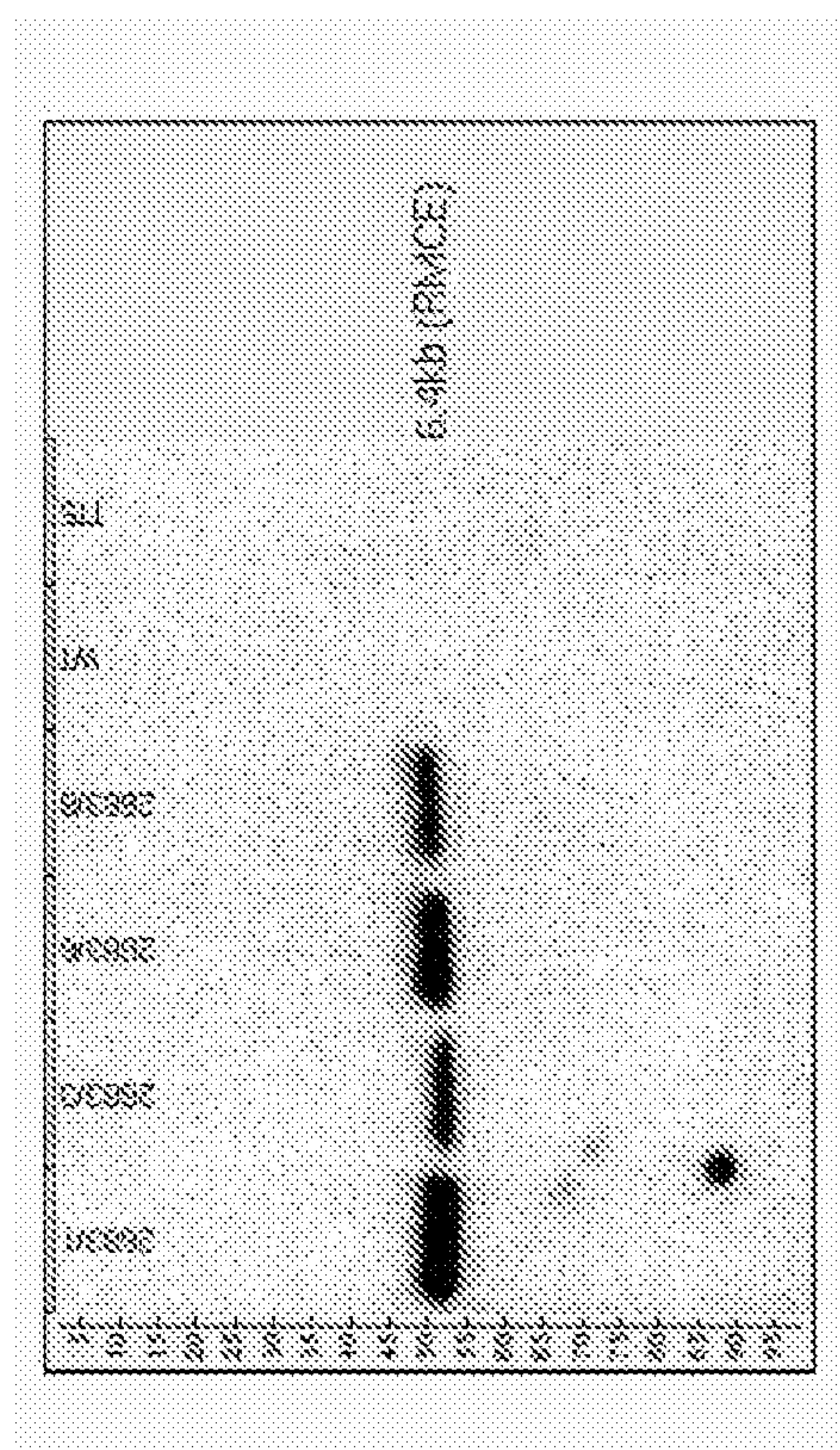


Figure 15

A

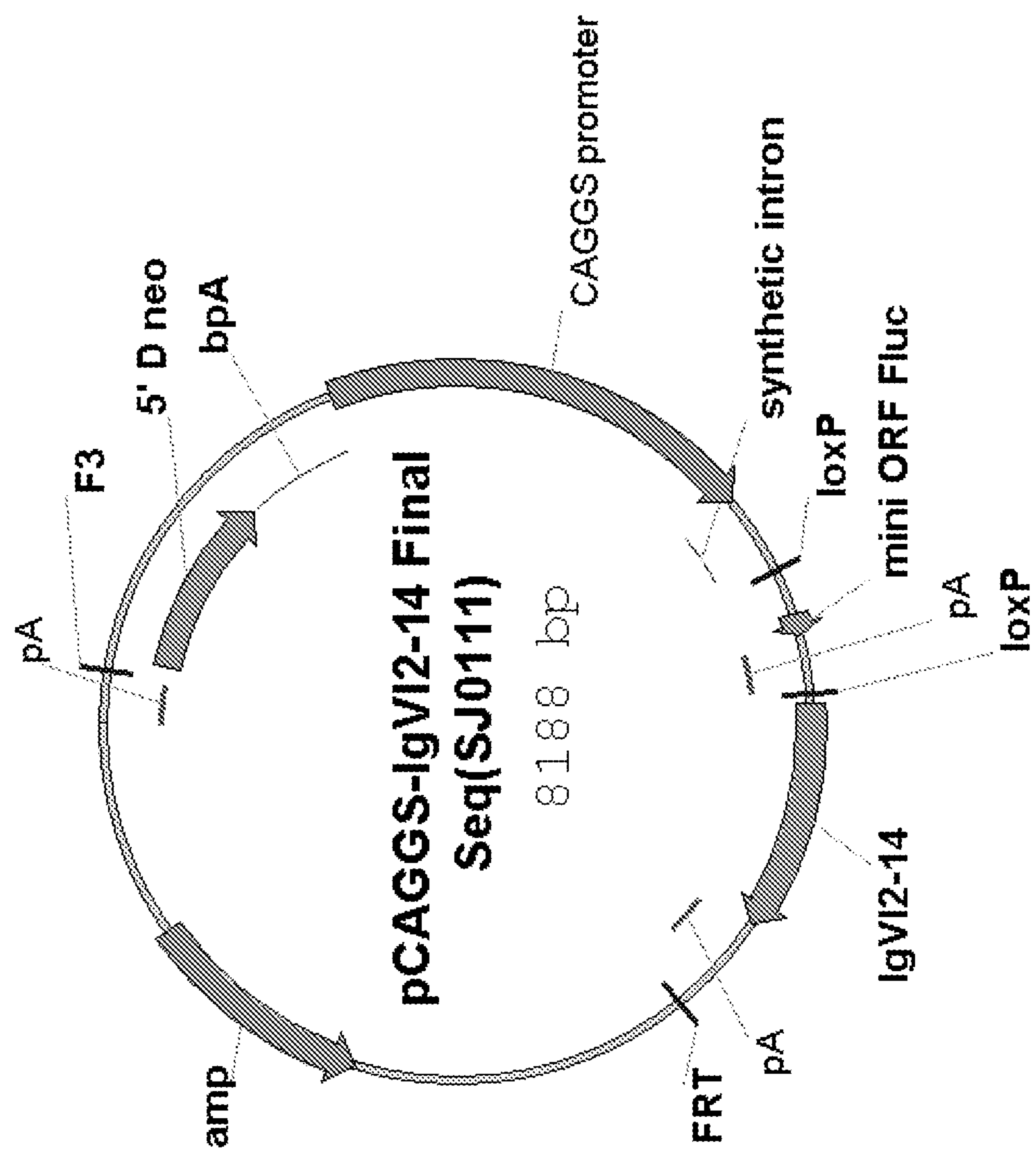


Figure 15 contd

B

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Figure 15 contd

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Figure 15 contd

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Figure 15 contd 48/72

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Figure 15 contd**49/72**

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Figure 15 contd **50/72**

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7681 agaacgtgga ctccaacgtc aaaggcgaa aaaccgtcta tcaggggcgat ggcccactac
7741 gtgaaccatc accctaataca agtttttttg ggtcgaggtg ccgtaaaagca ctaaatcggga
7801 accctaaaag gagccccga tttagagctt gacgggggaaa gccggcgaaac gtggcggagaa
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7921 tgcgcgtaac caccacacc gccgcgcta atgcgccgt acagggcgcg tccattcgc
7981 cattcaggct gcgcaactgt tgggaaggc gatcgggtgcg ggctctcttcg ctattacgcc
8041 agctggcgaa aggggggatgt gctgcaaggc gattaagtgt ggtaacgcca gggttttccc
8101 agtcacgacg ttgtaaaacg acggccagt agcgcgcgta atacgactca ctatagggcg
8161 aattgggggt aactaagtaa ggatcgag

Figure 15 contd 51/72

C

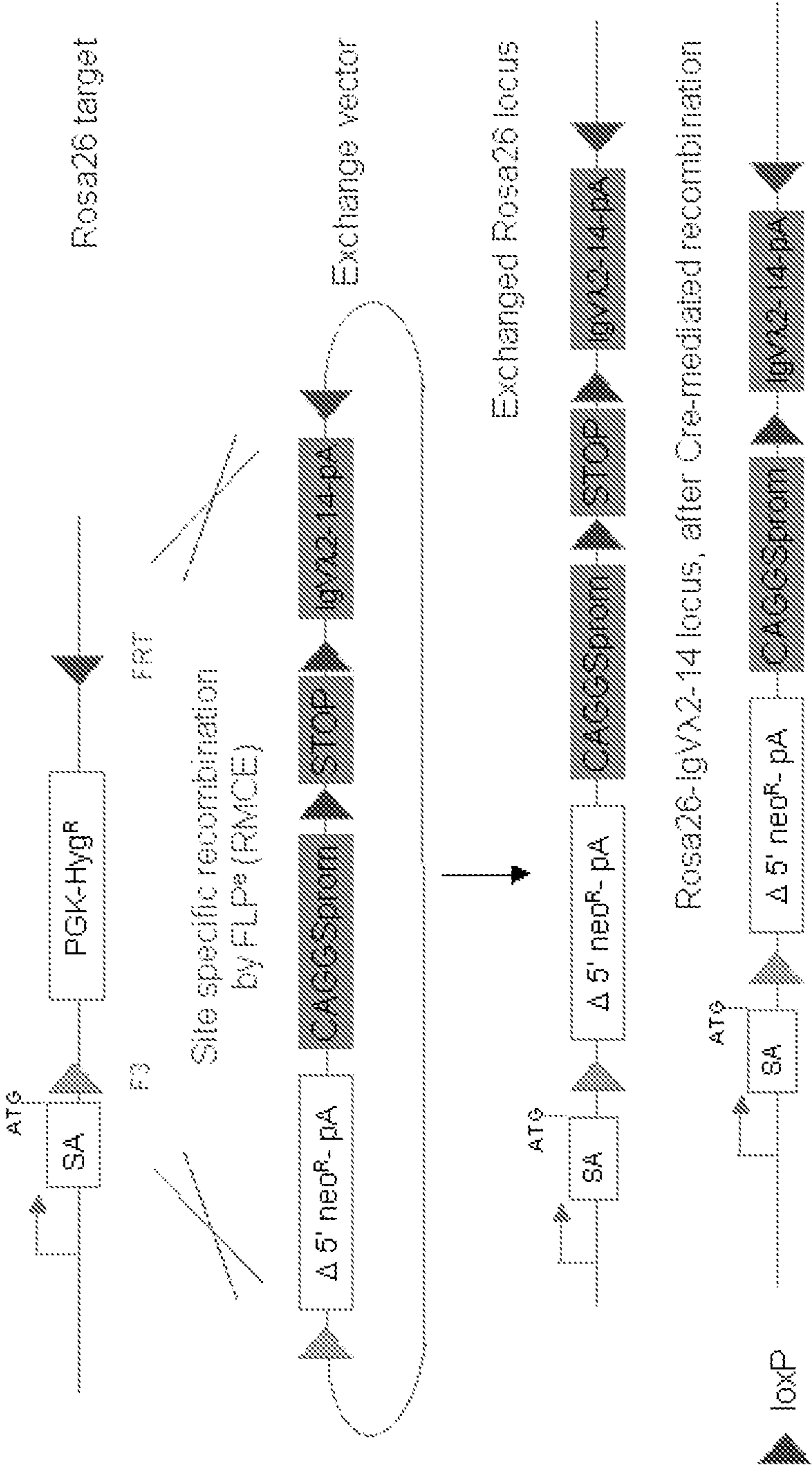


Figure 16 52/72

A

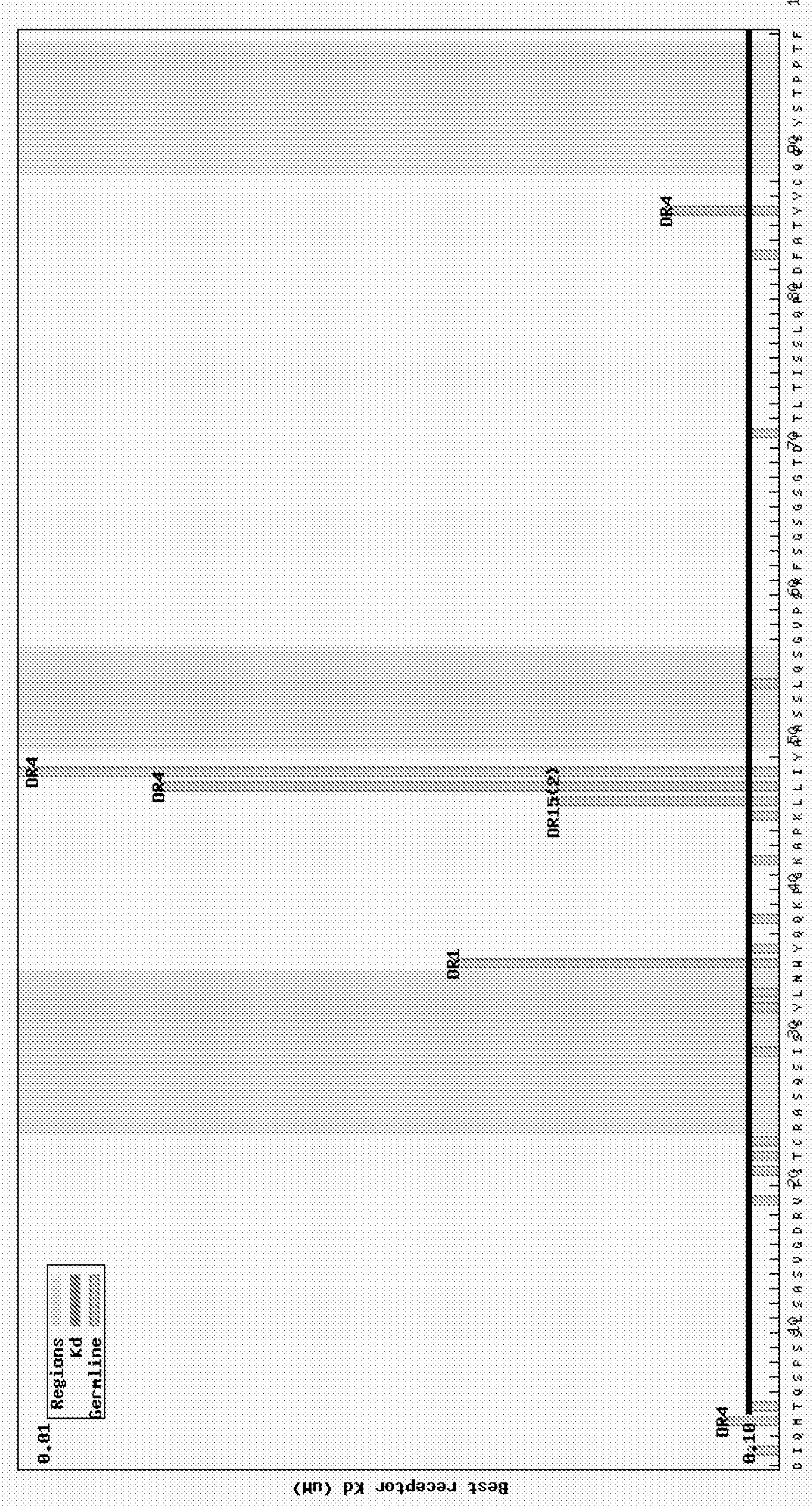


Figure 16 contd 53/72

B

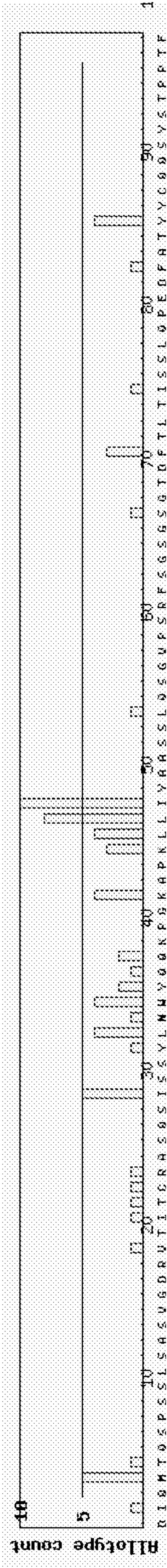
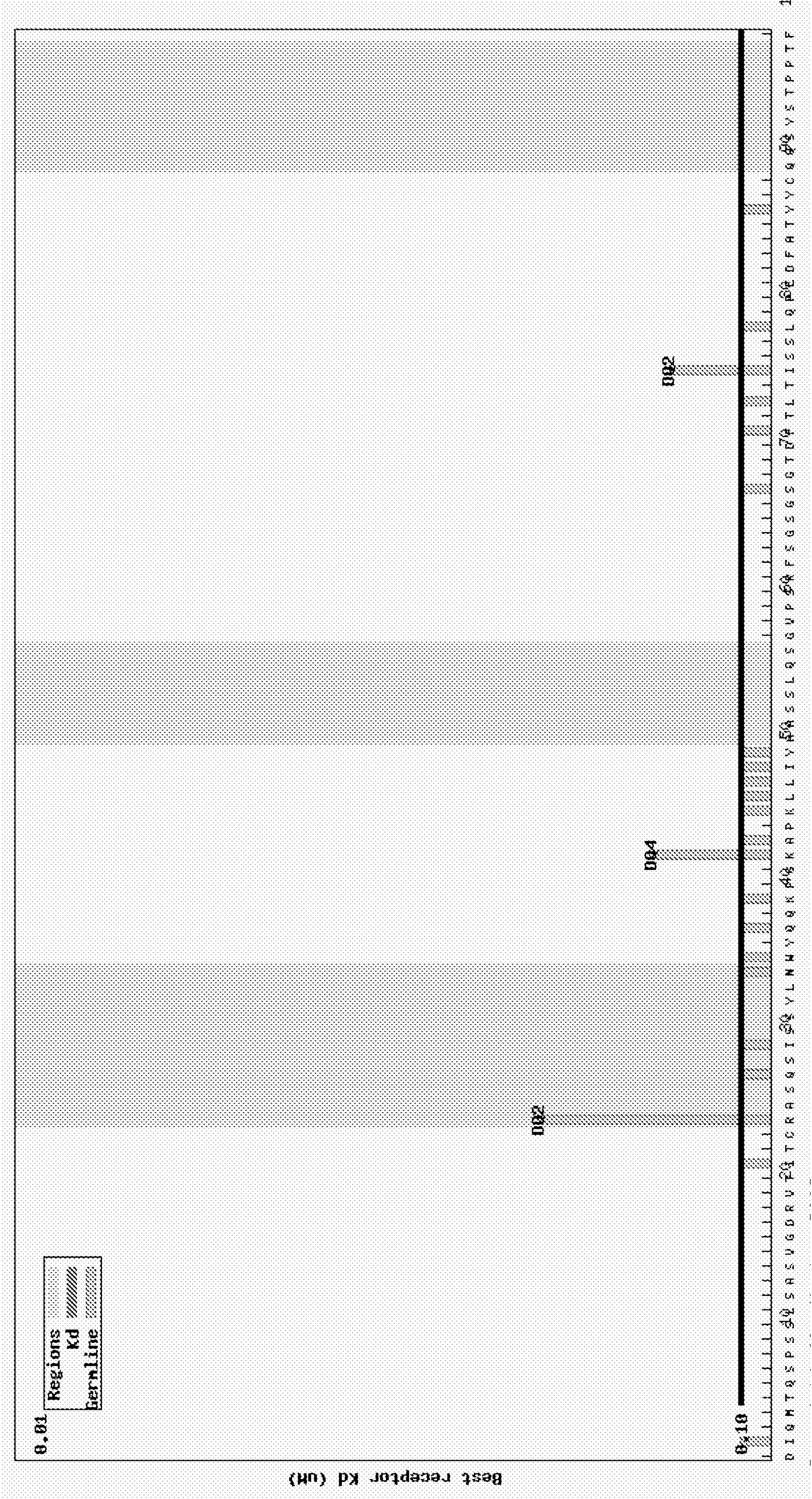


Figure 16 contd 54/72

C



55/72

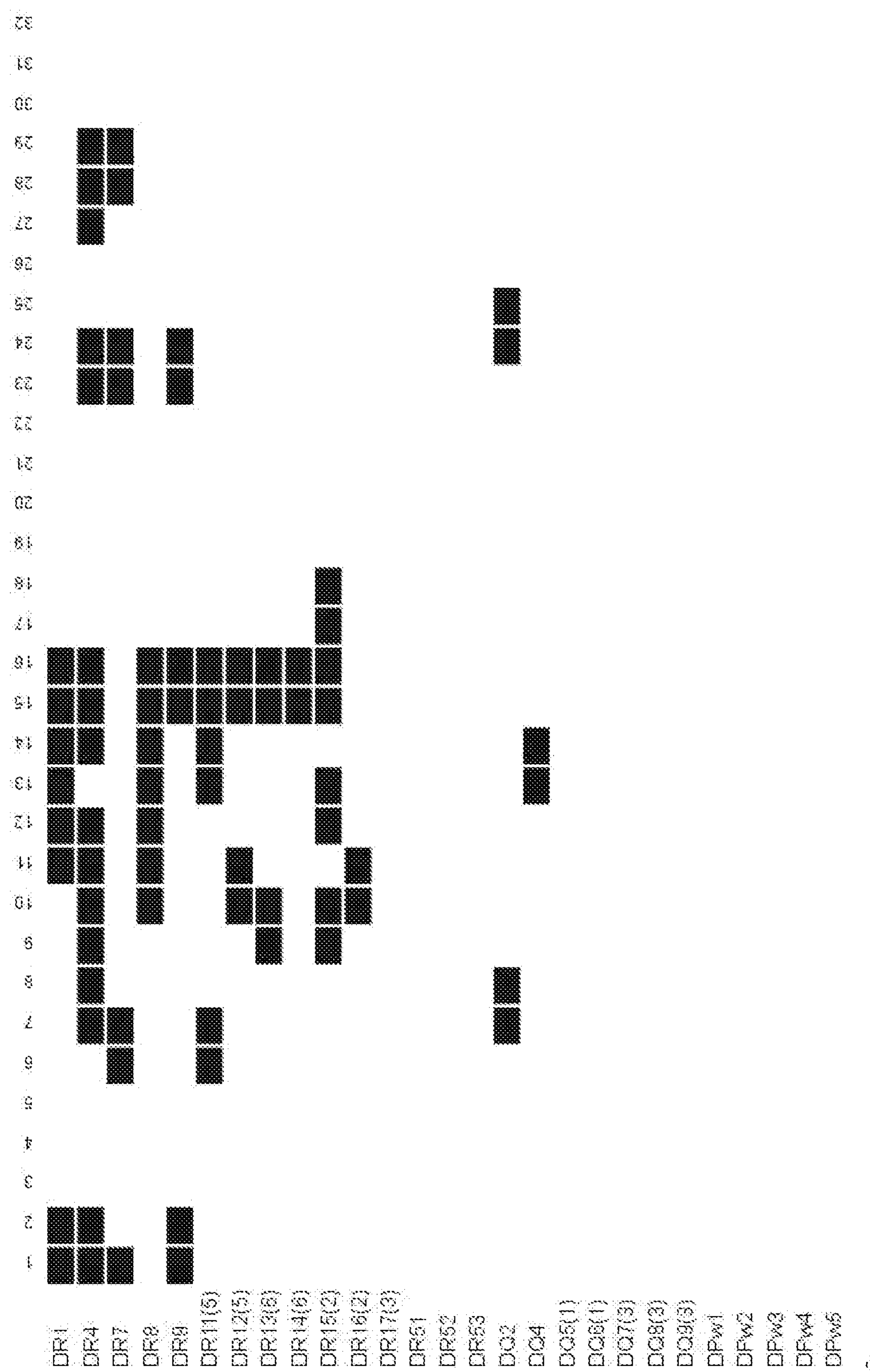


Figure 18 56/72

A

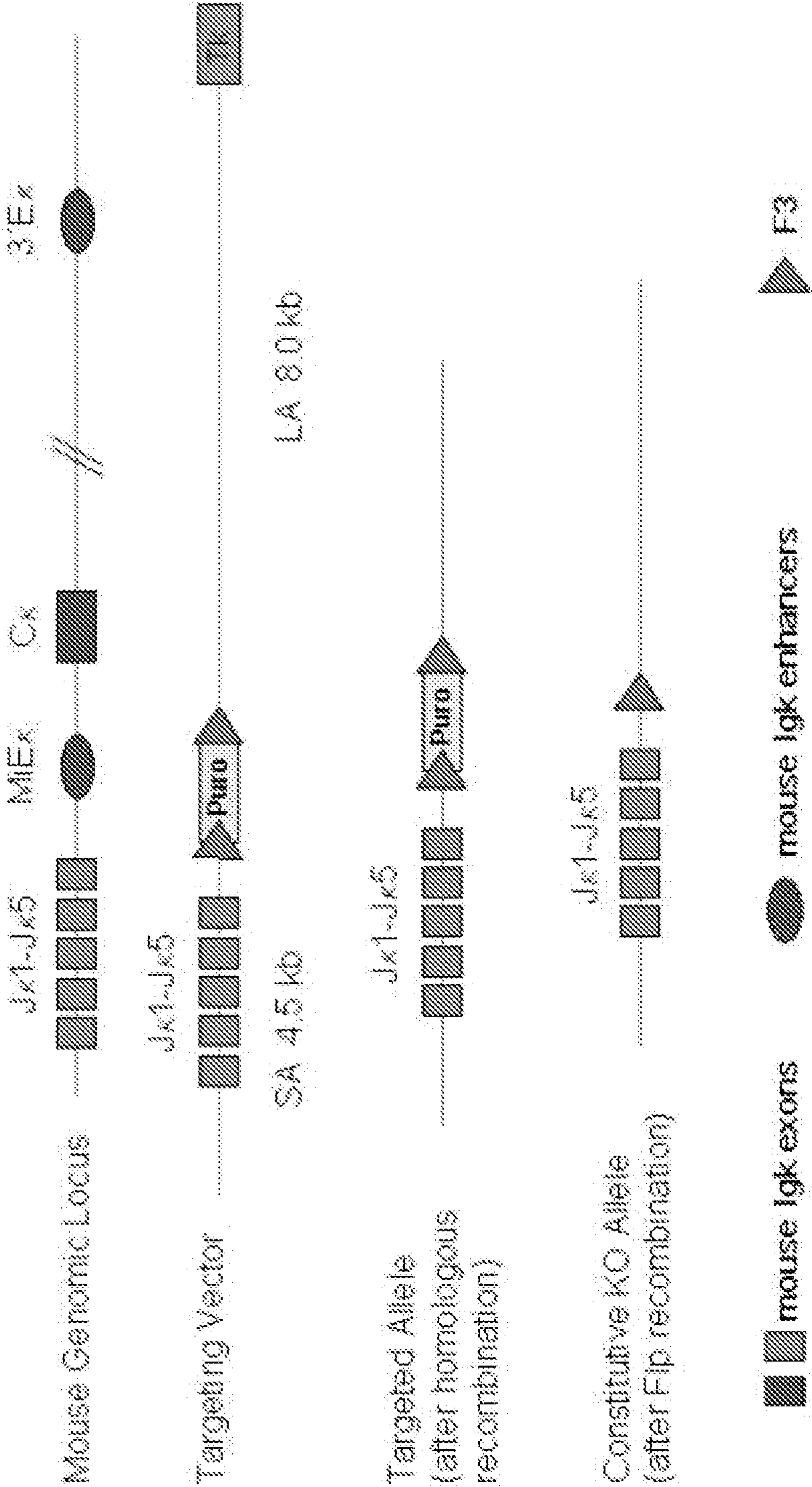


Figure 18 contd 57/72

B

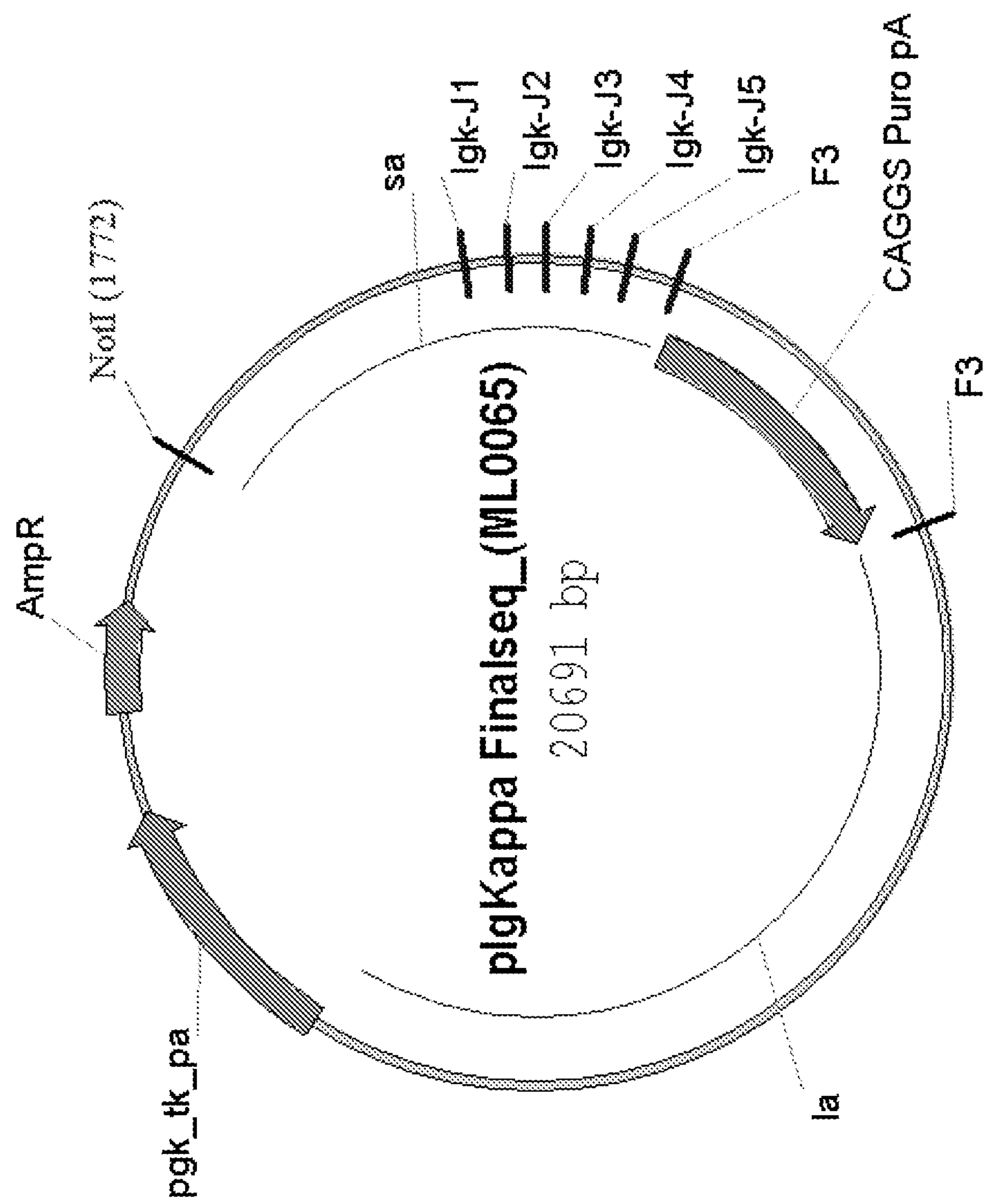


Figure 19 58/72

A

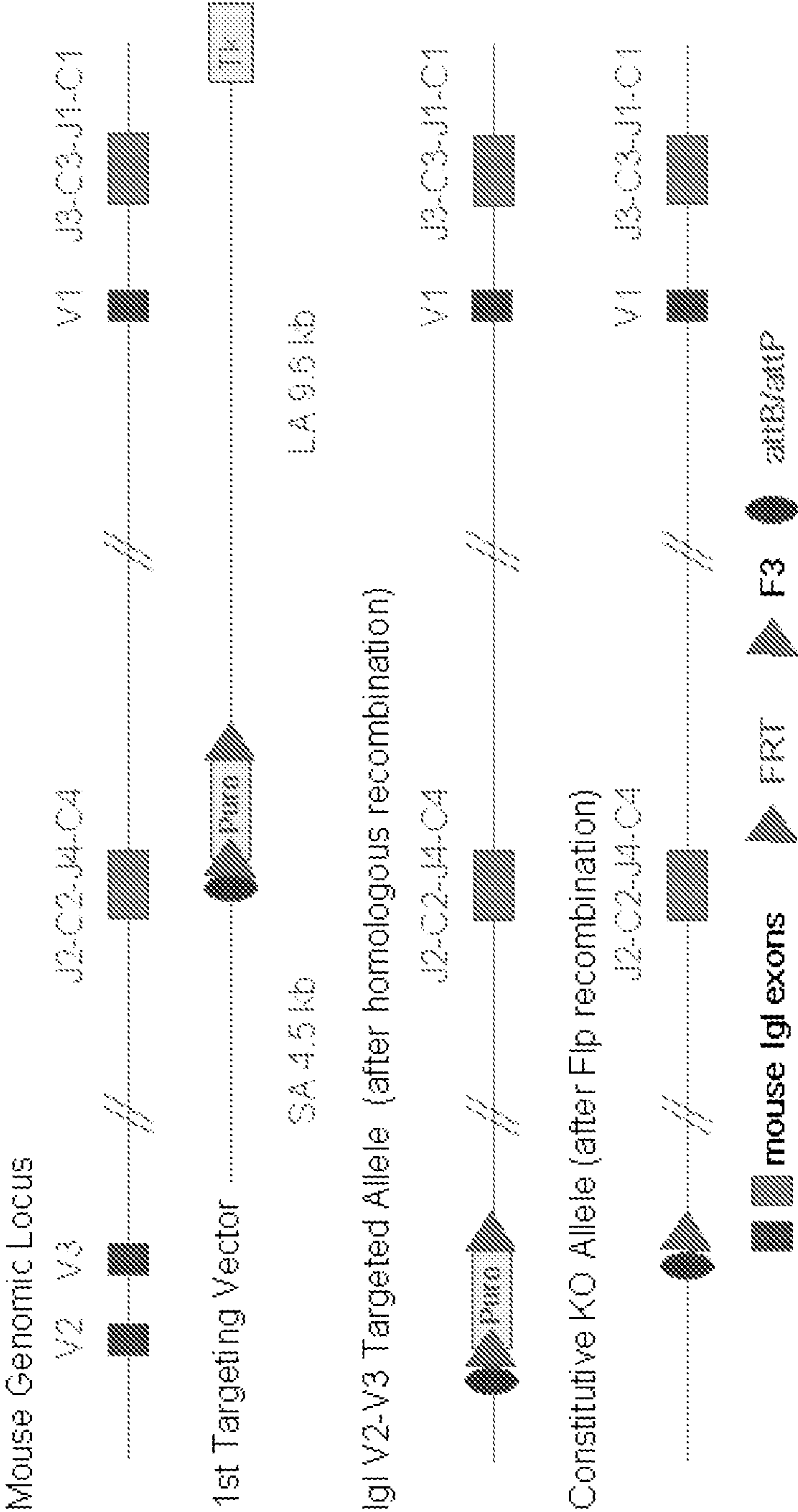


Figure 19 contd 59/72

B

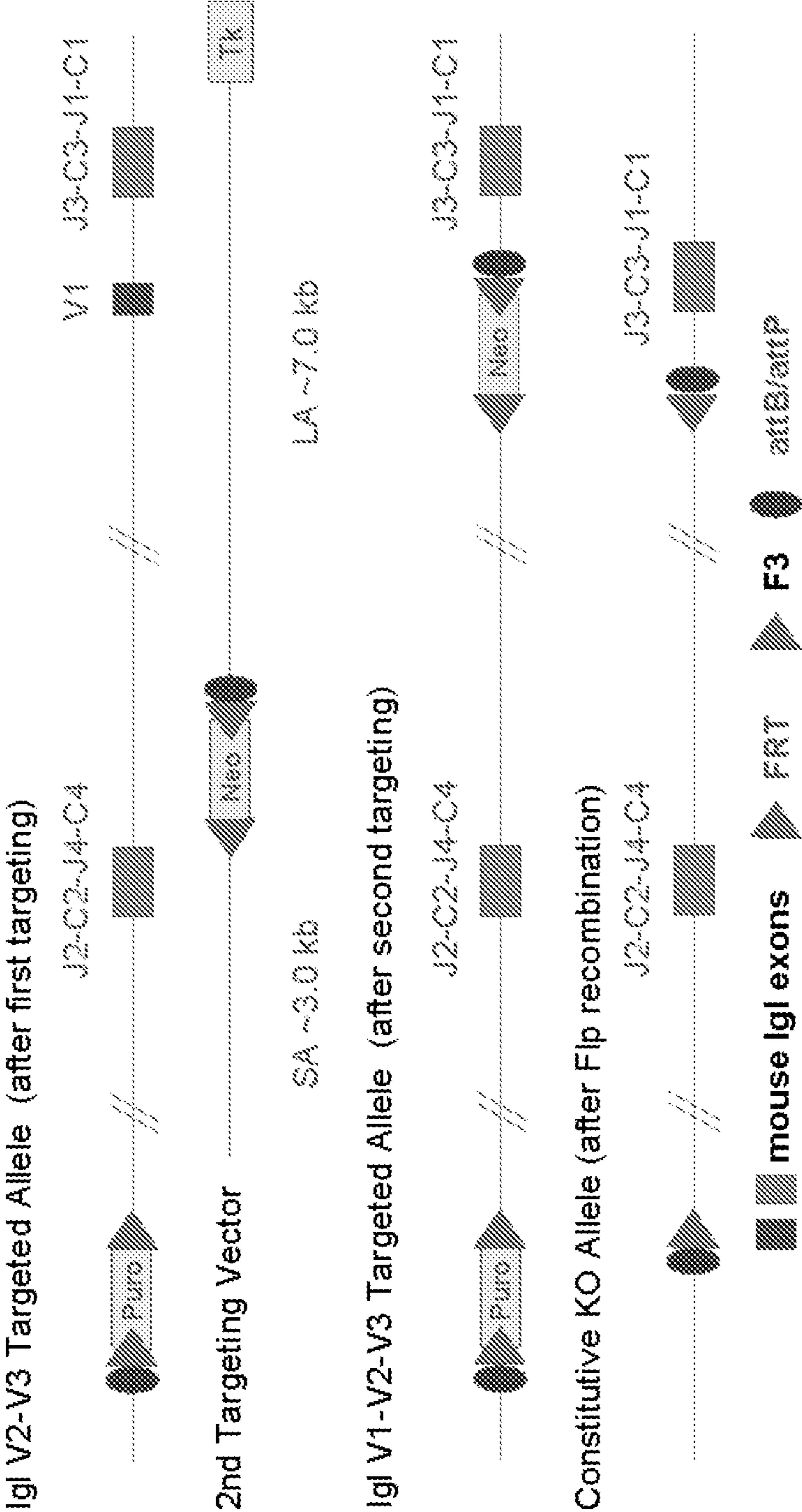


Figure 20

60/72

A

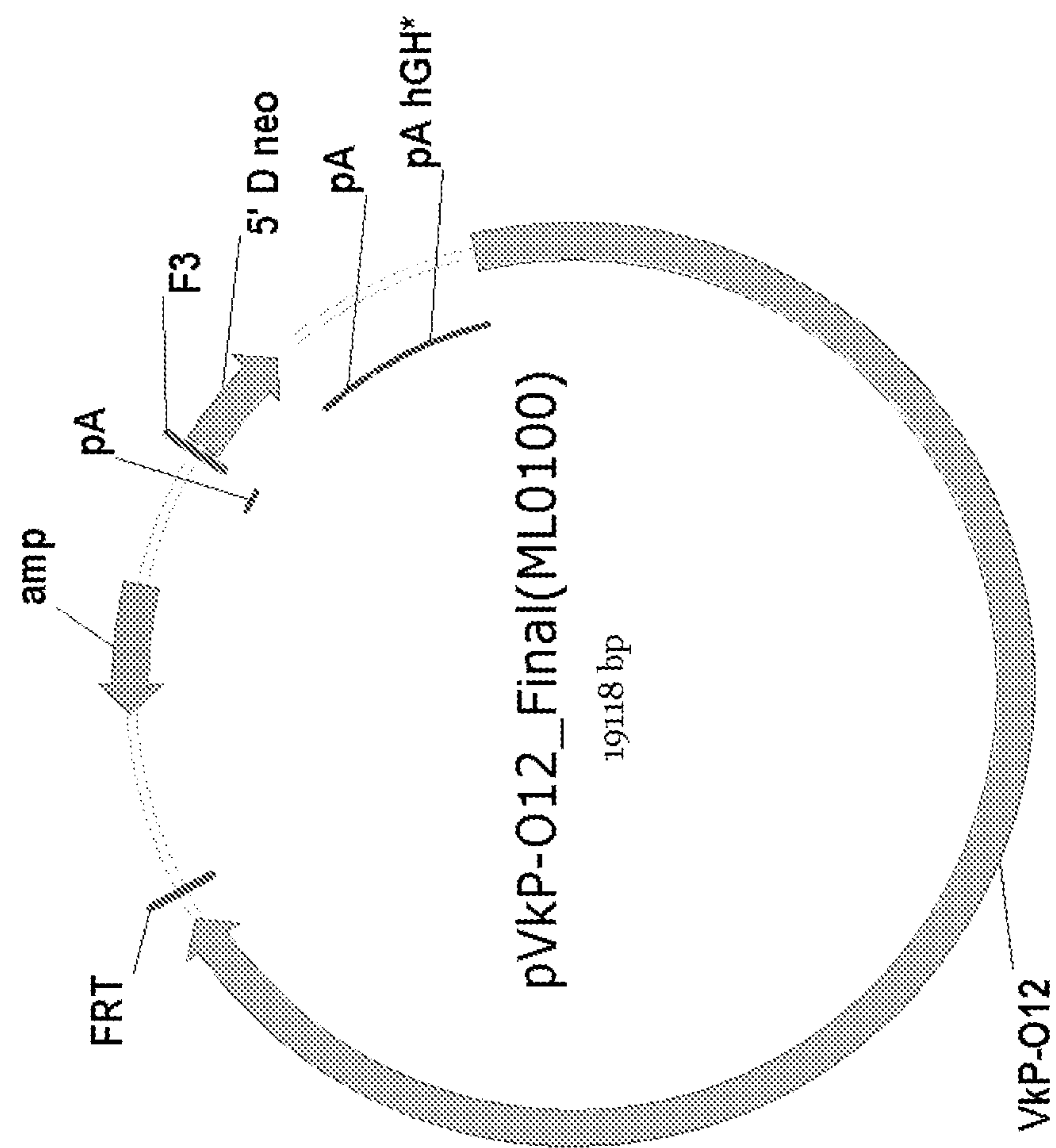


Figure 20 contd 61/72

B

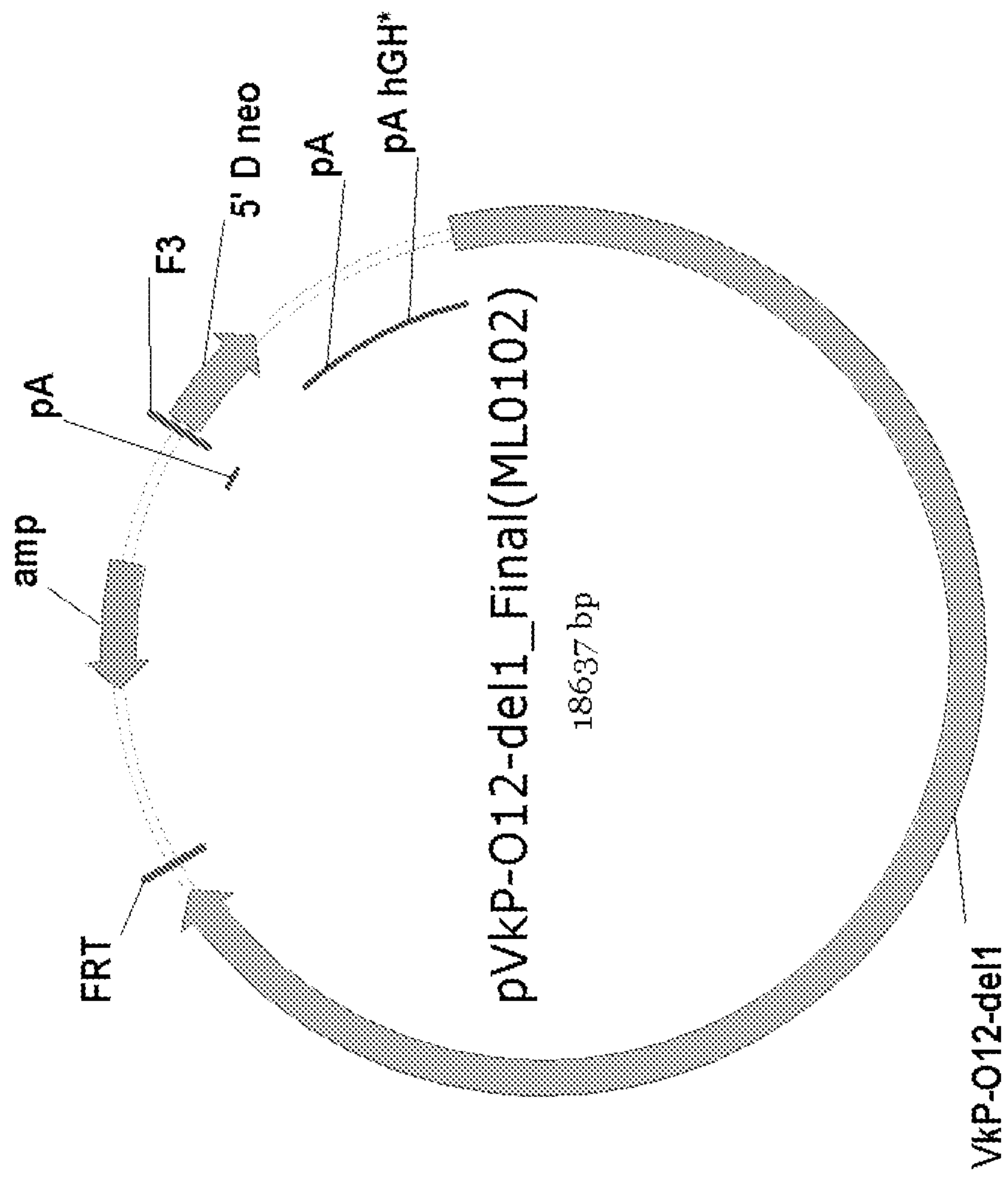


Figure 20 contd 62/72

C

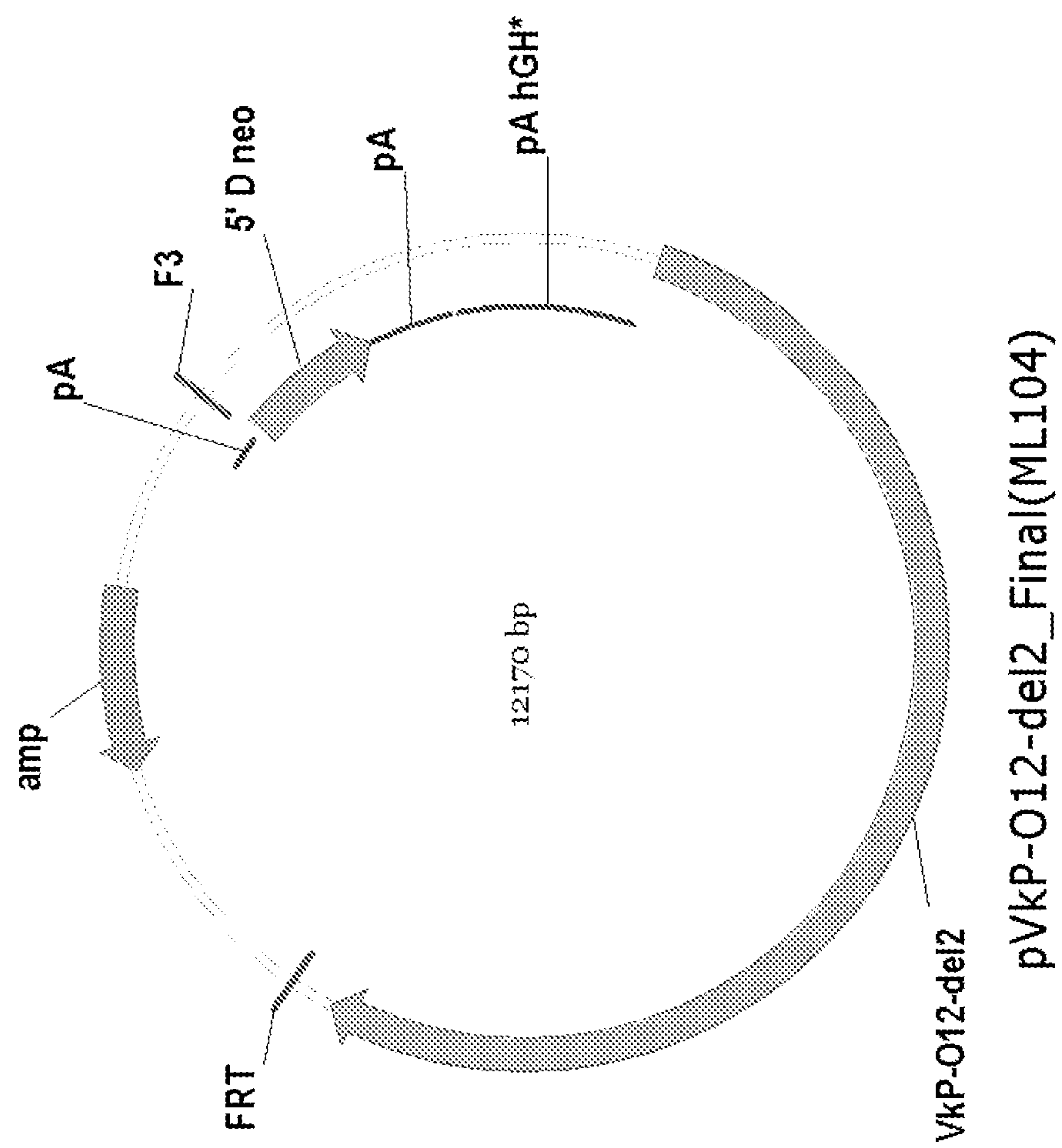


Figure 21 63/72

A

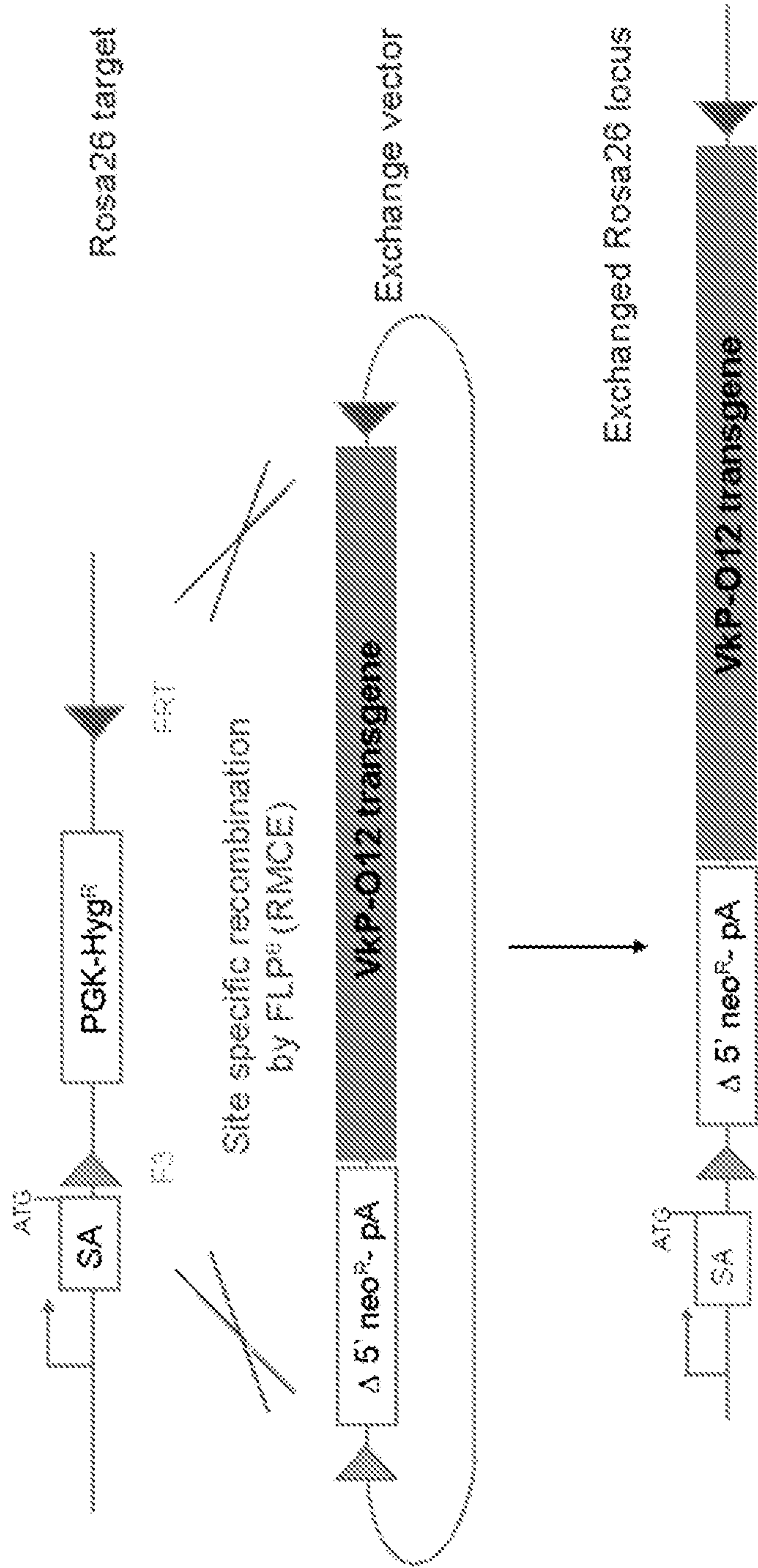


Figure 21 contd 64/72

B

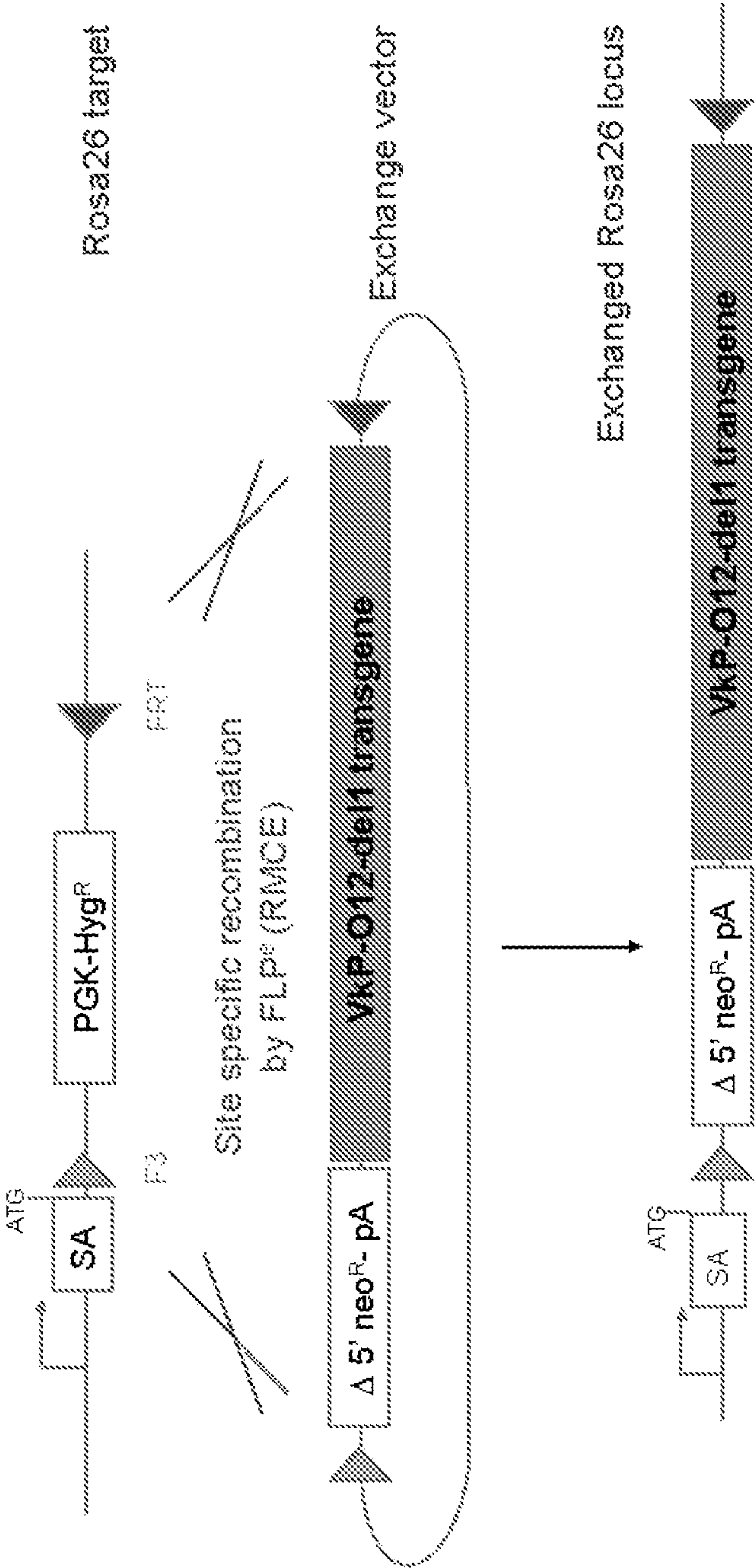


Figure 21 contd 65/72

C

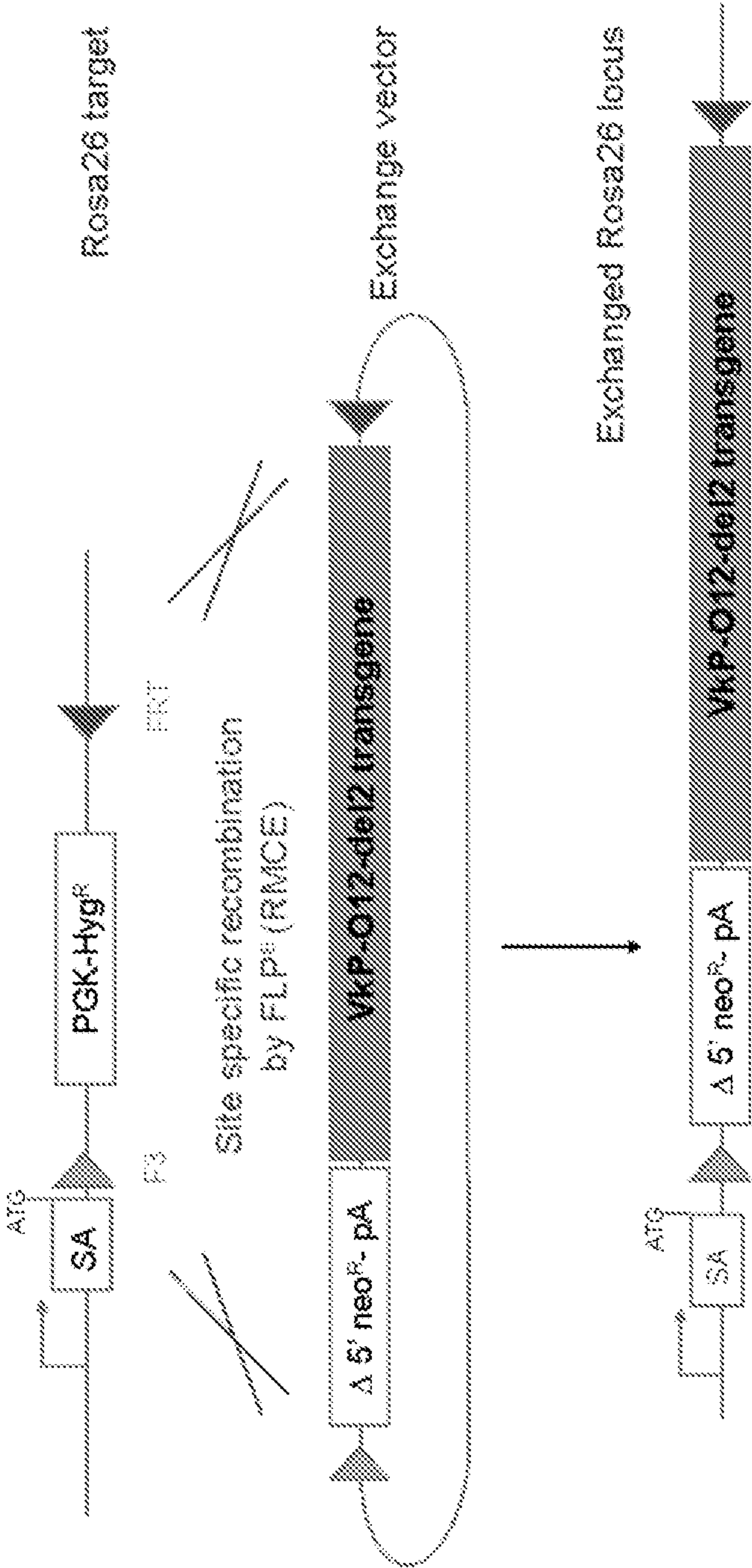


Figure 22

66/72

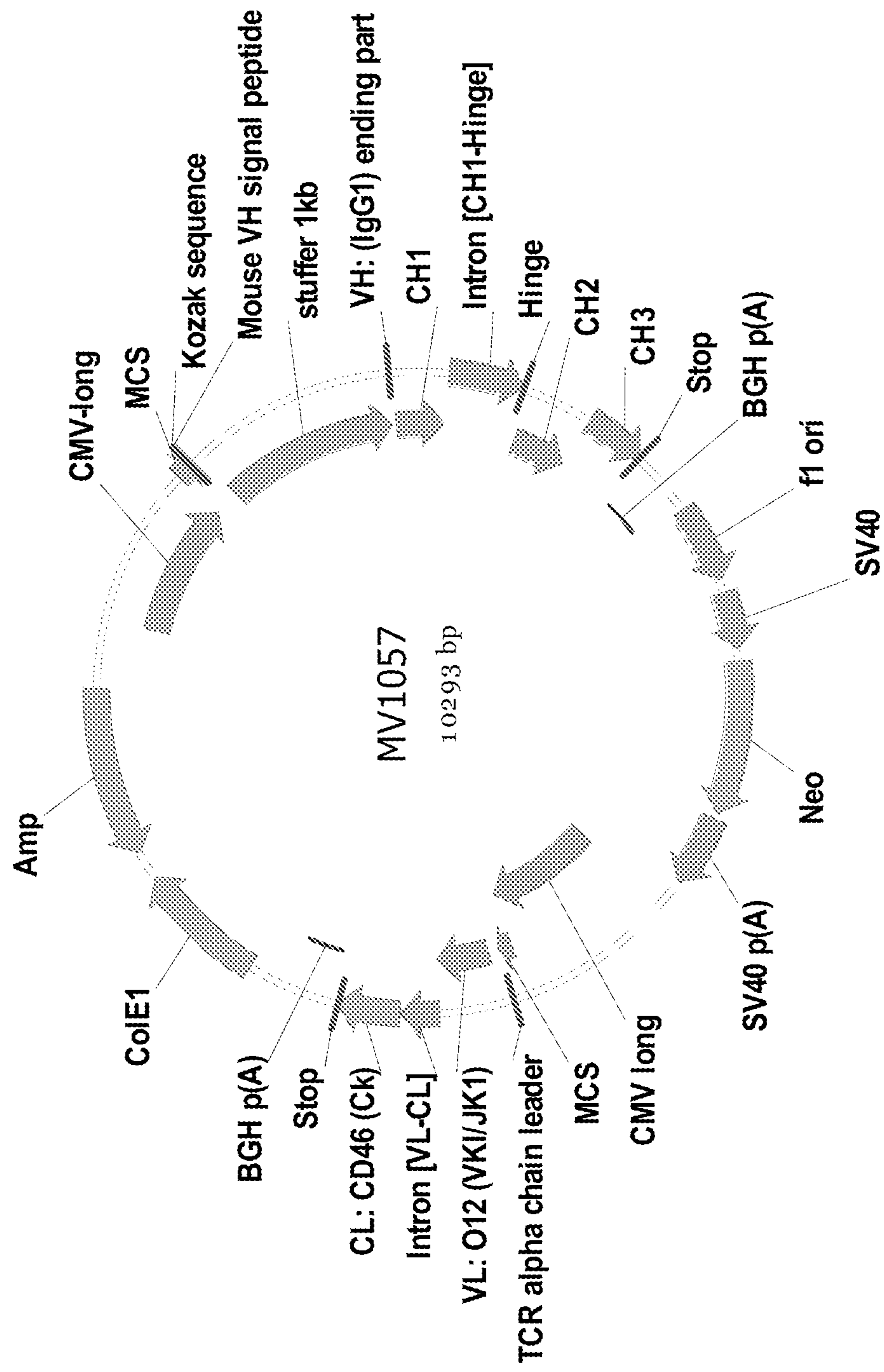


Figure 23

67/72

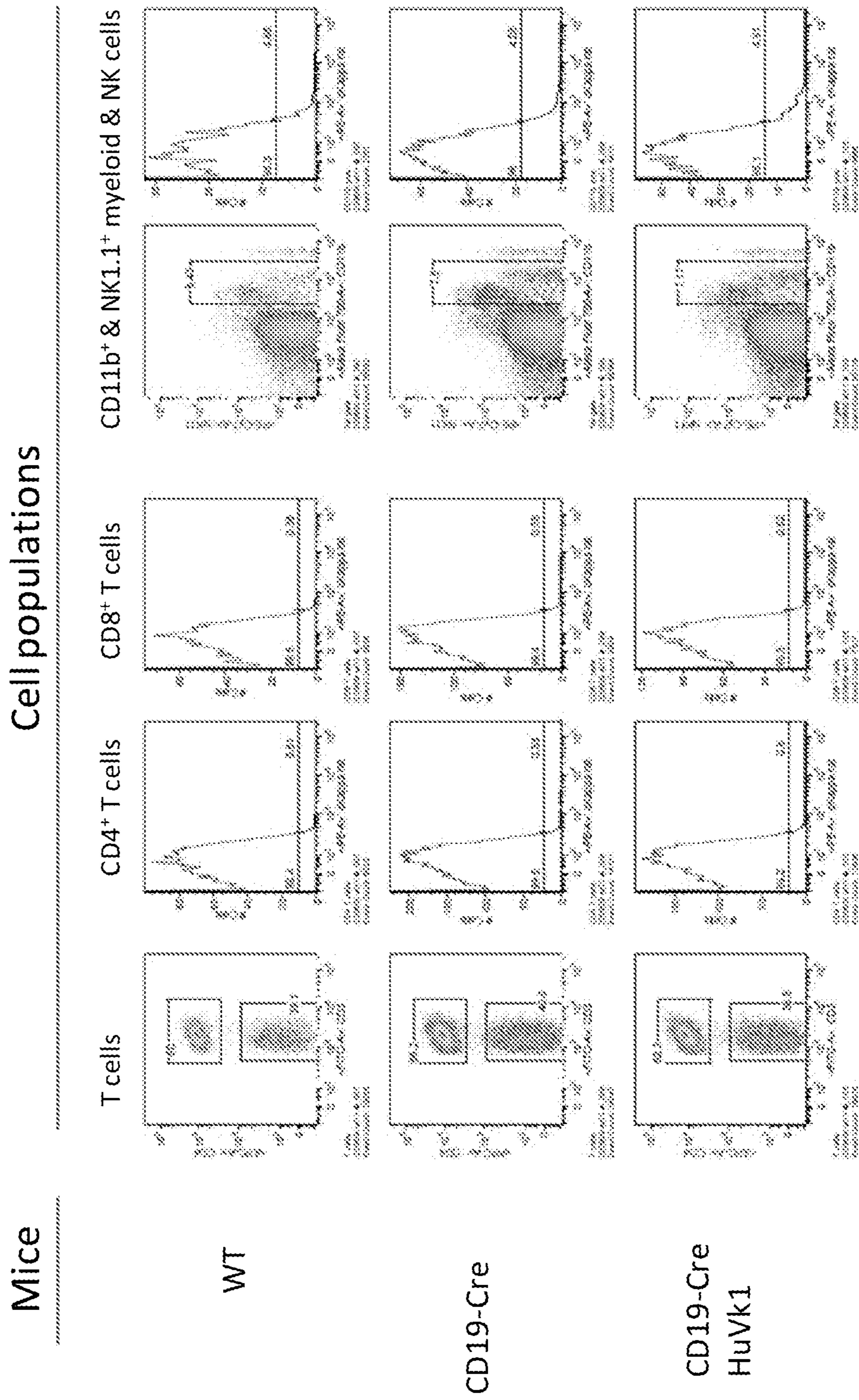


Figure 24 68/72

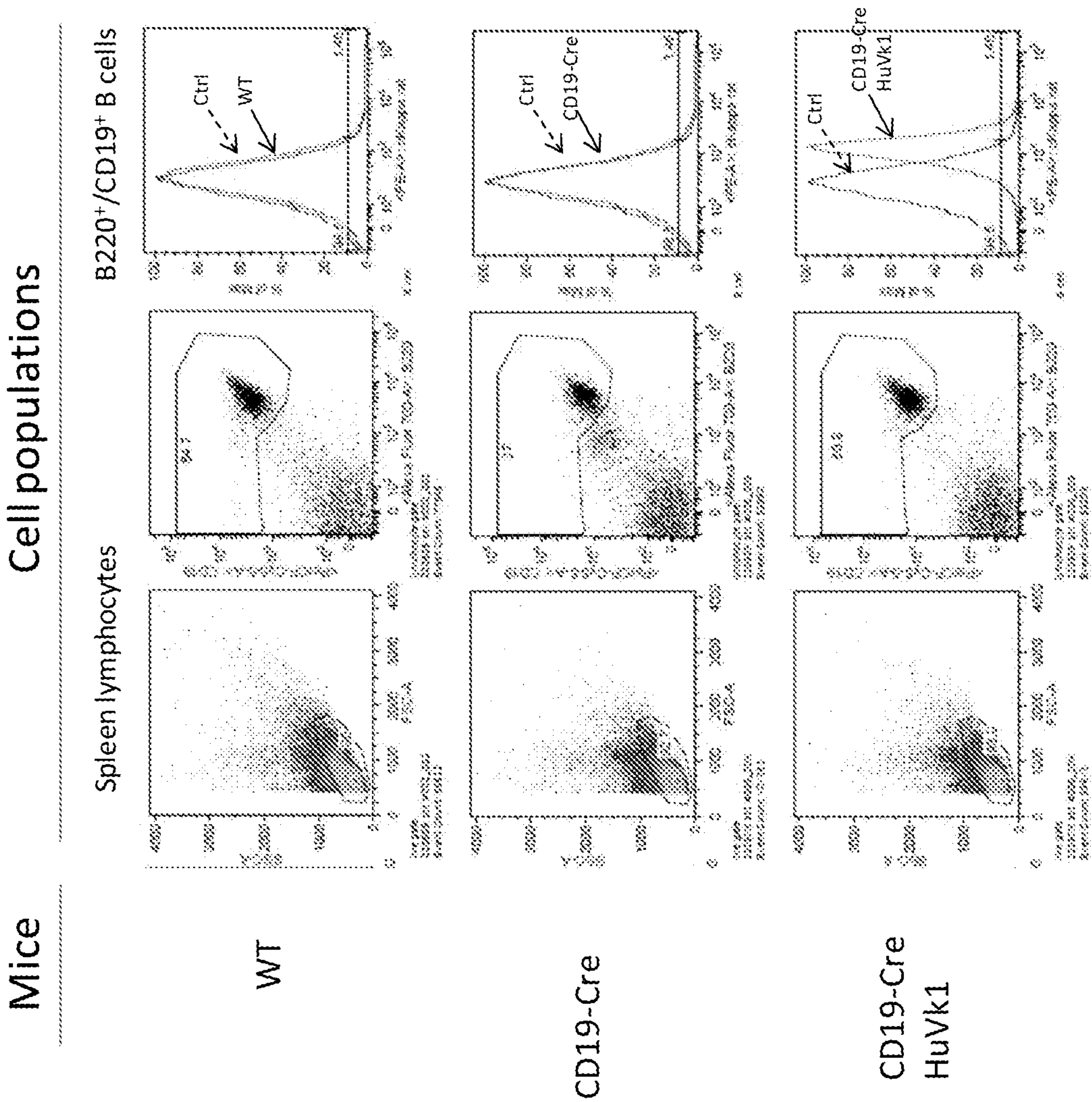


Figure 25

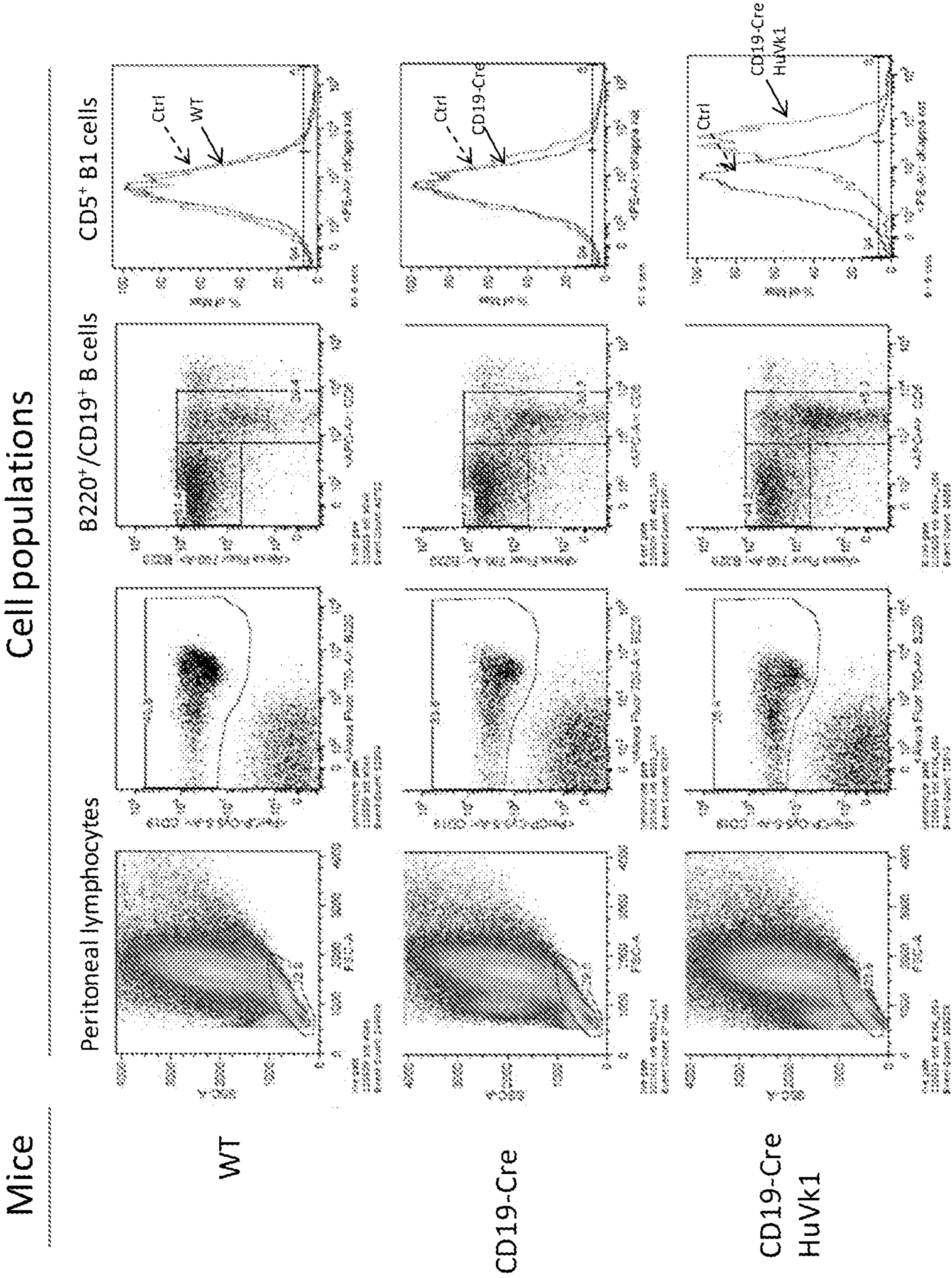


Figure 26

70/72

A

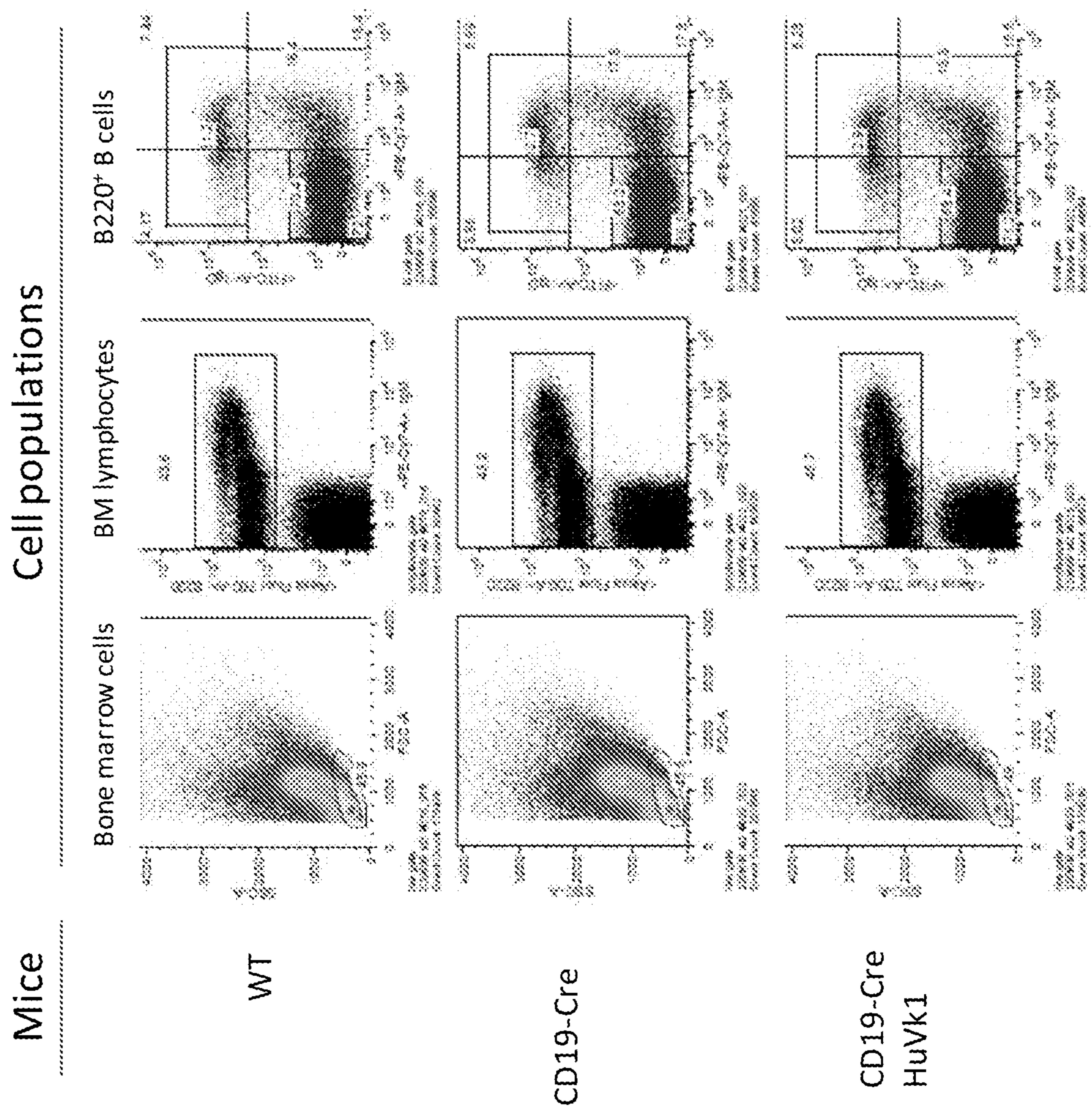


Figure 26 CONTD 71/72

B

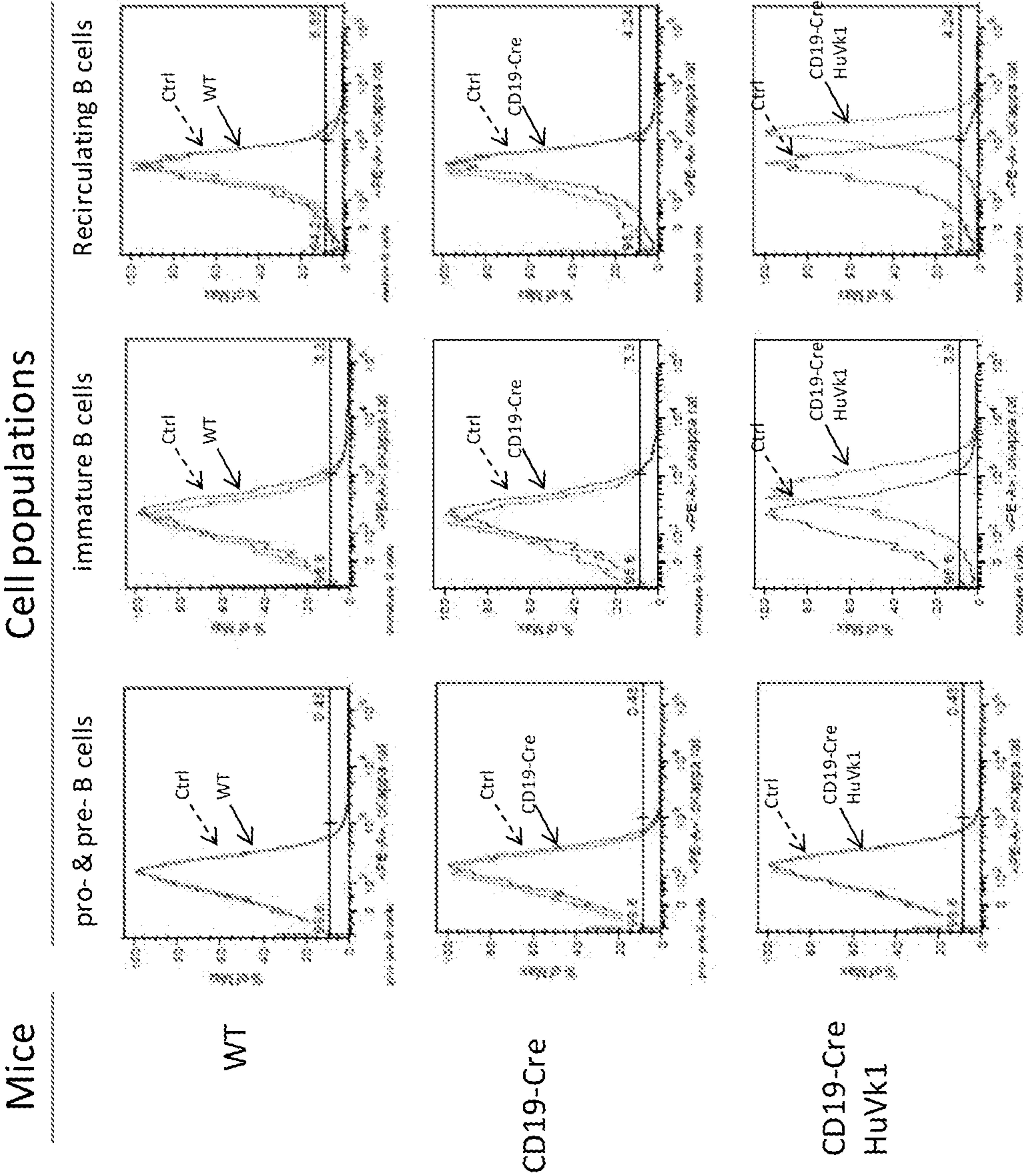


Figure 27

72/72

