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(56) Fremdragne publikationer:
WO-A1-2011/004192
WO-A1-2011/163311
WO-A1-2012/141798
US-A1- 2006 015 957
US-A1- 2012 167 237
CECIL HAN ET AL: "Comprehensive analysis of reproductive ADAMs: relationship of ADAM4 and ADAM6 with an ADAM complex required for fertilization in mice", BIOLOGY OF REPRODUCTION, NEW YORK, NY [U.A.] : ACADEM. PRESS, US, vol. 80, no. 5, 1 May 2009 (2009-05-01), pages 1001-1008, XP002677427, ISSN: 0006-3363, DOI: 10.1095/BIOLREPROD.108.073700 [retrieved on 2009-01-07]
KAREN FEATHERSTONE ET AL: "The Mouse Immunoglobulin Heavy Chain V-D Intergenic Sequence Contains Insulators That May Regulate Ordered V(D)J Recombination", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 285, no. 13, 26 March 2010 (2010-03-26), pages 9327-9338, XP002677428, ISSN: 0021-9258, DOI: 10.1074/JBC.M109.098251 [retrieved on 2010-01-25]
LONBERG NILS: "Human antibodies from transgenic animals", NATURE BIOTECHNOLOGY, NATURE

Fortsættes ...

PUBLISHING GROUP, NEW YORK, NY, US, vol. 23, no. 9, 1 September 2005 (2005-09-01), pages 1117-1125, XP002442149, ISSN: 1087-0156, DOI: 10.1038/NBT1135 cited in the application

DESCRIPTION

FIELD OF INVENTION

[0001] Genetically modified fertile mice that express human immunoglobulin λ light chain variable sequences cognate with human heavy chain variable sequences are described. Genetically modified mice, cells, embryos, and tissues that comprise a nucleic acid sequence encoding an ADAM6a functional in a mouse ADAM6 locus are described, wherein the mice, cells, embryos, and tissues comprise human immunoglobulin lambda light chain gene segments that are capable of rearranging to form a functional immunoglobulin light chain variable domain. Modifications include human and/or humanized immunoglobulin loci. Mice that comprise ADAM6 function are described, including mice that comprise an ectopic nucleic acid sequence that encodes an ADAM6 protein. Genetically modified male mice that comprise a genetic modification of an endogenous mouse immunoglobulin V_H region locus, and that further comprise ADAM6 activity are described, including mice that comprise an ectopic nucleic acid sequence that restores fertility to the male mouse.

[0002] Genetically modified fertile mice are described that comprise a deletion or a modification of an endogenous ADAM6 gene or homolog or ortholog thereof, and that comprise a genetic modification that restores ADAM6 (or homolog or ortholog thereof) function in whole or in part, wherein the mice express a human immunoglobulin λ variable sequence in the context of a κ light chain constant sequence.

BACKGROUND

[0003] Pharmaceutical applications for antibodies in the last two decades has fueled a great deal of research into making antibodies that are suitable for use as human therapeutics. Early antibody therapeutics, based on mouse antibodies, were not ideal as human therapeutics because repeatedly administering mouse antibodies to humans results in immunogenicity problems that can confound long-term treatment regimens. Solutions based on humanizing mouse antibodies to make them appear more human and less mouse-like were developed. Methods for expressing human immunoglobulin sequences for use in antibodies followed, mostly based on *in vitro* expression of human immunoglobulin libraries in phage, bacteria, or yeast. Finally, attempts were made to make useful human antibodies from human lymphoctyes *in vitro,* in mice engrafted with human hematopoietic cells, and in transchromosomal or transgenic mice with disabled endogenous immunoglobulin loci. In the transgenic mice, it was necessary to disable the endogenous mouse immunoglobulin genes so that the randomly integrated fully human transgenes would function as the source of immunoglobulin sequences expressed in the mouse. Such mice can make human antibodies suitable for use as human therapeutics, but these mice display substantial problems with their immune systems. These problems (1) make the mice impractical for generating a sufficiently diverse antibody

repertoire, (2) require the use of extensive re-engineering fixes, (3) provide a suboptimal clonal selection process likely due to incompatibility between human and mouse elements, and (4) render these mice an unreliable source of large and diverse populations of human variable sequences needed to be truly useful for making human therapeutics.

[0004] Transgenic mice that contain fully human antibody transgenes contain randomly inserted transgenes that contain unrearranged human immunoglobulin heavy chain variable sequences (V, D, and J sequences) linked to human heavy chain constant sequences, and unrearranged human immunoglobulin light chain variable sequences (V and J) linked to human light chain constant sequences. The mice therefore generate rearranged antibody genes from loci other than endogenous mouse loci, where the rearranged antibody genes are fully human. In general, the mice contain human heavy chain sequences and human κ light chain sequences, although mice with at least some human λ sequences have also been reported. The transgenic mice generally have damaged and nonfunctional endogenous immunoglobulin loci, or knockouts of endogenous immunoglobulin loci, so that the mice are incapable of rearranging human antibody sequences at an endogenous mouse immunoglobulin locus. The vagaries of such transgenic mice render them less than optimal for generating a sufficiently diverse human antibody repertoire in mice, likely due at least in part to a suboptimal clonal selection process that interfaces fully human antibody molecules within an endogenous mouse selection system.

[0005] There remains a need in the art for making improved genetically modified mice that are useful in generating immunoglobulin sequences, including human antibody sequences, and that are useful in generating a sufficiently diverse human antibody repertoire. There also remains a need for mice that are capable of rearranging immunoglobulin gene segments to form useful rearranged immunoglobulin genes, including human heavy chain variable domains that are cognate with human λ or human κ variable domains, or that are capable of making proteins from altered immunoglobulin loci, including loci that contain a sufficiently diverse selection of human λ and/or human κ light chain variable sequences. There is a need for mice that can generate antibody variable regions from both human κ and human λ segments, wherein the human κ and human λ segments are cognate with human heavy chain variable domains. There is also a need for increased usage in genetically modified mice of human λ sequences.

SUMMARY OF INVENTION

[0006] Genetically modified mice are described that comprise a modification that eliminates activity of an endogenous ADAM6 gene, wherein the modification results in a loss of fertility, and the mice further comprise a sequence that encodes an activity that complements or rescues the lost or reduced ADAM6 activity (or homolog or ortholog activity), and the mice further comprise modifications that enable them to express human immunoglobulin heavy chain variable regions that are cognate with human immunoglobulin λ light chain variable regions. Hence, a mouse is provided comprising (a) one or more unrearranged human Vλ

gene segments and one or more unrearranged human Jλ gene segments at an endogenous immunoglobulin kappa light chain locus of the mouse, wherein the immunoglobulin kappa light chain locus comprises a mouse Cκ region: (b) one or more human V_H gene segments, one or more human D_H gene segments and one or more human J_H gene segments at an endogenous immunoglobulin heavy chain locus of the mouse; and (c) a modification of an immunoglobulin heavy chain locus, wherein the modification eliminates endogenous ADAM6 function, which is associated with a reduction in fertility in male mice, the mouse further comprising a nucleic acid sequence encoding a mouse ADAM6a protein or an ortholog or homolog or functional fragment thereof, each of which is functional to improve or restore said reduction in fertility in a male mouse, and a nucleic acid sequence encoding a mouse ADAM6b protein, or an ortholog or homolog or functional fragment thereof, each of which is functional to improve or restore said reduction in fertility in a male mouse, wherein the nucleic acid sequences encoding the ADAM6 proteins, orthologs, homologs, or functional fragments thereof is present within the human heavy chain gene segments. The human immunoglobulin λ light chain variable regions are expressed fused to mouse κ constant regions.

[0007] In various aspects, the sequence that encodes ADAM6 activity is contiguous with a human immunoglobulin sequence. In various aspects, the sequence that encodes ADAM6 activity is contiguous with a non-human immunoglobulin sequence. The sequence is present on the same chromosome as the endogenous non-human immunoglobulin heavy chain locus of the mouse, present within the human heavy chain gene segments.

[0008] Genetically modified mice are described that comprise a modification that maintains activity of an ADAM6 gene or homolog or ortholog thereof, wherein the modification includes insertion of one or more human immunoglobulin heavy chain gene segments upstream of a non-human immunoglobulin heavy chain constant region, and the mice further comprise modifications that enable them to express human immunoglobulin λ light chain variable regions cognate with human immunoglobulin heavy chain variable regions. The human immunoglobulin λ light chain variable regions are expressed fused to mouse κ constant regions.

[0009] Exemplary disruptions, deletions and/or functionally silencing modifications include any modifications that result in an elimination of activity of the ADAM6 protein(s) encoded by the ADAM6 gene(s) of the mouse.

[0010] Also disclosed are nucleic acid constructs, cells, embryos, mice, and methods for making mice that comprise a modification that results in a nonfunctional endogenous mouse ADAM6 protein or ADAM6 gene (*e.g.,* a knockout of or a deletion in an endogenous ADAM6 gene), wherein the mice comprise a nucleic acid sequence that encodes an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse.

[0011] Also disclosed are nucleic acid constructs, cells, embryos, mice, and methods for making mice that comprise a modification of an endogenous mouse immunoglobulin locus, wherein the mice comprise an ADAM6 protein or ortholog or homolog or fragment thereof that

is functional in a male mouse. The endogenous mouse immunoglobulin locus is an immunoglobulin heavy chain locus, and the modification eliminates ADAM6 activity of a cell or tissue of a male mouse.

[0012] Also disclosed are mice that comprise an ectopic nucleotide sequence encoding a mouse ADAM6 or ortholog or homolog or functional fragment thereof; mice are also disclosed that comprise an endogenous nucleotide sequence encoding a mouse ADAM6 or ortholog or homolog or fragment thereof, and at least one genetic modification of a heavy chain immunoglobulin locus.

[0013] Also disclosed are methods making mice that comprise a modification of an endogenous mouse immunoglobulin locus, wherein the mice comprise an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse.

[0014] Also disclosed are methods for making mice that comprise a genetic modification of a heavy chain immunoglobulin locus, wherein application of the methods result in male mice that comprise a modified heavy chain immunoglobulin locus (or a deletion thereof), and the male mice are capable of generating offspring by mating. In one instance, the male mice are capable of producing sperm that can transit from a mouse uterus through a mouse oviduct to fertilize a mouse egg.

[0015] Also disclosed are methods for making mice that comprise a genetic modification of an immunoglobulin heavy chain locus and an immunoglobulin light chain locus, wherein application of the methods to modify the heavy chain locus result in male mice that exhibit a reduction in fertility, and the mice comprise a genetic modification that restores in whole or in part the reduction in fertility. In various embodiments, the reduction in fertility is characterized by an inability of the sperm of the male mice to migrate from a mouse uterus through a mouse oviduct to fertilize a mouse egg. In various embodiments, the reduction in fertility is characterized by sperm that exhibit an *in vivo* migration defect. In various embodiments, the genetic modification that restores in whole or in part the reduction in fertility is a nucleic acid sequence encoding a mouse ADAM6 gene or ortholog or homolog or fragment thereof that is functional in a male mouse.

[0016] In one embodiment, the genetic modification comprises replacing endogenous immunoglobulin heavy chain variable loci with human immunoglobulin heavy chain variable loci. In one embodiment, the genetic modification comprises insertion of human immunoglobulin heavy chain variable loci into endogenous immunoglobulin heavy chain variable loci. In one embodiment, the genetic modification comprises deletion of an endogenous immunoglobulin heavy chain variable locus in whole or in part, wherein the deletion results in a loss of endogenous ADAM6 function. In a specific embodiment, the loss of endogenous ADAM6 function is associated with a reduction in fertility in male mice.

[0017] Also disclosed is a genetic modification that comprises inactivation of an endogenous non-human immunoglobulin heavy chain variable locus in whole or in part, wherein the

inactivation does not result in a loss of endogenous ADAM6 function. Inactivation may include replacement or deletion of one or more endogenous mouse gene segments resulting in an endogenous mouse immunoglobulin heavy chain locus that is substantially incapable of rearrangement to encode a heavy chain of an antibody that comprises endogenous mouse gene segments. Inactivation may include other modifications that render the endogenous immunoglobulin heavy chain locus incapable of rearranging to encode the heavy chain of an antibody, wherein the modification does not include replacement or deletion of endogenous gene segments. Exemplary modifications include chromosomal inversions and/or translocations mediated by molecular techniques, *e.g.,* using precise placement of site-specific recombination sites (*e.g.,* Cre-lox technology). Other exemplary modifications include disabling the operable linkage between the mouse immunoglobulin variable gene segments and the non-human immunoglobulin constant regions.

[0018] In one embodiment, the genetic modification comprises inserting into the genome of the mouse a DNA fragment containing one or more human V_H gene segments, one or more human D_H gene segments and one or more human J_H gene segments operably linked to one or more constant region sequences (e.g., an IgM and/or an IgG gene). In one embodiment, the DNA fragment is capable of undergoing rearrangement in the genome of the mouse to form a sequence that encodes a human heavy chain variable domain of an antibody.

[0019] Disclosed are mice that comprise a modification that reduces or eliminates mouse ADAM6 expression from an endogenous ADAM6 allele such that a male mouse having the modification exhibits a reduced fertility (*e.g.,* a highly reduced ability to generate offspring by mating), or is essentially infertile, due to the reduction or elimination of endogenous ADAM6 function, wherein the mice further comprise an ectopic ADAM6 sequence or homolog or ortholog or functional fragment thereof. The modification that reduces or eliminates mouse ADAM6 expression may be a modification (*e.g.,* an insertion, a deletion, a replacement, *etc.*) in a mouse immunoglobulin locus.

[0020] Disclosed is that the reduction or loss of ADAM6 function may comprise an inability or substantial inability of the mouse to produce sperm that can travel from a mouse uterus through a mouse oviduct to fertilize a mouse egg. At least about 95%, 96%, 97%, 98%, or 99% of the sperm cells produced in an ejaculate volume of the mouse may be incapable of traversing through an oviduct in vivo following copulation and fertilizing a mouse ovum.

[0021] Disclosed is that the reduction or loss of ADAM6 function comprises an inability to form or substantial inability to form a complex of ADAM2 and/or ADAM3 and/or ADAM6 on a surface of a sperm cell of the mouse. In one embodiment, the loss of ADAM6 function comprises a substantial inability to fertilize a mouse egg by copulation with a female mouse.

[0022] Also disclosed is a mouse that lacks a functional endogenous ADAM6 gene, and comprises a protein (or an ectopic nucleotide sequence that encodes a protein) that confers ADAM6 functionality on the mouse. The mouse may be a male mouse and the functionality comprises enhanced fertility as compared with a mouse that lacks a functional endogenous

ADAM6 gene.

[0023] The protein is encoded by a genomic sequence located within an immunoglobulin locus in the germline of the mouse. The immunoglobulin locus is a heavy chain locus. The heavy chain locus comprises at least one human V_H, at least one human D_H and at least one human J_H gene segment.

[0024] In one embodiment, the mouse comprises a human or chimeric human/mouse or chimeric human/rat light chain (e.g., human variable, mouse or rat constant) and a chimeric human variable/mouse or rat constant heavy chain. In a specific embodiment, the mouse comprises a transgene that comprises a chimeric human variable/rat or mouse constant light chain gene operably linked to a transcriptionally active promoter, e.g., a ROSA26 promoter. In a further specific embodiment, the chimeric human/mouse or rat light chain transgene comprises a rearranged human light chain variable region sequence in the germline of the mouse.

[0025] The ectopic nucleotide sequence is located within an immunoglobulin locus in the germline of the mouse. The immunoglobulin locus is a heavy chain locus. The heavy chain locus comprises at least one human V_H, at least one human D_H and at least one human J_H gene segment.

[0026] Also disclosed is a mouse that lacks a functional endogenous ADAM6 gene, wherein the mouse comprises an ectopic nucleotide sequence that complements the loss of mouse ADAM6 function. The ectopic nucleotide sequence may confer upon the mouse an ability to produce offspring that is comparable to a corresponding wild-type mouse that contains a functional endogenous ADAM6 gene. The sequence may confer upon the mouse an ability to form a complex of ADAM2 and/or ADAM3 and/or ADAM6 on the surface of sperm cell of the mouse. The sequence may confer upon the mouse an ability to travel from a mouse uterus through a mouse oviduct to a mouse ovum to fertilize the ovum.

[0027] [Deleted]

[0028] [Deleted]

[0029] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence produces an average of at least about 2-fold, 3-fold, or 4-fold higher number of pups per litter in a 4- or 6-month breeding period than a mouse that lacks the functional endogenous ADAM6 gene and that lacks the ectopic nucleotide sequence, and that is bred for the same period of time.

[0030] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence is a male mouse, and the male mouse produces sperm that when recovered from oviducts at about 5-6 hours post-copulation reflects an oviduct migration that is at least 10-fold, at least 20-fold, at least 30-fold, at least 40-fold, at

least 50-fold, at least 60-fold, at least 70-fold, at least 80-fold, at least 90-fold, 100-fold, 110-fold, or 120-fold or higher than a mouse that lacks the functional endogenous ADAM6 gene and that lacks the ectopic nucleotide sequence.

[0031] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence when copulated with a female mouse generates sperm that is capable of traversing the uterus and entering and traversing the oviduct within about 6 hours at an efficiency that is about equal to sperm from a wild-type mouse.

[0032] In one embodiment, the mouse lacking the functional endogenous ADAM6 gene and comprising the ectopic nucleotide sequence produces about 1.5-fold, about 2-fold, about 3-fold, or about 4-fold or more litters in a comparable period of time than a mouse that lacks the functional ADAM6 gene and that lacks the ectopic nucleotide sequence.

[0033] In one aspect, the mouse provided comprises, in its germline, a non-mouse nucleic acid sequence that encodes an immunoglobulin protein is provided, wherein the non-mouse immunoglobulin sequence comprises an insertion of a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. The non-mouse immunoglobulin sequence comprises a human immunoglobulin sequence. The sequence comprises one or more human V gene segments, one or more human D gene segments, and one or more human J gene segments. In one embodiment, the one or more V, D, and J gene segments are unrearranged. In one embodiment, the one or more V, D, and J gene segments are rearranged. In one embodiment, following rearrangement of the one or more V, D, and J gene segments the mouse comprises in its genome at least one nucleic acid sequence encoding a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. In one embodiment, following rearrangement the mouse comprises in its genome at least two nucleic acid sequences encoding a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. In one embodiment, following rearrangement the mouse comprises in its genome at least one nucleic acid sequence encoding a mouse ADAM6 gene or homolog or ortholog or functional fragment thereof. In one embodiment, the mouse comprises the ADAM6 gene or homolog or ortholog or functional fragment thereof in a B cell. In one embodiment, the mouse comprises the ADAM6 gene or homolog or ortholog or functional fragment thereof in a non-B cell.

[0034] In one aspect, the mice provided express a human immunoglobulin heavy chain variable region or functional fragment thereof from an endogenous mouse immunoglobulin heavy chain locus, wherein the mice comprise an ADAM6 activity that is functional in a male mouse.

[0035] [Deleted]

[0036] Also disclosed are male mice that comprise an ectopic mouse ADAM6 sequence or homolog or ortholog or functional fragment thereof that encodes a protein that confers ADAM6 function.

[0037] In one embodiment, the male mice comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof at a location in the mouse genome that approximates the location of the endogenous mouse ADAM6 allele, *e.g.,* 3' of a V gene segment sequence and 5' of an initial D gene segment. In the embodiments described herein reference to "ADAM6" means "ADAM6 and ADAM6b".

[0038] Disclosed are male mice which comprise an ADAM6 sequence or homolog or ortholog or functional fragment thereof flanked upstream, downstream, or upstream and downstream (with respect to the direction of transcription of the ADAM6 sequence) of a nucleic acid sequence encoding an immunoglobulin variable gene segment. In a specific embodiment, the immunoglobulin variable gene segment is a human gene segment. In one embodiment, the immunoglobulin variable gene segment is a human gene segment, and the sequence encoding the mouse ADAM6 or ortholog or homolog or fragment thereof functional in a mouse is between human V gene segments; in one embodiment, the mouse comprises two or more human V gene segments, and the sequence is at a position between the final V gene segment and the penultimate V gene segment; in one embodiment, the sequence is at a position following the final V gene segment and the first D gene segment.

[0039] In one embodiment, the male mice comprise an ADAM6 homolog or ortholog or functional fragment thereof that is located at a position in an endogenous immunoglobulin locus that is the same or substantially the same as in a wild type male mouse. In a specific embodiment, the endogenous locus is incapable of encoding the heavy chain variable region of an antibody, wherein the variable region comprises or is derived from an endogenous mouse gene segment. In a specific embodiment, the endogenous locus is positioned at a location in the genome of the male mouse that renders it incapable of encoding the heavy chain variable region of an antibody. The male mice comprise an ADAM6 sequence located on the same chromosome as human immunoglobulin gene segments and the ADAM6 sequence encodes a functional ADAM6 protein.

[0040] Also disclosed is a male mouse that comprises a nonfunctional endogenous ADAM6 gene, or a deletion of an endogenous ADAM6 gene, in its germline; wherein sperm cells of the mouse are capable of transiting an oviduct of a female mouse and fertilizing an egg.

[0041] Also disclosed is a male mouse that comprises a functional endogenous ADAM6 gene and a modification to an endogenous immunoglobulin heavy chain locus. The modification may be made downstream, or 3', of the endogenous ADAM6 gene. The modification may be a replacement of one or more endogenous immunoglobulin heavy chain gene segments with one or more human immunoglobulin heavy chain gene segments. The modification may be an insertion of one or more human immunoglobulin heavy chain gene segments upstream of an endogenous immunoglobulin heavy chain constant region gene.

[0042] Also disclosed are mice that comprise a genetic modification that reduces endogenous mouse ADAM6 function, wherein the mouse comprises at least some ADAM6 functionality provided either by an endogenous unmodified allele that is functional in whole or in part (e.g., a

heterozygote), or by expression from an ectopic sequence that encodes an ADAM6 or an ortholog or homolog or functional fragment thereof that is functional in a male mouse.

[0043] In one embodiment, the mice comprise ADAM6 function sufficient to confer upon male mice the ability to generate offspring by mating, as compared with male mice that lack a functional ADAM6. ADAM6 homologs or orthologs or fragments thereof that are functional in a male mouse include those that restore, in whole or in part, the loss of ability to generate offspring observed in a male mouse that lacks sufficient endogenous mouse ADAM6 activity, *e.g.,* the loss in ability observed in an ADAM6 knockout mouse. In this sense ADAM6 knockout mice include mice that comprise an endogenous locus or fragment thereof, but that is not functional, *i.e.,* that does not express ADAM6 (ADAM6a and/or ADAM6b) at all, or that expresses ADAM6 (ADAM6a and/or ADAM6b) at a level that is insufficient to support an essentially normal ability to generate offspring of a wild-type male mouse. The loss of function can be due, *e.g.,* to a modification in a structural gene of the locus (*i.e.,* in an ADAM6a or ADAM6b coding region) or in a regulatory region of the locus (*e.g.,* in a sequence 5' to the ADAM6a gene, or 3' of the ADAM6a or ADAM6b coding region, wherein the sequence controls, in whole or in part, transcription of an ADAM6 gene, expression of an ADAM6 RNA, or expression of an ADAM6 protein). In various embodiments, orthologs or homologs or fragments thereof that are functional in a male mouse are those that enable a sperm of a male mouse (or majority of sperm cells in the ejaculate of a male mouse) to transit a mouse oviduct and fertilize a mouse ovum.

[0044] In one embodiment, male mice that express the human immunoglobulin variable region or functional fragment thereof comprise sufficient ADAM6 activity to confer upon the male mice the ability to generate offspring by mating with female mice and, in one embodiment, the male mice exhibit an ability to generate offspring when mating with female mice that is in one embodiment at least 25%, in one embodiment, at least 30%, in one embodiment at least 40%, in one embodiment at least 50%, in one embodiment at least 60%, in one embodiment at least 70%, in one embodiment at least 80%, in one embodiment at least 90%, and in one embodiment about the same as, that of mice with one or two endogenous unmodified ADAM6 alleles.

[0045] In one embodiment male mice express sufficient ADAM6 (or an ortholog or homolog or functional fragment thereof) to enable a sperm cell from the male mice to traverse a female mouse oviduct and fertilize a mouse egg.

[0046] [Deleted]

[0047] Disclosed are genetically modified mice and cells that comprise a modification of an endogenous immunoglobulin heavy chain locus, wherein the mice express at least a portion of an immunoglobulin heavy chain sequence, *e.g.,* at least a portion of a human sequence, wherein the mice comprise an ADAM6 activity that is functional in a male mouse. The modification may reduce or eradicate an ADAM6 activity of the mouse. The mouse may be modified such that both alleles that encode ADAM6 activity are either absent or express an

ADAM6 that does not substantially function to support normal mating in a male mouse. The mouse may further comprise an ectopic nucleic acid sequence encoding a mouse ADAM6 or ortholog or homolog or functional fragment thereof. The modification may maintain ADAM6 activity of the mouse and renders an endogenous immunoglobulin heavy chain locus incapable of encoding a heavy chain variable region of an antibody. The modification may include chromosomal inversions and or translocations that render the endogenous immunoglobulin heavy chain variable gene segments incapable of rearranging to encode a heavy chain variable region of antibody that is operably linked to a heavy chain constant region.

[0048] Disclosed are modified mice and cells that comprise a modification of an endogenous immunoglobulin heavy chain locus, wherein the modification reduces or eliminates ADAM6 activity expressed from an ADAM6 sequence of the locus, and wherein the mice comprise an ADAM6 protein or ortholog or homolog or functional fragment thereof. The ADAM6 protein or fragment thereof may be encoded by an ectopic ADAM6 sequence. The ADAM6 protein or fragment thereof may be expressed from an endogenous ADAM6 allele. The mouse may comprise a first immunoglobulin heavy chain allele comprises a first modification that reduces or eliminates expression of a functional ADAM6 from the first immunoglobulin heavy chain allele, and the mouse may comprise a second immunoglobulin heavy chain allele that comprises a second modification that does not substantially reduce or does not eliminate expression of a functional ADAM6 from the second immunoglobulin heavy chain allele.

[0049] The modification may be the insertion of one or more human immunoglobulin heavy chain gene segments upstream, or 5', of an endogenous immunoglobulin heavy chain constant region gene. The modification may maintain the endogenous ADAM6 gene located at the endogenous immunoglobulin heavy chain locus.

[0050] The second modification may be located 3' (with respect to the transcriptional directionality of the mouse V gene segment) of a final mouse V gene segment and located 5' (with respect to the transcriptional directionality of the constant sequence) of a mouse (or chimeric human/mouse) immunoglobulin heavy chain constant gene or fragment thereof (*e.g.,* a nucleic acid sequence encoding a human and/or mouse: C_H1 and/or hinge and/or C_H2 and/or C_H3).

[0051] The modification may be at a first immunoglobulin heavy chain allele at a first locus that encodes a first ADAM6 allele, and the ADAM6 function results from expression of an endogenous ADAM6 at a second immunoglobulin heavy chain allele at a second locus that encodes a functional ADAM6, wherein the second immunoglobulin heavy chain allele comprises at least one modification of a V, D, and/or J gene segment. The at least one modification of the V, D, and or J gene segment may be a deletion, a replacement with a human V, D, and/or J gene segment, a replacement with a camelid V, D, and/or J gene segment, a replacement with a humanized or camelized V, D, and/or J gene segment, a replacement of a heavy chain sequence with a light chain sequence, and a combination thereof. The at least one modification may be the deletion of one or more heavy chain V, D, and/or J gene segments and a replacement with one or more light chain V and/or J gene

segments (*e.g.,* a human light chain V and/or J gene segment) at the heavy chain locus.

[0052] [Deleted]

[0053] [Deleted]

[0054] In one embodiment, the modification is at a first immunoglobulin heavy chain allele at a first locus and a second immunoglobulin heavy chain allele at a second locus, and the ADAM6 function results from expression of an ectopic ADAM6 sequence at the first immunoglobulin heavy chain allele. In one embodiment, the modification is at a first immunoglobulin heavy chain allele at a first locus and a second immunoglobulin heavy chain allele at a second locus, and the ADAM6 function or activity results from expression of an ectopic ADAM6 at the second immunoglobulin heavy chain allele.

[0055] Disclosed is a mouse comprising a heterozygous or a homozygous knockout of ADAM6 The mouse may further comprise a modified immunoglobulin sequence that is a human or a humanized immunoglobulin sequence, or a camelid or camelized human or mouse immunoglobulin sequence. The modified immunoglobulin sequence may be present at the endogenous heavy chain immunoglobulin locus. The modified immunoglobulin sequence may comprise a human heavy chain variable gene sequence at an endogenous heavy chain immunoglobulin locus. The human heavy chain variable gene sequence may replace an endogenous heavy chain variable sequence at the endogenous immunoglobulin heavy chain locus.

[0056] Also disclosed is a mouse incapable of expressing a functional endogenous mouse ADAM6 from an endogenous mouse ADAM6 locus. The mouse may comprise an ectopic nucleic acid sequence that encodes an ADAM6, or functional fragment thereof, that is functional in the mouse. The ectopic nucleic acid sequence may encode a protein that rescues a loss in the ability to generate offspring exhibited by a male mouse that is homozygous for an ADAM6 knockout. The ectopic nucleic acid sequence may encode a mouse ADAM6 protein.

[0057] Described is a mouse that lacks a functional endogenous ADAM6 locus, and that comprises an ectopic nucleic acid sequence that confers upon the mouse ADAM6 function. The nucleic acid sequence may comprise an endogenous mouse ADAM6 sequence or functional fragment thereof. The endogenous mouse ADAM6 sequence comprises ADAM6a- and ADAM6b-encoding sequence located in a wild-type mouse between the 3'-most mouse immunoglobulin heavy chain V gene segment (V_H) and the 5'-most mouse immunoglobulin heavy chain D gene segment (D_H).

[0058] Described is a nucleic acid sequence encoding mouse ADAM6a or functional fragment thereof and/or a sequence encoding mouse ADAM6b or functional fragment thereof, wherein the ADAM6a and/or ADAM6b or functional fragment(s) thereof is operably linked to a promoter. The promoter may be a human promoter. The promoter may be the mouse ADAM6 promoter. The ADAM6 promoter comprises sequence may be located between the first codon

of the first ADAM6 gene closest to the mouse 5'-most D_H gene segment and the recombination signal sequence of the 5'-most D_H gene segment, wherein 5' is indicated with respect to direction of transcription of the mouse immunoglobulin genes. The promoter may be a viral promoter. The viral promoter may be a cytomegalovirus (CMV) promoter. The promoter may be a ubiquitin promoter.

[0059] The promoter may be an inducible promoter. The inducible promoter may regulate expression in non-reproductive tissues. The inducible promoter may regulate expression in reproductive tissues. The expression of the mouse ADAM6a and/or ADAM6b sequences or functional fragment(s) thereof may be developmentally regulated by the inducible promoter in reproductive tissues.

[0060] In one embodiment, the mouse ADAM6a and/or ADAM6b are selected from the ADAM6a of SEQ ID NO:1 and/or ADAM6b of sequence SEQ ID NO:2. In one embodiment, the mouse ADAM6 promoter is a promoter of SEQ ID NO:3. In a specific embodiment, the mouse ADAM6 promoter comprises the nucleic acid sequence of SEQ ID NO:3 directly upstream (with respect to the direction of transcription of ADAM6a) of the first codon of ADAM6a and extending to the end of SEQ ID NO:3 upstream of the ADAM6 coding region. In another specific embodiment, the ADAM6 promoter is a fragment extending from within about 5 to about 20 nucleotides upstream of the start codon of ADAM6a to about 0.5kb, 1kb, 2kb, or 3kb or more upstream of the start codon of ADAM6a.

[0061] In one embodiment, the nucleic acid sequence comprises SEQ ID NO:3 or a fragment thereof that when placed into a mouse that is infertile or that has low fertility due to a lack of ADAM6, improves fertility or restores fertility to about a wild-type fertility. In one embodiment, SEQ ID NO:3 or a fragment thereof confers upon a male mouse the ability to produce a sperm cell that is capable of traversing a female mouse oviduct in order to fertilize a mouse egg.

[0062] In one embodiment, the nucleic acid sequence is any sequence encoding an ADAM6 gene or homolog or ortholog or functional fragment thereof that when placed into or maintained in a mouse yields a level of fertility that is the same or comparable to a wild-type mouse. An exemplary level of fertility may be demonstrated by the ability of a male mouse to produce a sperm cell that is capable of traversing a female mouse oviduct in order to fertilize a mouse egg.

[0063] Also disclosed is a mouse that comprises a deletion of an endogenous nucleotide sequence that encodes an ADAM6 protein, a replacement of an endogenous mouse V_H gene segment with a human V_H gene segment, and an ectopic nucleotide sequence that encodes a mouse ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse.

[0064] In one instance, the mouse comprises an immunoglobulin heavy chain locus that comprises a deletion of an endogenous immunoglobulin locus nucleotide sequence that

comprises an endogenous ADAM6 gene, comprises a nucleotide sequence encoding one or more human immunoglobulin gene segments, and wherein the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within or directly adjacent to the nucleotide sequence encoding the one or more human immunoglobulin gene segments.

[0065] In one embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H gene segments with a nucleotide sequence encoding one or more human V_H gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within the nucleotide sequence encoding the one or more human V_H gene segments. In one embodiment, the mouse further comprises a replacement of one or more endogenous D_H gene segments with one or more human D_H gene segments at the endogenous D_H gene locus. In one embodiment, the mouse further comprises a replacement of one or more endogenous J_H gene segments with one or more human J_H gene segments at the endogenous J_H gene locus. In one embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H, D_H, and J_H gene segments and a replacement at the endogenous V_H, D_H, and J_H gene loci with human V_H, D_H, and J_H gene segments, wherein the mouse comprises an ectopic sequence encoding a mouse ADAM6 protein. In one embodiment, the mouse comprises an insertion of human V_H, D_H and J_H gene segments at an endogenous immunoglobulin heavy chain locus, wherein the mouse comprises an ADAM6 gene that is functional in the mouse. In a specific embodiment, the ectopic sequence encoding the mouse ADAM6 protein is placed between the penultimate 3'-most V_H gene segment of the human V_H gene segments present, and the ultimate 3' V_H gene segment of the human V_H gene segments present. In a specific embodiment, the mouse comprises a deletion of all or substantially all mouse V_H gene segments, and a replacement with all or substantially all human V_H gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is placed downstream of human gene segment V_H1-2 and upstream of human gene segment V_H6-1.

[0066] In a specific embodiment, the mouse comprises a replacement of all or substantially all endogenous V_H gene segments with a nucleotide sequence encoding one or more human V_H gene segments, and the ectopic nucleotide sequence encoding the mouse ADAM6 protein is within the nucleotide sequence encoding the one or more human V_H gene segments.

[0067] [Deleted]

[0068] Described is a mouse that comprises a modification of an endogenous immunoglobulin heavy chain locus, wherein the mouse expresses a B cell that comprises a rearranged immunoglobulin sequence operably linked to a heavy chain constant region gene sequence, and the B cell comprises in its genome (*e.g.,* on a B cell chromosome) a gene encoding an ADAM6 or ortholog or homolog or fragment thereof that is functional in a male mouse. The rearranged immunoglobulin sequence may be operably linked to the heavy chain constant region gene sequence comprises a human heavy chain V, D, and/or J sequence; a mouse

heavy chain V, D, and/or J sequence; a human or mouse light chain V and/or J sequence. In one embodiment, the heavy chain constant region gene sequence comprises a human or a mouse heavy chain sequence selected from the group consisting of a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

[0069] In one aspect, the mouse provided comprises a functionally silenced endogenous immunoglobulin heavy chain variable gene locus, wherein ADAM6 function is maintained in the mouse, and further comprises an insertion of one or more human immunoglobulin gene segments upstream or 5' of one or more mouse heavy chain constant region. The one or more human immunoglobulin gene segments include one or more human V_H gene segments, one or more human D_H gene segments and one or more human J_H gene segments. In a specific embodiment, the mouse further comprises a functionally silenced endogenous light chain locus, wherein the mouse comprises an ADAM6 activity that is the same or comparable to a wild-type mouse, and further comprises an insertion of one or more human λ light chain gene segments upstream or 5' of a mouse light chain constant region. In one embodiment, the human λ light chain gene segments comprise 12 human Vλ gene segments and one or more human Jλ gene segments. In one embodiment, the human λ light chain gene segments comprise 12 human Vλ gene segments and four human Jλ gene segments. In one embodiment, the human λ light chain gene segments comprise 28 human Vλ gene segments and one or more human Jλ gene segments. In one embodiment, the human λ light chain gene segments comprises 28 human Vλ gene segments and four human Jλ gene segments. In one embodiment, the human λ light chain gene segments comprises 40 human Vλ gene segments and one or more human Jλ gene segments. In one embodiment, the human λ light chain gene segments comprise 40 human Vλ gene segments and four human Jλ gene segments. In various embodiments, the four human Jλ gene segments include Jλ1, Jλ2, Jλ3 and Jλ7. The mouse light chain constant region is a mouse CK.

[0070] In one aspect, in the genetically modified mouse provided, the mouse comprises a functionally silenced immunoglobulin light chain gene, and further comprises a replacement of one or more endogenous immunoglobulin heavy chain variable region gene segments with one or more human immunoglobulin heavy chain variable region gene segments, wherein the mouse lacks a functional endogenous ADAM6 locus, and wherein the mouse comprises an ectopic nucleotide sequence that expresses a mouse ADAM6 protein or an ortholog or homolog or fragment thereof that is functional in a male mouse.

[0071] In one aspect, the mouse provided lacks a functional endogenous mouse ADAM6 locus or sequence and comprises an ectopic nucleotide sequence encoding a mouse ADAM6 locus or functional fragment of a mouse ADAM6 locus or sequence, wherein the mouse is capable of mating with a mouse of the opposite sex to produce a progeny that comprises the ectopic ADAM6 locus or sequence. In one embodiment, the mouse is male. In one embodiment, the mouse is female.

[0072] In one aspect, the genetically modified mouse provided comprises a human

immunoglobulin heavy chain variable region gene segment at an endogenous mouse immunoglobulin heavy chain variable region gene locus, the mouse lacks an endogenous functional ADAM6 sequence at the endogenous mouse immunoglobulin heavy chain variable region gene locus, and wherein the mouse comprises an ectopic nucleotide sequence that expresses a mouse ADAM6 protein or an ortholog or homolog or fragment thereof that is functional in a male mouse.

[0073] [Deleted] In one embodiment, the ectopic nucleotide sequence that expresses the mouse ADAM6 protein is integrated at one or more loci in a genome of the mouse which is a heavy chain immunoglobulin locus.

[0074] Described is a mouse that expresses an immunoglobulin heavy chain sequence from a modified endogenous mouse immunoglobulin heavy chain locus, wherein the heavy chain is derived from a human V gene segment, a D gene segment, and a J gene segment, wherein the mouse comprises an ADAM6 activity that is functional in the mouse.

[0075] In one embodiment, the mouse comprises a plurality of human V gene segments, a plurality of human D gene segments, and a plurality of human J gene segments. In one embodiment, the mouse further comprises a humanized heavy chain constant region sequence, wherein the humanization comprises replacement of a sequence selected from a C_H1, hinge, C_H2, C_H3, and a combination thereof. In a specific embodiment, the heavy chain is derived from a human V gene segment, a human D gene segment, a human J gene segment, a human C_H1 sequence, a human or mouse hinge sequence, a mouse C_H2 sequence, and a mouse C_H3 sequence. In another specific embodiment, the mouse further comprises a human light chain constant sequence.

[0076] In one embodiment, the mouse comprises an ADAM6 gene that is flanked 5' and 3' by endogenous immunoglobulin heavy chain gene segments. In a specific embodiment, the endogenous immunoglobulin heavy chain gene segments are incapable of encoding a heavy chain of an antibody.

[0077] In one embodiment, the V gene segment is flanked 5' (with respect to transcriptional direction of the V gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse.

[0078] In one embodiment, the V gene segment is flanked 3' (with respect to transcriptional direction of the V gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse.

[0079] In one embodiment, the D gene segment is flanked 5' (with respect to transcriptional direction of the D gene segment) by a sequence encoding an ADAM6 activity that is functional in the mouse.

[0080] In one embodiment, the ADAM6 activity that is functional in the mouse results from

expression of a nucleotide sequence located 5' of the 5'-most D gene segment and 3' of the 3'-most V gene segment (with respect to the direction of transcription of the V gene segment) of the modified endogenous mouse heavy chain immunoglobulin locus.

[0081] In one embodiment, the ADAM6 activity that is functional in the mouse results from expression of a nucleotide sequence located between two human V gene segments in the modified endogenous mouse heavy chain immunoglobulin locus. In one embodiment, the two human V gene segments are a human V_H1-2 gene segment and a V_H6-1 gene segment.

[0082] The nucleotide sequence comprises a sequence selected from a mouse ADAM6b sequence or functional fragment thereof and a mouse ADAM6a sequence or functional fragment thereof.

[0083] In one embodiment, the nucleotide sequence between the two human V gene segments is placed in opposite transcription orientation with respect to the human V gene segments. In a specific embodiment, nucleotide sequence encodes, from 5' to 3' with respect to the direction of transcription of ADAM6 genes, and ADAM6a sequence followed by an ADAM6b sequence.

[0084] In one embodiment, the mouse comprises a replacement of a human ADAM6 pseudogene sequence between human V gene segments V_H1-2 and V_H6-1 with a mouse ADAM6 sequence or a functional fragment thereof.

[0085] In one embodiment, the sequence encoding the ADAM6 activity that is functional in the mouse is a mouse ADAM6 sequence or functional fragment thereof.

[0086] Disclosed is a mouse comprising an endogenous mouse DFL16.1 gene segment (e.g., in a mouse heterozygous for the modified endogenous mouse immunoglobulin heavy chain locus), or a human D_H1-1 gene segment. The D gene segment of the immunoglobulin heavy chain expressed by the mouse may be derived from an endogenous mouse DFL16.1 gene segment or a human D_H1-1 gene segment.

[0087] Described is a mouse that comprises a nucleic acid sequence encoding a mouse ADAM6 (or homolog or ortholog or functional fragment thereof) in a DNA-bearing cell of non-rearranged B cell lineage, but does not comprise the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) in a B cell that comprise rearranged immunoglobulin loci, wherein the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) occurs in the genome at a position that is different from a position in which a mouse ADAM6 gene appears in a wild-type mouse. The nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) may be present in all or substantially all DNA-bearing cells that are not of rearranged B cell lineage; the nucleic acid sequence may be present in germline cells of the mouse, but not in a chromosome of a rearranged B cell.

[0088] Described is a mouse that comprises a nucleic acid sequence encoding a mouse ADAM6 (or homolog or ortholog or functional fragment thereof) in all or substantially all DNA-bearing cells, including B cells that comprise rearranged immunoglobulin loci, wherein the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) occurs in the genome at a position that is different from a position in which a mouse ADAM6 gene appears in a wild-type mouse. The nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) may be on a nucleic acid that is contiguous with the rearranged immunoglobulin locus. The nucleic acid that may be contiguous with the rearranged immunoglobulin locus is a chromosome. The chromosome may be a chromosome that is found in a wild-type mouse and the chromosome comprises a modification of a mouse immunoglobulin locus.

[0089] Described is a genetically modified mouse, wherein the mouse comprises a B cell that comprises in its genome an ADAM6 sequence or ortholog or homolog thereof. The ADAM6 sequence or ortholog or homolog thereof may be at an immunoglobulin heavy chain locus. The ADAM6 sequence or ortholog or homolog thereof may be at a locus that is not an immunoglobulin locus. The ADAM6 sequence may be on a transgene driven by a heterologous promoter. The heterologous promoter may be a non-immunoglobulin promoter. Described is a B cell which expresses an ADAM6 protein or ortholog or homolog thereof.

[0090] 90% or more of the B cells of the mouse may comprise a gene encoding an ADAM6 protein or an ortholog thereof or a homolog thereof or a fragment thereof that is functional in the mouse. The mouse may be a male mouse.

[0091] Described is a B cell genome comprising a first allele and a second allele comprising the ADAM6 sequence or ortholog or homolog thereof. The B cell genome may comprise a first allele but not a second allele comprising the ADAM6 sequence or ortholog or homolog thereof.

[0092] Described is a mouse that comprises a modification at one or more endogenous immunoglobulin heavy chain alleles, wherein the modification maintains one or more endogenous ADAM6 alleles and the mouse further comprises an insertion of one or more human Vλ gene segments and one or more human Jλ gene segments upstream of a mouse light chain constant region. The mouse light chain constant region is a mouse Cκ.

[0093] The modification may render the mouse incapable of expressing a functional heavy chain that comprises rearranged endogenous heavy chain gene segments from at least one heavy chain allele and maintains an endogenous ADAM6 allele located within the at least one endogenous immunoglobulin heavy chain allele.

[0094] The mice may be incapable of expressing a functional heavy chain that comprises rearranged endogenous heavy chain gene segments from at least one of the endogenous immunoglobulin heavy chain alleles, and the mice express and ADAM6 protein from an endogenous ADAM6 allele. The mice may be incapable of expressing a functional heavy chain that comprises rearranged endogenous heavy chain gene segments from two endogenous

immunoglobulin heavy chain alleles, and the mice may express an ADAM6 protein from one or more endogenous ADAM6 alleles.

[0095] The mice may be incapable of expressing a functional heavy chain from each endogenous heavy chain allele, and the mice comprise an functional ADAM6 allele located within 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 or more Mbp upstream (with respect to the direction of transcription of the mouse heavy chain locus) of a mouse immunoglobulin heavy chain constant region sequence. The functional ADAM6 allele is at the endogenous immunoglobulin heavy chain locus (*e.g.,* in an intergenic V-D region, between two V gene segments, between a V and a D gene segment, between a D and a J gene segment, *etc.*). In a specific embodiment, the functional ADAM6 allele is located within a 90 to 100 kb intergenic sequence between the final mouse V gene segment and the first mouse D gene segment.

[0096] Described is a mouse that comprises a modification at one or more endogenous ADAM6 alleles.

[0097] The modification may render the mouse incapable of expressing a functional ADAM6 protein from at least one of the one or more endogenous ADAM6 alleles. The mouse may be incapable of expressing a functional ADAM6 protein from each of the endogenous ADAM6 alleles.

[0098] The mice may be incapable of expressing a functional ADAM6 protein from each endogenous ADAM6 allele, and the mice comprise an ectopic ADAM6 sequence.

[0099] The mice may be incapable of expressing a functional ADAM6 protein from each endogenous ADAM6 allele, and the mice comprise an ectopic ADAM6 sequence located within 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 or more kb upstream (with respect to the direction of transcription of the mouse heavy chain locus) of a mouse immunoglobulin heavy chain constant region sequence. The ectopic ADAM6 sequence may be at the endogenous heavy chain locus (*e.g.,* in an intergenic V-D region, between two V gene segments, between a V and a D gene segment, between a D and a J gene segment, *etc.*). The ectopic ADAM6 sequence may be located within a 90 to 100 kb intergenic sequence between the final mouse V gene segment and the first mouse D gene segment. The endogenous 90 to 100 kb intergenic V-D sequence may be removed, and the ectopic ADAM6 sequence is placed between the final V and the first D gene segment.

[0100] Described is an infertile male mouse, wherein the mouse comprises a deletion of two or more endogenous ADAM6 alleles. Also described is a female mouse that is a carrier of a male infertility trait, wherein the female mouse comprises in its germline a nonfunctional ADAM6 allele or a knockout of an endogenous ADAM6 allele.

[0101] Described is a mouse comprising an endogenous immunoglobulin heavy chain V, D, and or J gene segment that are incapable of rearranging to encode an heavy chain of an

antibody, wherein the majority of the B cells of the mouse comprise an functional ADAM6 gene. The majority of the B cells of the mouse may further comprise one or more human Vλ gene segments and one or more human Jλ gene segments upstream of a mouse immunoglobulin light chain constant region. The mouse immunoglobulin light chain constant region is a mouse CK.

[0102] In one embodiment, the mouse comprises an intact endogenous immunoglobulin heavy chain V, D, and J gene segments that are incapable of rearranging to encode a functional heavy chain of an antibody. In one embodiment, the mouse comprises at least one and up to 89 V gene segments, at least one and up to 13 D gene segments, at least one and up to four J gene segments, and a combination thereof; wherein the at least one and up to 89 V gene segments, at least one and up to 13 D gene segments, at least one and up to four J gene segments are incapable of rearranging to encode a heavy chain variable region of an antibody. In a specific embodiment, the mouse comprises a functional ADAM6 gene located within the intact endogenous immunoglobulin heavy chain V, D, and J gene segments. In one embodiment, the mouse comprises an endogenous heavy chain locus that includes an endogenous ADAM6 locus, wherein the endogenous heavy chain locus comprises 89 V gene segments, 13 D gene segments, and four J gene segments, wherein the endogenous heavy chain gene segments are incapable of rearranging to encode a heavy chain variable region of an antibody and the ADAM6 locus encodes an ADAM6 protein that is functional in the mouse.

[0103] Described is a mouse that lacks an endogenous immunoglobulin heavy chain V, D, and J gene segment, wherein a majority of the B cells of the mouse comprise an ADAM6 sequence or ortholog or homolog thereof. The majority of the B cells of the mouse may express a immunoglobulin light chain comprising a human lambda variable domain and an endogenous immunoglobulin light chain constant region.

[0104] In one embodiment, the mouse lacks endogenous immunoglobulin heavy chain gene segments selected from two or more V gene segments, two or more D gene segments, two or more J gene segments, and a combination thereof. In one embodiment, the mouse lacks immunoglobulin heavy chain gene segments selected from at least one and up to 89 V gene segments, at least one and up to 13 D gene segments, at least one and up to four J gene segments, and a combination thereof. In one embodiment, the mouse lacks a genomic DNA fragment from chromosome 12 comprising about three megabases of the endogenous immunoglobulin heavy chain locus. In a specific embodiment, the mouse lacks all functional endogenous heavy chain V, D, and J gene segments. In a specific embodiment, the mouse lacks 89 V_H gene segments, 13 D_H gene segments and four J_H gene segments.

[0105] Described is a mouse wherein the mouse has a genome in the germline comprising a modification of an immunoglobulin heavy chain locus, wherein the modification to the immunoglobulin heavy chain locus comprises the replacement of one or more mouse immunoglobulin variable region sequences with one or more human immunoglobulin variable region sequences, and wherein the mouse comprises a nucleic acid sequence encoding a mouse ADAM6 protein. The D_H and J_H sequences and at least 3, at least 10, at least 20, at

least 40, at least 60, or at least 80 V_H sequences of the immunoglobulin heavy chain locus may be replaced by human immunoglobulin variable region sequences. The D_H, J_H, and all V_H sequences of the immunoglobulin heavy chain locus may be replaced by human immunoglobulin variable region sequences. The human immunoglobulin variable region sequences can be non-rearranged. The human immunoglobulin variable region sequences may comprise complete non-rearranged D_H and J_H regions and at least 3, at least 10, at least 20, at least 40, at least 60, or at least 80 non-rearranged V_H sequences which are human. In a further preferred embodiment, the non-mouse immunoglobulin variable region sequences comprise the complete human variable region, including all V_H, D_H, and J_H regions.

[0106] Described is a mouse that expresses an antibody that comprises at least one human variable domain/non-human constant domain immunoglobulin polypeptide, wherein the mouse expresses a mouse ADAM6 protein or ortholog or homolog thereof from a locus other than an immunoglobulin locus.

[0107] The ADAM6 protein or ortholog or homolog thereof may be expressed in a B cell of the mouse, wherein the B cell comprises a rearranged immunoglobulin sequence that comprises a human variable sequence and a non-human constant sequence.

[0108] In one embodiment, the non-human constant sequence is a rodent sequence. In one embodiment, the rodent is selected from a mouse, a rat, and a hamster.

[0109] Described is a method for making an infertile male mouse, comprising rendering an endogenous ADAM6 allele of a donor ES cell nonfunctional (or knocking out said allele), introducing the donor ES cell into a host embryo, gestating the host embryo in a surrogate mother, and allowing the surrogate mother to give birth to progeny derived in whole or in part from the donor ES cell. The method may further comprise breeding progeny to obtain an infertile male mouse.

[0110] Described is a method for making a mouse with a genetic modification of interest, wherein the mouse is infertile, the method comprising the steps of (a) making a genetic modification of interest in a genome; (b) modifying the genome to knockout an endogenous ADAM6 allele, or render an endogenous ADAM6 allele nonfunctional; and, (c) employing the genome in making a mouse. The genome may be from an ES cell or used in a nuclear transfer experiment.

[0111] Described is a mouse made using a targeting vector, nucleotide construct, or cell as described herein.

[0112] Described is a progeny of a mating of a mouse as described herein with a second mouse that is a wild-type mouse or genetically modified.

[0113] Described is a method for maintaining a mouse strain, wherein the mouse strain

comprises a replacement of a mouse immunoglobulin heavy chain sequence with one or more heterologous immunoglobulin heavy chain sequences which are human immunoglobulin heavy chain sequences.

[0114] In one embodiment, the mouse strain comprises a deletion of one or more mouse V_H, D_H, and/or J_H gene segments. The mouse further comprises one or more human V_H gene segments, one or more human D_H gene segments, and/or one or more human J_H gene segments. In one embodiment, the mouse comprises at least 3, at least 10, at least 20, at least 40, at least 60, or at least 80 human V_H segments, at least 27 human D_H gene segments, and at least six J_H gene segments. In a specific embodiment, the mouse comprises at least 3, at least 10, at least 20, at least 40, at least 60, or at least 80 human V_H segments, the at least 27 human D_H gene segments, and the at least six J_H gene segments are operably linked to a constant region gene. In one embodiment, the constant region gene is a mouse constant region gene. In one embodiment, the constant region gene comprises a mouse constant region gene sequence selected from a C_H1, a hinge, a C_H2, a C_H3, and/or a C_H4 or a combination thereof.

[0115] Described is a method comprising generating a male mouse heterozygous for the replacement of the mouse immunoglobulin heavy chain sequence, and breeding the heterozygous male mouse with a wild-type female mouse or a female mouse that is homozygous or heterozygous for the human heavy chain sequence. The method may comprise maintaining the strain by repeatedly breeding heterozygous males with females that are wild type or homozygous or heterozygous for the human heavy chain sequence.

[0116] The method may comprise obtaining cells from male or female mice homozygous or heterozygous for the human heavy chain sequence, and employing those cells as donor cells or nuclei therefrom as donor nuclei, and using the cells or nuclei to make genetically modified animals using host cells and/or gestating the cells and/or nuclei in surrogate mothers.

[0117] [Deleted]

[0118] In one embodiment, the mouse further comprises a replacement of λ and/or κ light chain variable sequences at an endogenous immunoglobulin light chain locus with heterologous immunoglobulin light chain sequences. The heterologous immunoglobulin light chain sequences are human immunoglobulin λ light chain variable sequences and may also include κ light chain variable sequences.

[0119] [Deleted]

[0120] Described is a nucleic acid construct, comprising an upstream homology arm and a downstream homology arm, wherein the upstream homology arm comprises a sequence that is identical or substantially identical to a human immunoglobulin heavy chain variable region sequence, the downstream homology arm comprises a sequence that is identical or

substantially identical to a human or mouse immunoglobulin variable region sequence, and disposed between the upstream and downstream homology arms is a sequence that comprises a nucleotide sequence encoding a mouse ADAM6 protein. The sequence may encode the mouse ADAM6 gene is operably linked with a mouse promoter with which the mouse ADAM6 is linked in a wild type mouse.

[0121] Described is a targeting vector, comprising (a) a nucleotide sequence that is identical or substantially identical to a human variable region gene segment nucleotide sequence; and, (b) a nucleotide sequence encoding a mouse ADAM6 or ortholog or homolog or fragment thereof that is functional in a mouse.

[0122] The targeting vector may further comprise a promoter operably linked to the sequence encoding the mouse ADAM6. In a specific embodiment, the promoter is a mouse ADAM6 promoter.

[0123] Described is a nucleotide construct for modifying a mouse immunoglobulin heavy chain variable locus, wherein the construct comprises at least one site specific recombinase recognition site and a sequence encoding an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a mouse.

[0124] In one aspect, mouse cells and mouse embryos are provided, including but not limited to ES cells, pluripotent cells, and induced pluripotent cells, that comprise genetic modifications as described herein. Cells that are XX and cells that are XY are provided. Cells that comprise a nucleus containing a modification as described herein are also provided, *e.g.,* a modification introduced into a cell by pronuclear injection. Cells, embryos, and mice that comprise a virally introduced ADAM6 gene are also described, *e.g.,* cells, embryos, and mice comprising a transduction construct comprising an ADAM6 gene that is functional in the mouse are also described.

[0125] Described is a genetically modified mouse cell, wherein the cell lacks a functional endogenous mouse ADAM6 locus, and the cell comprises an ectopic nucleotide sequence that encodes a mouse ADAM6 protein or functional fragment thereof. The cell may further comprises a modification of an endogenous immunoglobulin heavy chain variable gene sequence. The modification of the endogenous immunoglobulin heavy chain variable gene sequence may comprise a deletion selected from a deletion of a mouse V_H gene segment, a deletion of a mouse D_H gene segment, a deletion of a mouse J_H gene segment, and a combination thereof. In a specific embodiment, the mouse comprises a replacement of one or more mouse immunoglobulin V_H, D_H, and/or J_H sequences with a human immunoglobulin sequence. The human immunoglobulin sequence may be selected from a human V_H, a human V_L, a human D_H, a human J_H, a human J_L, and a combination thereof.

[0126] In one embodiment, the cell is a totipotent cell, a pluripotent cell, or an induced pluripotent cell. In a specific embodiment, the cell is a mouse ES cell.

[0127] In one aspect, a mouse B cell is provided, wherein the B cell is isolated from the mouse provided, where the mouse B cell comprises a rearranged immunoglobulin heavy chain gene, wherein the B cell comprises on a chromosome of the B cell a nucleic acid sequence encoding an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse. In one embodiment, the mouse B cell comprises two alleles of the nucleic acid sequence.

[0128] In one embodiment, the nucleic acid sequence is on a nucleic acid molecule (*e.g.,* a B cell chromosome) that is contiguous with the rearranged mouse immunoglobulin heavy chain locus.

[0129] [Deleted]

[0130] In one embodiment, the mouse B cell comprises a rearranged non-mouse immunoglobulin variable gene sequence operably linked to a mouse or human immunoglobulin constant region gene, wherein the B cell comprises a nucleic acid sequence that encodes an ADAM6 protein or ortholog or homolog or fragment thereof that is functional in a male mouse.

[0131] In one aspect, a somatic mouse cell is provided, where the cell has been isolated from the mouse provided, the cell comprising a chromosome that comprises a modified immunoglobulin heavy chain locus, and a nucleic acid sequence encoding a mouse ADAM6 or ortholog or homolog or fragment thereof that is functional in a male mouse. The nucleic acid sequence is on the same chromosome as the modified immunoglobulin heavy chain locus. In one embodiment, the somatic cell comprises a single copy of the nucleic acid sequence. In one embodiment, the somatic cell comprises at least two copies of the nucleic acid sequence. In a specific embodiment, the somatic cell is a B cell. In a specific embodiment, the cell is a germ cell. In a specific embodiment, the cell is a stem cell.

[0132] In one aspect, a mouse germ cell is provided, where the cell has been isolated from the mouse provided, the cell comprising a nucleic acid sequence encoding a mouse ADAM6 (or homolog or ortholog or functional fragment thereof) on a chromosome of the germ cell, wherein the nucleic acid sequence encoding the mouse ADAM6 (or homolog or ortholog or functional fragment thereof) is at a position in the chromosome that is different from a position in a chromosome of a wild-type mouse germ cell. The nucleic acid sequence is at a mouse heavy chain immunoglobulin locus. In one embodiment, the mouse immunoglobulin locus comprises a replacement of at least one mouse immunoglobulin sequence with at least one human immunoglobulin sequence.

[0133] In one aspect, a pluripotent, induced pluripotent, or totipotent cell derived from a mouse as described herein is provided. In a specific embodiment, the cell is a mouse embryonic stem (ES) cell.

[0134] In one aspect, a cell or tissue derived from a mouse as described herein is provided. In one embodiment, the cell or tissue is derived from spleen, lymph node or bone marrow of a

mouse as described herein. In one embodiment, the cell is a B cell. In one embodiment the cell is an embryonic stem cell. In one embodiment, the cell is a germ cell.

[0135] In one embodiment, the tissue is selected from connective, muscle, nervous and epithelial tissue. In a specific embodiment, the tissue is reproductive tissue.

[0136] In one embodiment, the cell and/or tissue derived from a mouse as described herein is isolated for use in one or more *ex vivo* assays. In various embodiments, the one or more ex *vivo* assays include measurements of physical, thermal, electrical, mechanical or optical properties, a surgical procedure, measurements of interactions of different tissue types, the development of imaging techniques, or a combination thereof.

[0137] In aspect, use of cell or tissue derived from a mouse as described herein to make an antibody is provided. In one aspect, use of a cell or tissue derived from a mouse as described herein to make a hybridoma or quadroma is provided.

[0138] In one aspect, a mouse cell comprising a chromosome or fragment thereof of a mouse as described herein. In one embodiment, the mouse cell comprises a nucleus of a mouse as described herein. In one embodiment, the mouse cell comprises the chromosome or fragment thereof as the result of a nuclear transfer.

[0139] Described is a nucleus derived from a mouse as described herein. The nucleus may be from a diploid cell that is not a B cell.

[0140] Described is a nucleotide sequence encoding an immunoglobulin variable region made in a mouse as described.

[0141] Described is an immunoglobulin heavy chain or immunoglobulin light chain variable region amino acid sequence of an antibody made in a mouse as described herein.

[0142] Described is an immunoglobulin heavy chain or immunoglobulin light chain variable region nucleotide sequence encoding a variable region of an antibody made in a mouse as described herein.

[0143] Described is an antibody or antigen-binding fragment thereof (*e.g.,* Fab, $F(ab)_2$, scFv) made in a mouse as described herein.

[0144] Described is a method for making a genetically modified mouse, comprising replacing one or more immunoglobulin heavy chain gene segments upstream (with respect to transcription of the immunoglobulin heavy chain gene segments) of an endogenous ADAM6 locus of the mouse with one or more human immunoglobulin heavy chain gene segments, and replacing one or more immunoglobulin gene segments downstream (with respect to transcription of the immunoglobulin heavy chain gene segments) of the ADAM6 locus of the mouse with one or more human immunoglobulin heavy chain or light chain gene segments.

The one or more human immunoglobulin gene segments replacing one or more endogenous immunoglobulin gene segments upstream of an endogenous ADAM6 locus of the mouse may include V gene segments. The human immunoglobulin gene segments replacing one or more endogenous immunoglobulin gene segments upstream of an endogenous ADAM6 locus of the mouse may include V and D gene segments. The one or more human immunoglobulin gene segments may replace one or more endogenous immunoglobulin gene segments downstream of an endogenous ADAM6 locus of the mouse include J gene segments. The one or more human immunoglobulin gene segments may replace one or more endogenous immunoglobulin gene segments downstream of an endogenous ADAM6 locus of the mouse include D and J gene segments. The one or more human immunoglobulin gene segments may replace one or more endogenous immunoglobulin gene segments downstream of an endogenous ADAM6 locus of the mouse include V, D and J gene segments.

[0145] The one or more immunoglobulin heavy chain gene segments upstream and/or downstream of the ADAM6 gene may be replaced in a pluripotent, induced pluripotent, or totipotent cell to form a genetically modified progenitor cell; the genetically modified progenitor cell is introduced into a host; and, the host comprising the genetically modified progenitor cell is gestated to form a mouse comprising a genome derived from the genetically modified progenitor cell. In one embodiment, the host is an embryo. The host may be selected from a mouse pre-morula (*e.g.,* 8- or 4-cell stage), a tetraploid embryo, an aggregate of embryonic cells, or a blastocyst.

[0146] Described is a method for making a genetically modified mouse, comprising replacing a mouse nucleotide sequence that comprises a mouse immunoglobulin gene segment and a mouse ADAM6 (or ortholog or homolog or fragment thereof functional in a male mouse) nucleotide sequence with a sequence comprising a human immunoglobulin gene segment to form a first chimeric locus, then inserting a sequence comprising a mouse ADAM6-encoding sequence (or a sequence encoding an ortholog or homolog or functional fragment thereof) into the sequence comprising the human immunoglobulin gene segment to form a second chimeric locus.

[0147] The second chimeric locus may comprise a human immunoglobulin heavy chain variable (V_H) gene segment. The second chimeric locus may comprise a human immunoglobulin light chain variable (V_L) gene segment. In a specific embodiment, the second chimeric locus comprises a human V_H gene segment or a human V_L gene segment operably linked to a human D_H gene segment and a human J_H gene segment. The second chimeric locus may be operably linked to a third chimeric locus that comprises a human C_H1 sequence, or a human C_H1 and human hinge sequence, fused with a mouse C_H2 + C_H3 sequence.

[0148] Described is the use of a mouse that comprises an ectopic nucleotide sequence comprising a mouse ADAM6 locus or sequence to make a fertile male mouse, wherein the use comprises mating the mouse comprising the ectopic nucleotide sequence that comprises the mouse ADAM6 locus or sequence to a mouse that lacks a functional endogenous mouse

ADAM6 locus or sequence, and obtaining a progeny that is a female capable of producing progeny having the ectopic ADAM6 locus or sequence or that is a male that comprises the ectopic ADAM6 locus or sequence, and the male exhibits a fertility that is approximately the same as a fertility exhibited by a wild-type male mouse.

[0149] In one aspect, use of a mouse as described herein to make an immunoglobulin variable region nucleotide sequence is provided.

[0150] In one aspect, use of a mouse as described herein to make a fully human Fab or a fully human $F(ab)_2$ is provided.

[0151] Described is the use of a mouse as described herein to make an immortalized cell line.

[0152] Described is the use of a mouse as described herein to make a hybridoma or quadroma.

[0153] Described is the use of a mouse as described herein to make a phage library containing human heavy chain variable regions and human light chain variable regions.

[0154] In one aspect, use of a mouse as described herein to generate a variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating a lymphocyte from the immunized mouse of (a), (c) exposing the lymphocyte to one or more labeled antibodies, (d) identifying a lymphocyte that is capable of binding to the antigen of interest, and (e) amplifying one or more variable region nucleic acid sequence from the lymphocyte thereby generating a variable region sequence.

[0155] In one embodiment, the lymphocyte is derived from the spleen of the mouse. In one embodiment, the lymphocyte is derived from a lymph node of the mouse. In one embodiment, the lymphocyte is derived from the bone marrow of the mouse.

[0156] In one embodiment, the labeled antibody is a fluorophore-conjugated antibody. In one embodiment, the one or more fluorophore-conjugated antibodies are selected from an IgM, an IgG, and/or a combination thereof.

[0157] In one embodiment, the lymphocyte is a B cell.

[0158] In one embodiment, the one or more variable region nucleic acid sequence comprises a heavy chain variable region sequence. In one embodiment, the one or more variable region nucleic acid sequence comprises a light chain variable region sequence. In a specific embodiment, the light chain variable region sequence is an immunoglobulin κ light chain variable region sequence. In one embodiment, the one or more variable region nucleic acid sequence comprises a heavy chain and a κ light chain variable region sequence.

[0159] In one embodiment, use of a mouse as described herein to generate a heavy and a κ light chain variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating the spleen from the immunized mouse of (a), (c) exposing B lymphocytes from the spleen to one or more labeled antibodies, (d) identifying a B lymphocyte of (c) that is capable of binding to the antigen of interest, and (e) amplifying a heavy chain variable region nucleic acid sequence and a κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the heavy chain and κ light chain variable region sequences.

[0160] In one embodiment, use of a mouse as described herein to generate a heavy and a κ light chain variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating one or more lymph nodes from the immunized mouse of (a), (c) exposing B lymphocytes from the one or more lymph nodes to one or more labeled antibodies, (d) identifying a B lymphocyte of (c) that is capable of binding to the antigen of interest, and (e) amplifying a heavy chain variable region nucleic acid sequence and a κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the heavy chain and κ light chain variable region sequences.

[0161] In one embodiment, use of a mouse as described herein to generate a heavy and a κ light chain variable region sequence for making a human antibody is provided, comprising (a) immunizing a mouse as described herein with an antigen of interest, (b) isolating bone marrow from the immunized mouse of (a), (c) exposing B lymphocytes from the bone marrow to one or more labeled antibodies, (d) identifying a B lymphocyte of (c) that is capable of binding to the antigen of interest, and (e) amplifying a heavy chain variable region nucleic acid sequence and a κ light chain variable region nucleic acid sequence from the B lymphocyte thereby generating the heavy chain and κ light chain variable region sequences. In various embodiments, the one or more labeled antibodies are selected from an IgM, an IgG, and/or a combination thereof.

[0162] In various embodiments, use of a mouse as described herein to generate a heavy and κ light chain variable region sequence for making a human antibody is provided, further comprising fusing the amplified heavy and light chain variable region sequences to human heavy and light chain constant region sequences, expressing the fused heavy and light chain sequences in a cell, and recovering the expressed heavy and light chain sequences thereby generating a human antibody.

[0163] In various embodiments, the human heavy chain constant regions are selected from IgM, IgD, IgA, IgE and IgG. In various specific embodiments, the IgG is selected from an IgG1, an IgG2, an IgG3 and an IgG4. In various embodiments, the human heavy chain constant region comprises a C_H1, a hinge, a C_H2, a C_H3, a C_H4, or a combination thereof. In various embodiments, the light chain constant region is an immunoglobulin κ constant region. In various embodiments, the cell is selected from a HeLa cell, a DU145 cell, a Lncap cell, a MCF-7 cell, a MDA-MB-438 cell, a PC3 cell, a T47D cell, a THP-1 cell, a U87 cell, a SHSY5Y (human neuroblastoma) cell, a Saos-2 cell, a Vero cell, a CHO cell, a GH3 cell, a PC12 cell, a human retinal cell (e.g., a PER.C6™ cell), and a MC3T3 cell. In a specific embodiment, the cell is a

CHO cell.

[0164] In one aspect, a method for generating a reverse-chimeric rodent-human antibody specific against an antigen of interest is provided, comprising the steps of immunizing a mouse as described herein with the antigen, isolating at least one cell from the mouse producing a reverse-chimeric mouse-human antibody specific against the antigen, culturing at least one cell producing the reverse-chimeric mouse-human antibody specific against the antigen, and obtaining said antibody.

[0165] In one embodiment, the reverse-chimeric mouse-human antibody comprises a human heavy chain variable domain fused with a mouse or rat heavy chain constant gene, and a human light chain variable domain fused with a mouse or rat or human light chain constant gene.

[0166] In one embodiment, culturing at least one cell producing the reverse-chimeric rodent-human antibody specific against the antigen is performed on at least one hybridoma cell generated from the at least one cell isolated from the mouse.

[0167] In one aspect, a method for generating a fully human antibody specific against an antigen of interest is provided, comprising the steps of immunizing a mouse as described herein with the antigen, isolating at least one cell from the mouse producing a reverse-chimeric rodent-human antibody specific against the antigen, generating at least one cell producing a fully human antibody derived from the reverse-chimeric rodent-human antibody specific against the antigen, and culturing at least one cell producing the fully human antibody, and obtaining said fully human antibody.

[0168] In various embodiments, the at least one cell isolated from the mouse producing a reverse-chimeric rodent-human antibody specific against the antigen is a splenocyte or a B cell.

[0169] In various embodiments, the antibody is a monoclonal antibody.

[0170] In various embodiments, immunization with the antigen of interest is carried out with protein, DNA, a combination of DNA and protein, or cells expressing the antigen.

[0171] In one aspect, use of a mouse as described herein to make a nucleic acid sequence encoding an immunoglobulin variable region or fragment thereof is provided. In one embodiment, the nucleic acid sequence is used to make a human antibody or antigen-binding fragment thereof. In one embodiment, the mouse is used to make an antigen-binding protein selected from an antibody, a multi-specific antibody (*e.g.,* a bi-specific antibody), an scFv, a bi-specific scFv, a diabody, a triabody, a tetrabody, a V-NAR, a V_{HH}, a V_L, a F(ab), a $F(ab)_2$, a DVD (*i.e.,* dual variable domain antigen-binding protein), a an SVD (*i.e.,* single variable domain antigen-binding protein), or a bispecific T-cell engager (BiTE).

[0172] Described is use of a mouse as described herein to introduce an ectopic ADAM6 sequence into a mouse that lacks a functional endogenous mouse ADAM6 sequence wherein the use comprises mating a mouse as described herein with the mouse that lacks the functional endogenous mouse ADAM6 sequence.

[0173] Described is the use of genetic material from a mouse as described herein to make a mouse having an ectopic ADAM6 sequence. The use may comprise nuclear transfer using a nucleus of a cell of a mouse as described herein. The use may comprise cloning a cell of a mouse as described herein to produce an animal derived from the cell. The use may comprise employing a sperm or an egg of a mouse as described herein in a process for making a mouse comprising the ectopic ADAM6 sequence.

[0174] Described is a method for making a fertile male mouse comprising a modified immunoglobulin heavy chain locus, comprising fertilizing a first mouse germ cell that comprises a modification of an endogenous immunoglobulin heavy chain locus with a second mouse germ cell that comprises an ADAM6 gene or ortholog or homolog or fragment thereof that is functional in a male mouse; forming a fertilized cell; allowing the fertilized cell to develop into an embryo; and, gestating the embryo in a surrogate to obtain a mouse.

[0175] The fertilization may be achieved by mating a male mouse and a female mouse. The female mouse may comprise the ADAM6 gene or ortholog or homolog or fragment thereof. The male mouse may comprise the ADAM6 gene or ortholog or homolog or fragment thereof.

[0176] Described is the use of a nucleic acid sequence encoding a mouse ADAM6 protein or an ortholog or homolog thereof or a functional fragment of the corresponding ADAM6 protein for restoring or enhancing the fertility of a mouse having a genome comprising a modification of an immunoglobulin heavy chain locus, wherein the modification reduces or eliminates endogenous ADAM6 function.

[0177] The nucleic acid sequence is integrated into the genome of the mouse at an endogenous immunoglobulin locus which is a heavy chain locus.

[0178] Described herein is the use of the mouse as described herein for the manufacture of a medicament (*e.g.,* an antigen-binding protein), or for the manufacture of a sequence encoding a variable sequence of a medicament (*e.g.,* an antigen-binding protein), for the treatment of a human disease or disorder.

[0179] Described is a genetically modified mouse cell, wherein the cell is incapable of expressing a heavy chain comprising rearranged endogenous immunoglobulin heavy chain gene segments, and the cell comprises a functional ADAM6 gene that encodes a mouse ADAM6 protein or functional fragment thereof. The cell may further comprise an insertion of human immunoglobulin gene segments. The human immunoglobulin gene segments may be heavy chain gene segments that are operably linked to mouse heavy chain constant regions such that upon rearrangement encode a functional heavy chain of an antibody that comprises

a human variable region.

[0180] Described are genetically mice, embryos, cells, tissues, as well as nucleic acid constructs for modifying the mice, and methods and compositions for making and using them. Also described are mice and cells that generate lambda (λ) variable regions (human or non-human) in the context of a kappa (κ) light chain, wherein the mice and cells comprise a modification of a heavy chain immunoglobulin locus that eliminates or reduces activity of an ADAM6 protein or homolog or ortholog thereof, wherein the mice further comprise a genetic modification that restores in whole or in part ADAM6 activity (or the activity of the homolog or ortholog thereof). Also described are mice that are fertile and express a human λ variable domain cognate with a human heavy chain variable domain, wherein the human λ variable domain is expressed in the mouse contiguous with a mouse κ constant region, and in various embodiments the κ constant region is an endogenous (mouse) constant region. Also described are mice and cells that generate human λ variable regions in the context of a mouse κ light chain, e.g., from an endogenous mouse light chain locus. Also described are methods for making antibodies that comprise lambda variable regions. Methods for selecting heavy chains that express with cognate lambda variable regions are also described.

[0181] Described are chimeric and human antigen-binding proteins (*e.g.,* antibodies), and nucleic acids encoding them, that comprise somatically mutated variable regions, including antibodies that have light chains comprising a variable domain derived from a human Vλ and a human Jλ gene segment fused to a mouse κ light chain constant domain.

[0182] Described is a mouse that expresses a human λ variable region sequence on a light chain that comprises a κ constant region. Described is a mouse is that expresses from an endogenous mouse light chain locus a light chain that comprises a human λ variable region sequence. Described is a mouse that comprises a rearranged light chain gene that comprises a human λ variable sequence linked to a mouse κ constant sequence.

[0183] Described is a genetically modified mouse, wherein the mouse comprises an unrearranged human λ light chain variable gene segment (hVλ) and a human λ joining gene segment (hJλ). The unrearranged hVλ and hJλ are at a mouse κ light chain locus. The mouse may be capable of making an immunoglobulin that comprises a light chain that is derived from an unrearranged hVλ sequence and a hJλ sequence and a mouse κ light chain constant region (Cκ) nucleic acid sequence. Methods and compositions for making and using genetically modified mice are also described. Antibodies are described that comprise (a) a human heavy chain variable domain (hV_H) fused to a mouse heavy chain constant region, and (b) a human VL fused to a mouse Cκ domain; including wherein one or more of the variable domains are somatically mutated, e.g., during antibody or immune cell selection in a mouse of the invention. The unrearranged hVλ and unrearranged hJλ are operably linked with a mouse κ constant region (CK).

[0184] Described is a mouse that comprises in its germline, at an endogenous mouse κ light chain locus, a human λ light chain variable region sequence, wherein the human lambda

variable region sequence is expressed in a light chain that comprises a mouse immunoglobulin κ light chain constant region gene sequence.

[0185] [Deleted]

[0186] In one embodiment, the mouse lacks an endogenous light chain variable sequence at the endogenous mouse light chain locus.

[0187] In one embodiment, all or substantially all endogenous mouse light chain variable region gene segments are replaced with one or more human λ variable region gene segments.

[0188] In one embodiment, the human λ light chain variable region sequence comprises a human Jλ sequence. In one embodiment, the human Jλ sequence is selected from the group consisting of Jλ1, Jλ2, Jλ3, Jλ7, and a combination thereof.

[0189] In one embodiment, the human λ light chain variable region sequence comprises a fragment of cluster A of the human light chain locus. In a specific embodiment, the fragment of cluster A of the human λ light chain locus extends from hVλ3-27 through hVλ3-1.

[0190] In one embodiment, the human λ light chain variable region sequence comprises a fragment of cluster B of the human light chain locus. In a specific embodiment, the fragment of cluster B of the human λ light chain locus extends from hVλ5-52 through hVλ1-40.

[0191] In one embodiment, the human λ light chain variable region sequence comprises a genomic fragment of cluster A and a genomic fragment of cluster B. In a one embodiment, the human λ light chain variable region sequence comprises at least one gene segment of cluster A and at least one gene segment of cluster B.

[0192] In one embodiment, more than 10% of the light chain naive repertoire of the mouse is derived from at least two hVλ gene segments selected from 2-8, 2-23, 1-40, 5-45, and 9-49. In one embodiment, more than 20% of the light chain naive repertoire of the mouse is derived from at least three hVλ gene segments selected from 2-8, 2-23, 1-40, 5-45, and 9-49. In one embodiment, more than 30% of the light chain naive repertoire of the mouse is derived from at least four hVλ gene segments selected from 2-8, 2-23, 1-40, 5-45, and 9-49.

[0193] Described is a mouse that expresses an immunoglobulin light chain that comprises a human λ variable sequence fused with a mouse κ constant region, wherein the mouse exhibits a κ usage to λ usage ratio of about 1:1.

[0194] The immunoglobulin light chain is expressed from an endogenous mouse light chain locus.

[0195] Described is a mouse that comprises a λ light chain variable region sequence (Vλ) and at least one J sequence (J), contiguous with a mouse κ light chain constant region sequence.

[0196] The mouse may lack a functional mouse Vκ and/or mouse Jκ gene segment.

[0197] The Vλ is a human Vλ (hVλ), and the J is a human Jλ (hJλ). The hVλ and the hJλ are unrearranged gene segments.

[0198] In one embodiment, the mouse comprises a plurality of unrearranged human hVλ gene segments and at least one human hJλ gene segment. In a specific embodiment, the plurality of unrearranged hVλ gene segments are at least 12 gene segments, at least 28 gene segments, or at least 40 gene segments.

[0199] In one embodiment, the at least one hJλ gene segment is selected from the group consisting of Jλ1, Jλ2, Jλ3, Jλ7, and a combination thereof.

[0200] In one embodiment, an endogenous mouse λ light chain locus is deleted in whole or in part.

[0201] The mouse κ light chain constant region sequence is at an endogenous mouse κ light chain locus.

[0202] In one embodiment, about 10% to about 45% of the B cells of the mouse express an antibody that comprises a light chain comprising a human λ light chain variable (Vλ) domain and a mouse κ light chain constant (Cκ) domain.

[0203] In one embodiment, the human λ variable domain is derived from a rearranged hVλ/hJλ sequence selected from the group consisting of 3-1/1, 3-1/7, 4-3/1, 4-3/7, 2-8/1, 3-9/1, 3-10/1, 3-10/3, 3-10/7, 2-14/1, 3-19/1, 2-23/1, 3-25/1, 1-40/1, 1-40/2, 1-40/3, 1-40/7, 7-43/1, 7-43/3, 1-44/1, 1-44/7, 5-45/1, 5-45/2, 5-45/7, 7-46/1, 7-46/2, 7-46/7, 9-49/1, 9-49/2, 9-49/7 and 1-51/1.

[0204] In one embodiment, the mouse further comprises a human Vκ-Jκ intergenic region from a human κ light chain locus, wherein the human Vκ-Jκ intergenic region is contiguous with the Vλ sequence and the J sequence. In a specific embodiment, the human Vκ-Jκ intergenic region is placed between the Vλ sequence and the J sequence.

[0205] Described is a mouse that comprises (a) at least 12 to at least 40 unrearranged human λ light chain variable region gene segments and at least one human Jλ gene segment at an endogenous mouse light chain locus; (b) a human Vκ-Jκ intergenic sequence located between the at least 12 to at least 40 human light chain variable region gene segments and the at least one human Jλ sequence; wherein the mouse express an antibody that comprises a light chain comprising a human Vλ domain and a mouse Cκ domain.

[0206] Described is a mouse that expresses an antibody comprising a light chain that comprises a λ variable sequence and a mouse κ constant sequence.

[0207] In one embodiment, the mouse exhibits a κ usage to λ usage ratio of about 1:1.

[0208] In one embodiment, a population of immature B cells obtained from bone marrow of the mouse exhibits a κ usage to λ usage ratio of about 1:1.

[0209] Described is a genetically modified mouse, wherein the mouse comprises an unrearranged immunoglobulin Vλ and a Jλ gene segment operably linked to a mouse κ light chain locus that comprises a mouse Cκ gene.

[0210] The Vλ and Jλ gene segments are human gene segments.

[0211] The endogenous mouse light chain locus is a κ light chain locus.

[0212] The unrearranged Vλ and Jλ gene segments are at an endogenous mouse κ light chain locus.

[0213] [Deleted]

[0214] In one embodiment, the mouse further comprises a replacement of one or more heavy chain V, D, and/or J gene segments with one or more human V, D, and/or J gene segments at an endogenous mouse heavy chain immunoglobulin locus.

[0215] In one embodiment, the mouse comprises an unrearranged immunoglobulin Vλ and a Jλ gene segment at an endogenous mouse κ light chain locus that comprises a mouse Cκ gene.

[0216] In one embodiment, the mouse further comprises an unrearranged human immunoglobulin λ light chain variable gene segment (Vλ) and a λ joining gene segment (Jλ) at an endogenous mouse λ light chain locus that comprises a mouse Cλ gene.

[0217] The light chain variable gene locus (the "V_L locus") comprises at least one human Vλ (hVλ) gene segment. The V_L locus comprises at least one human Jλ (hJλ) gene segment. In another embodiment, V_L locus comprises up to four hJλ
gene segments. In one embodiment, the V_L locus comprises a contiguous sequence comprising human λ and human κ genomic sequence.

[0218] The κ light chain variable gene locus (the "κ locus") comprises at least one human Vλ (hVλ) gene segment. The κ locus comprises at least one human Jλ (hJλ) gene segment. In one embodiment, the κ locus comprises up to four hJλ gene segments. In one embodiment, the κ locus comprises at least one human hVλ and at least one human hJλ, and lacks or substantially lacks a functional Vκ region gene segment and lacks or substantially lacks a functional Jκ region gene segment. In one embodiment, the mouse comprises no functional Vκ region gene segment. In one embodiment, the mouse comprises no functional Jκ region gene

segment.

[0219] In one embodiment, the λ light chain variable gene locus (the "λ locus") comprises at least one hVλ gene segment. In one embodiment, the λ locus comprises at least one human Jλ (hJλ) gene segment. In another embodiment, the λ locus comprises up to four hJλ gene segments.

[0220] In one embodiment, the V_L locus comprises a plurality of hVλs. In one embodiment, the plurality of hVλs are selected so as to result in expression of a λ light chain variable region repertoire that reflects about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% or more of the Vλ usage observed in a human. In one embodiment, the V_L locus comprises gene segments hVλ 1-40, 1-44, 2-8, 2-14, 3-21, and a combination thereof.

[0221] In one embodiment, the hVλs include 3-1, 4-3, 2-8, 3-9, 3-10, 2-11, and 3-12. In a specific embodiment, the V_L locus comprises a contiguous sequence of the human λ light chain locus that spans from Vλ3-12 to Vλ3-1. In one embodiment, the V_L locus comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hVλs. In a specific embodiment, the hVλs include 3-1, 4-3, 2-8, 3-9, 3-10, 2-11, and 3-12. In a specific embodiment, the V_L locus comprises a contiguous sequence of the human λ locus that spans from Vλ3-12 to Vλ3-1. In one embodiment, the V_L locus is at the endogenous κ locus. In a specific embodiment, the V_L locus is at the endogenous κ locus and the endogenous λ light chain locus is deleted in part or completely. In one embodiment, the V_L locus is at the endogenous λ locus. In a specific embodiment, the V_L locus is at the endogenous λ locus and the endogenous κ locus is deleted in part or completely.

[0222] In one embodiment, the V_L locus comprises 13 to 28 or more hVλs. In a specific embodiment, the hVλs include 2-14, 3-16, 2-18, 3-19, 3-21, 3-22, 2-23, 3-25, and 3-27. In a specific embodiment, the κ locus comprises a contiguous sequence of the human λ locus that spans from Vλ3-27 to Vλ3-1. In one embodiment, the V_L locus is at the endogenous κ locus. In a specific embodiment, the V_L locus is at the endogenous κ locus and the endogenous λ light chain locus is deleted in part or completely. In another embodiment, the V_L locus is at the endogenous λ locus. In a specific embodiment, the V_L locus is at the endogenous λ locus and the endogenous κ locus is deleted in part or completely.

[0223] In one embodiment, the V_L locus comprises 29 to 40 hVλs. In a specific embodiment, the κ locus comprises a contiguous sequence of the human λ locus that spans from Vλ3-29 to Vλ3-1, and a contiguous sequence of the human λ locus that spans from Vλ5-52 to Vλ1-40. In a specific embodiment, all or substantially all sequence between hVλ1-40 and hVλ3-29 in the genetically modified mouse consists essentially of a human λ sequence of approximately 959 bp found in nature (*e.g.,* in the human population) downstream of the hVλ1-40 gene segment (downstream of the 3' untranslated portion), a restriction enzyme site (*e.g.,* PI-SceI), followed

by a human λ sequence of approximately 3,431 bp upstream of the hVλ3-29 gene segment found in nature. In one embodiment, the V_L locus is at the endogenous mouse κ locus. In a specific embodiment, the V_L locus is at the endogenous mouse κ locus and the endogenous mouse λ light chain locus is deleted in part or completely. In another embodiment, the V_L locus is at the endogenous mouse λ locus. In a specific embodiment, the V_L locus is at the endogenous mouse λ locus and the endogenous mouse κ locus is deleted in part or completely.

[0224] The V_L locus comprises at least one hJλ. In one embodiment, the V_L locus comprises a plurality of hJλs. In one embodiment, the V_L locus comprises at least 2, 3, 4, 5, 6, or 7 hJλ. In a specific embodiment, the V_L locus comprises four hJλ. In a specific embodiment, the four hJλs are hJλ1, hJλ2, hJλ3, and hJλ7. In one embodiment, the V_L locus is a κ locus. In a specific embodiment, the V_L locus is at the endogenous κ locus and the endogenous λ light chain locus is deleted in part or completely. In one embodiment, the V_L locus comprises one hJλ. In a specific embodiment, the one hJλ is hJλ1. In one embodiment, the V_L locus is at the endogenous κ locus. In a specific embodiment, the V_L locus is at the endogenous κ locus and the endogenous λ light chain locus is deleted in part or completely. In another embodiment, the V_L locus is at the endogenous λ locus. In a specific embodiment, the V_L locus is at the endogenous λ locus and the endogenous κ locus is deleted in part or completely.

[0225] The Vκ locus comprises at least one hVλ, at least one hJλ, and a mouse Cκ gene.

[0226] In one embodiment, the mouse comprises a replacement at the endogenous mouse κ locus of endogenous mouse Vκ gene segments with one or more hVλ gene segments, wherein the hVλ gene segments are operably linked to an endogenous mouse Cκ region gene, such that the mouse rearranges the human Vλ gene segments and expresses a reverse chimeric immunoglobulin light chain that comprises a human Vλ domain and a mouse Cκ. In one embodiment, 90-100% of unrearranged mouse Vκ gene segments are replaced with at least one unrearranged hVλ gene segment. In a specific embodiment, all or substantially all of the endogenous mouse Vκ gene segments are replaced with at least one unrearranged hVλ gene segment. In one embodiment, the replacement is with at least 12, at least 28, or at least 40 unrearranged hVλ gene segments. In one embodiment, the replacement is with at least 7 functional unrearranged hVλ gene segments, at least 16 functional unrearranged hVλ gene segments, or at least 27 functional unrearranged hVλ gene segments. In one embodiment, the mouse comprises a replacement of all mouse Jκ gene segments with at least one unrearranged hJλ gene segment. In one embodiment, the at least one unrearranged hJλ gene segment is selected from Jλ1, Jλ2, Jλ3, Jλ4, Jλ5, Jλ6, Jλ7, and a combination thereof. In a specific embodiment, the one or more hVλ gene segment is selected from a 3-1, 4-3, 2-8, 3-9, 3-10, 2-11, 3-12, 2-14, 3-16, 2-18, 3-19, 3-21, 3-22, 2-23, 3-25, 3-27, 1-40, 7-43, 1-44, 5-45, 7-46, 1-47, 5-48, 9-49, 1-50, 1-51, a 5-52 hVλ gene segment, and a combination thereof. In a specific embodiment, the at least one unrearranged hJλ gene segment is selected from Jλ1, Jλ2, Jλ3, Jλ7, and a combination thereof.

[0227] In one embodiment, the mouse further comprises a replacement of endogenous mouse Vλ gene segments at the endogenous mouse λ locus with one or more human Vλ gene segments at the endogenous mouse λ locus, wherein the hVλ gene segments are operably linked to a mouse Cλ region gene, such that the mouse rearranges the hVλ gene segments and expresses a reverse chimeric immunoglobulin light chain that comprises a hVλ domain and a mouse Cλ. In a specific embodiment, the mouse Cλ gene is Cλ2. In a specific embodiment, the mouse Cλ gene is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2. In one embodiment, 90-100% of unrearranged mouse Vλ gene segments are replaced with at least one unrearranged hVλ gene segment. In a specific embodiment, all or substantially all of the endogenous mouse Vλ gene segments are replaced with at least one unrearranged hVλ gene segment. In one embodiment, the replacement is with at least 12, at least 28, or at least 40 unrearranged hVλ gene segments. In one embodiment, the replacement is with at least 7 functional unrearranged hVλ gene segments, at least 16 functional unrearranged hVλ gene segments, or at least 27 functional unrearranged hVλ gene segments. In one embodiment, the mouse comprises a replacement of all mouse Jλ gene segments with at least one unrearranged hJλ gene segment. In one embodiment, the at least one unrearranged hJλ gene segment is selected from Jλ1, Jλ2, Jλ3, Jλ4, Jλ5, Jλ6, Jλ7, and a combination thereof. In a specific embodiment, the one or more hVλ gene segment is selected from a 3-1, 4-3, 2-8, 3-9, 3-10, 2-11, 3-12, 2-14, 3-16, 2-18, 3-19, 3-21, 3-22, 2-23, 3-25, 3-27, 1-40, 7-43, 1-44, 5-45, 7-46, 1-47, 5-48, 9-49, 1-50, 1-51, a 5-52 hVλ gene segment, and a combination thereof. In a specific embodiment, the at least one unrearranged hJλ gene segment is selected from Jλ1, Jλ2, Jλ3, Jλ7, and a combination thereof.

[0228] In one aspect, a genetically modified mouse is provided that comprises a human Vκ-Jκ intergenic region sequence located at an endogenous mouse κ light chain locus.

[0229] In one embodiment, the human Vκ-Jκ intergenic region sequence is at an endogenous κ light chain locus of a mouse that comprises a hVλ and hJλ gene segment, and the human Vκ-Jκ intergenic region sequence is disposed between the hVλ and hJλ gene segments. In a specific embodiment, the hVλ and hJλ gene segments are capable of recombining to form a functional human λ light chain variable domain in the mouse.

[0230] In one embodiment, a mouse is provided that comprises a plurality of hVλ's and one or more hJλ's, and the human Vκ-Jκ intergenic region sequence is disposed, with respect to transcription, downstream of the proximal or 3' most hVλ sequence and upstream or 5' of the first hJλ sequence.

[0231] In one embodiment, the human Vκ-Jκ intergenic region is a region located about 130 bp downstream or 3' of a human Vκ4-1 gene segment, about 130 bp downstream of the 3' untranslated region of the human Vκ4-1 gene segment, and spans to about 600 bp upstream or 5' of a human Jκ1 gene segment. In a specific embodiment, the human Vκ-Jκ intergenic region is about 22.8 kb in size. In one embodiment, the Vκ-Jκ intergenic region is about 90% or

more, 91% or more, 92% or more, 93% or more, 94% or more, or about 95% or more identical with a human Vκ-Jκ intergenic region extending from the end of the 3' untranslated region of a human Vκ4-1 gene segment to about 600 bp upstream of a human Jκ1 gene segment. In one embodiment, the Vκ-Jκ intergenic region comprises SEQ ID NO:158. In a specific embodiment, the Vκ-Jκ intergenic region comprises a functional fragment of SEQ ID NO:158. In a specific embodiment, the Vκ-Jκ intergenic region is SEQ ID NO:158.

[0232] Described is a non-human animal, a non-human cell (*e.g.,* an ES cell or a pluripotent cell), a non-human embryo, or a non-human tissue that comprise the recited human Vκ-Jκ intergenic region sequence, wherein the intergenic region sequence is ectopic. The ectopic sequence may be placed at a humanized endogenous non-human immunoglobulin locus. The non-human animal is a mouse.

[0233] Described is an isolated nucleic acid construct that comprises the recited human Vκ-Jκ intergenic region sequence. The nucleic acid construct may comprise targeting arms to target the human Vκ-Jκ intergenic region sequence to a mouse light chain locus. The mouse light chain locus may be a κ locus. The targeting arms may target the human Vκ-Jκ intergenic region to a modified endogenous mouse κ locus, wherein the targeting is to a position between a hVλ sequence and a hJλ sequence.

[0234] Described is a genetically modified mouse, wherein the mouse comprises no more than two light chain alleles, wherein the light chain alleles comprise (a) an unrearranged immunoglobulin human Vλ and a Jλ gene segment at an endogenous mouse κ light chain locus that comprises a mouse Cκ gene; and, (b) an unrearranged immunoglobulin V_L and a J_L gene segment at an endogenous mouse light chain locus that comprises a mouse C_L gene.

[0235] In another embodiment, the endogenous mouse light chain locus that comprises a mouse C_L gene is a λ locus.

[0236] The no more than two light chain alleles may be selected from a κ allele and a λ allele, two κ alleles, and two λ alleles. In a specific embodiment, one of the two light chain alleles is a λ allele that comprises a Cλ2 gene.

[0237] The mouse may comprise one functional immunoglobulin light chain locus and one nonfunctional light chain locus, wherein the functional light chain locus comprises an unrearranged immunoglobulin human Vλ and a Jλ gene segment at an endogenous mouse κ light chain locus that comprises a mouse Cκ gene.

[0238] Also disclosed is a mouse that comprises one functional immunoglobulin light chain locus and one nonfunctional light chain locus, wherein the functional light chain locus comprises an unrearranged immunoglobulin human Vλ and a Jλ gene segment at an endogenous mouse λ light chain locus that comprises a mouse Cλ gene. The Cλ gene may be Cλ2. The mouse Cλ gene may be at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2.

[0239] The mouse further comprises at least one immunoglobulin heavy chain allele. In one embodiment, the at least one immunoglobulin heavy chain allele comprises a human V_H gene segment, a human D_H gene segment, and a human J_H gene segment at an endogenous mouse heavy chain locus that comprises a human heavy chain gene that expresses a human/mouse heavy chain. In a specific embodiment, the mouse comprises two immunoglobulin heavy chain alleles, and the mouse expresses a human/mouse heavy chain.

[0240] In one embodiment, the mouse comprises a first light chain allele that comprises an unrearranged hVκ and an unrearranged hJκ, at an endogenous mouse κ locus that comprises an endogenous Cκ gene; and a second light chain allele that comprises an unrearranged hVλ and an unrearranged hJλ, at an endogenous mouse κ locus that comprises an endogenous Cκ gene. In a specific embodiment, the first and the second light chain alleles are the only functional light chain alleles of the genetically modified mouse. In a specific embodiment, the mouse comprises a nonfunctional λ locus. In one embodiment, the genetically modified mouse does not express a light chain that comprises a λ constant region.

[0241] In one embodiment, the mouse comprises a first light chain allele that comprises an unrearranged hVκ and an unrearranged hJκ, at an endogenous mouse κ locus that comprises an endogenous Cκ gene; and a second light chain allele that comprises an unrearranged hVλ and an unrearranged hJλ, at an endogenous mouse λ locus that comprises an endogenous Cλ gene. In a specific embodiment, the first and the second light chain alleles are the only functional light chain alleles of the genetically modified mouse. In one embodiment, the endogenous Cλ gene is Cλ2. In a specific embodiment, the mouse Cλ gene is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2.

[0242] In one embodiment, the mouse comprises six immunoglobulin alleles, wherein the first allele comprises an unrearranged immunoglobulin Vλ and Jλ gene segment at an endogenous mouse κ light chain locus that comprises a mouse Cκ gene, the second comprises an unrearranged immunoglobulin Vκ and Jκ gene segment at an endogenous mouse κ light chain locus that comprises a mouse Cκ gene, the third comprises an unrearranged immunoglobulin Vλ and Jλ gene segment at an endogenous mouse λ light chain locus that comprises a mouse Cλ gene, the fourth and fifth each independently comprise an unrearranged V_H and D_H and J_H gene segment at an endogenous mouse heavy chain locus that comprises a mouse heavy chain gene, and the sixth comprises either (a) an unrearranged immunoglobulin Vλ and Jλ gene segment at an endogenous mouse λ light chain locus that comprises a mouse Cλ gene, (b) a λ locus that is nonfunctional, or (c) a deletion in whole or in part of the λ locus.

[0243] In one embodiment, the first allele comprises an unrearranged hVλ and hJλ. In one embodiment, the second allele comprises an unrearranged hVκ and hJκ. In one embodiment, the third allele comprises an unrearranged hVλ and hJλ. In one embodiment, the fourth and fifth each independently comprise an unrearranged hV_H and hD_H and hJ_H. In one embodiment, the sixth allele comprises an endogenous mouse λ locus that is deleted in whole or in part.

[0244] In one embodiment, the mouse comprises six immunoglobulin alleles, wherein the first allele comprises an unrearranged immunoglobulin Vλ and Jλ gene segment at an endogenous mouse λ light chain locus that comprises a mouse Cλ gene, the second comprises an unrearranged immumoglobulin Vλ and Jλ gene segment at an endogenous mouse λ light chain locus that comprises a mouse Cλ gene, the third comprises an unrearranged immunoglobulin Vκ and Jκ gene segment at an endogenous mouse κ light chain locus that comprises a mouse Cκ gene, the fourth and fifth each independently comprise an unrearranged V_H and D_H and J_H gene segment at an endogenous mouse heavy chain locus that comprises a mouse heavy chain gene, and the sixth comprises either (a) an unrearranged immunoglobulin Vκ and Jκ gene segment at an endogenous mouse κ light chain locus that comprises a mouse Cκ gene, (b) a κ locus that is nonfunctional, or (c) a deletion of one or more elements of the κ locus.

[0245] In one embodiment, the first allele comprises an unrearranged hVλ and hJλ gene segment. In one embodiment, the second allele comprises an unrearranged hVλ and hJλ gene segment. In one embodiment, the third allele comprises an unrearranged hVκ and hJκ gene segment. In one embodiment, the fourth and fifth each independently comprise an unrearranged hV_H and hD_H and hJ_H gene segment. In one embodiment, the sixth allele comprises an endogenous mouse κ locus that is functionally silenced.

[0246] In one embodiment, the genetically modified mouse comprises a B cell that comprises a rearranged antibody gene comprising a rearranged hVλ domain operably linked to a mouse C_L domain. In one embodiment, the mouse C_L domain is selected from a mouse Cκ and a mouse Cλ domain. In a specific embodiment, the mouse Cλ domain is derived from a Cλ2 gene. In a specific embodiment, the mouse Cλ domain is derived from a Cλ domain that is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2.

[0247] Described is a genetically modified mouse that expresses a Vλ region on a C_L that is a Cκ. Described is a genetically modified mouse that expresses a hVλ region on a C_L selected from a human Cκ, a human Cλ, or a mouse Cκ. Described is a genetically modified mouse that expresses a hVλ region on a mouse Cκ.

[0248] In one embodiment, about 10-50% of the splenocytes of the mouse are B cells (*i.e.,* CD19-positive), or which about 9-28% express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0249] In a specific embodiment, about 23-34% of the splenocytes of the mouse are B cells (*i.e.,* CD19-positive), or which about 9-11% express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0250] In a specific embodiment, about 19-31% of the splenocytes of the mouse are B cells (*i.e.,* CD19-positive), or which about 9-17% express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0251] In a specific embodiment, about 21-38% of the splenocytes of the mouse are B cells (*i.e.,* CD19-positive), or which about 24-27% express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0252] In a specific embodiment, about 10-14% of the splenocytes of the mouse are B cells (*i.e.,* CD19-positive), or which about 9-13% express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0253] In a specific embodiment, about 31-48% of the splenocytes of the mouse are B cells *(i.e.,* CD19-positive), or which about 15-21% express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain. In a specific embodiment, about 30-38% of the splenocytes of the mouse are B cells (*i.e.,* CD19-positive), of which about 33-48% express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0254] In one embodiment, about 52-70% of the bone marrow of the mouse are B cells (*i.e.,* **CD**19-positive), or which about 31-47% of the immature B cells (*i.e.,* CD19-positive/B220-intermediate positive/IgM-positive) express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0255] In one embodiment, about 60% of the bone marrow of the mouse are B cells (*i.e.,* CD19-positive), or which about 38.3% of the immature B cells (*i.e.,* CD19-positive/B220-intermediate positive/IgM-positive) express an immunoglobulin light chain comprising a hVλ domain fused to a mouse Cκ domain.

[0256] In one embodiment, the mouse expresses an antibody comprising a light chain that comprises a variable domain derived from a human V and a human J gene segment, and a constant domain derived from a mouse constant region gene. In one embodiment, the mouse constant region gene is a Cκ gene. In another embodiment, the mouse constant region gene is a Cλ gene. In a specific embodiment, the Cλ region is Cλ2. In a specific embodiment, the mouse Cλ gene is derived from a Cλ gene that is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2. In a specific embodiment, the antibody further comprises a heavy chain comprising a variable domain derived from a human V, a human D and a human J gene segment, and a heavy chain constant domain derived from a mouse heavy chain constant region gene. In one embodiment, the mouse heavy chain constant region gene comprises a hinge-CH2-CH3 sequence of a heavy chain constant domain. In another embodiment, the mouse heavy chain constant region gene comprises a CH1-hinge-CH2-CH3 sequence of a heavy chain constant domain. In another embodiment, the mouse heavy chain constant region gene comprises a CH1-CH2-CH3-CH4 sequence of a heavy chain constant domain. In another embodiment, the mouse heavy chain constant region gene comprises a CH2-CH3-CH4 sequence of a heavy chain constant domain.

[0257] In one embodiment, the mouse expresses an antibody comprising a light chain that comprises a rearranged human Vλ-Jλ sequence and a mouse Cκ sequence. In one embodiment, the rearranged human Vλ-Jλ sequence is derived from a rearrangement of hVλ

gene segments selected from a 3-1, 4-3, 2-8, 3-9, 3-10, 2-14, 3-19, 2-23, 3-25, 1-40, 7-43, 1-44, 5-45, 7-46, 1-47, 9-49, and a 1-51 gene segment. In one embodiment, the rearranged human Vλ-Jλ sequence is derived from a rearrangement of hJλ gene segments selected from Jλ1, Jλ2, Jλ3, and a Jλ7 gene segment.

[0258] In one embodiment, the mouse expresses an antibody comprising a light chain that comprises a rearranged immunoglobulin λ light chain variable region comprising a human Vλ/Jλ sequence selected from 3-1/1, 3-1/7, 4-3/1, 4-3/7, 2-8/1, 3-9/1, 3-10/1, 3-10/3, 3-10/7, 2-14/1, 3-19/1, 2-23/1, 3-25/1, 1-40/1, 1-40/2, 1-40/3, 1-40/7, 7-43/1, 7-43/3, 1-44/1, 1-44/7, 5-45/1, 5-45/2, 5-45/7, 7-46/1, 7-46/2, 7-46/7, 9-49/1, 9-49/2, 9-49/7 and 1-51/1. In a specific embodiment, the B cell expresses an antibody comprising a human immunoglobulin heavy chain variable domain fused with a mouse heavy chain constant domain, and a human immunoglobulin λ light chain variable domain fused with a mouse κ light chain constant domain.

[0259] Described is a mouse that expresses an antibody comprising (a) a heavy chain comprising a heavy chain variable domain derived from an unrearranged human heavy chain variable region gene segment, wherein the heavy chain variable domain is fused to a mouse heavy chain constant (C_H) region; and, (b) a light chain comprising a light chain variable domain derived from an unrearranged hVλ and a hJλ, wherein the light chain variable domain is fused to a mouse C_L region.

[0260] In one embodiment, the mouse comprises (i) a heavy chain locus that comprises a replacement of all or substantially all functional endogenous mouse V, D and J gene segments with all or substantially all functional human V, D, and J gene segments, a mouse C_H gene, (ii) a first κ light chain locus comprising a replacement of all or substantially all functional endogenous mouse Vκ and Jκ gene segments with all, substantially all, or a plurality of, functional hVλ and hJλ gene segments, and a mouse C| gene, (iii) a second κ light chain locus comprising a replacement of all or substantially all functional endogenous mouse Vκ and Jκ gene segments with all, substantially all, or a plurality of, functional hVκ and hJκ gene segments, and a mouse Cκ gene. In one embodiment, the mouse does not express an antibody that comprises a Cλ region. In one embodiment, the mouse comprises a deletion of a Cλ gene and/or a Vλ and/or a Jλ gene segment. In one embodiment, the mouse comprises a nonfunctional λ light chain locus. In a specific embodiment, the λ light chain locus is deleted in whole or in part.

[0261] In one embodiment, the mouse comprises (i) a heavy chain locus that comprises a replacement of all or substantially all functional endogenous mouse V, D and J gene segments with all or substantially all functional human V, D, and J gene segments, a mouse C_H gene, (ii) a first λ light chain locus comprising a replacement of all or substantially all functional endogenous mouse Vλ and Jλ gene segments with all, substantially all, or a plurality of, functional hVλ and hJλ gene segments, and a mouse Cλ gene, (iii) a second λ light chain locus comprising a replacement of all or substantially all functional endogenous mouse Vλ and Jλ

gene segments with all, substantially all, or a plurality of, functional hVλ and hJλ gene segments, and a mouse Cλ gene. In a specific embodiment, the mouse Cλ gene is Cλ2. In a specific embodiment, the mouse Cλ gene is derived from a Cλ gene that is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2.

[0262] In one embodiment, the mouse comprises a deletion of a Cκ gene and/or a Vκ and/or a Jκ gene segment. In one embodiment, the mouse comprises a nonfunctional κ light chain locus.

[0263] Described is a genetically modified mouse that expresses an antibody, wherein greater than 10%, greater than 15%, greater than 20%, greater than 25%, greater than 30%, greater than 35%, greater than 40%, greater than 60%, greater than 70%, greater than 80%, or greater than 90% of total IgG antibody produced by the mouse comprises a λ-derived variable domain, and wherein the mouse expresses antibodies comprising a κ-derived variable domain fused with a mouse Cκ region. About 15-40%, 20-40%, 25-40%, 30-40%, or 35-40% of total antibody produced by the mouse may comprise a λ-derived variable domain.

[0264] The λ-derived variable domain is derived from a hVλ and a hJλ. The λ-derived variable domain is in a light chain that comprises a mouse Cκ region. In one embodiment, the κ-derived variable domain is derived from a hVκ and a hJκ, and in a specific embodiment is in a light chain that comprises a mouse Cκ region.

[0265] Described is an isolated DNA construct that comprises an upstream homology arm and a downstream homology arm, wherein the upstream and the downstream homology arms target the construct to a mouse κ locus, and the construct comprises a functional unrearranged hVλ segment and a functional unrearranged hJλ segment, and a selection or marker sequence.

[0266] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the direction of transcription, a targeting arm for targeting a mouse λ sequence upstream of mouse Vλ2, a selection cassette flanked 5' and 3' with recombinase recognition sites, and a targeting arm for targeting a mouse λ sequence 3' of mouse Jλ2. In one embodiment, the selection cassette is a Frt'ed Hyg-TK cassette. The 3' targeting arm may comprise mouse Cλ2. Jλ4, Cλ4, and mouse enhancer 2.4.

[0267] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the direction of transcription, a targeting arm for targeting the mouse λ locus 5' with respect to Vλ1, a selection cassette flanked 5' and 3' with recombinase recognition sites, and a 3' targeting arm for targeting a mouse λ sequence 3' with respect to mouse Cλ1. The selection cassette may be a loxed neomycin cassette. The 3' targeting arm may comprise the mouse λ 3' enhancer and mouse λ 3' enhancer 3.1.

[0268] Described is an isolated DNA construct, comprising from 5' to 3' with respect to the direction of transcription, a targeting arm for targeting the mouse λ locus 5' with respect to Vλ2,

a selection cassette flanked 5' and 3' with recombinase recognition sites, and a 3' targeting arm for targeting a mouse λ sequence 3' with respect to mouse Jλ2 and 5' with respect to mouse Cλ2. The selection cassette may be a Frt'ed hygromycin-TK cassette. The 3' targeting arm may comprise the mouse Cλ2-Jλ4-Cλ4 gene segments and mouse λ enhancer 2.4.

[0269] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the direction of transcription, a targeting arm for targeting the mouse λ locus 5' with respect to Vλ2, a selection cassette flanked 5' and 3' with recombinase recognition sites, a human genomic fragment comprising a contiguous region of the human λ light chain locus from hVλ3-12 downstream to the end of hJλ1, and a 3' targeting arm for targeting a mouse λ sequence 3' with respect to mouse Jλ2. The selection cassette is a Frt'ed neomycin cassette. The 3' targeting arm comprises the mouse Cλ2-Jλ4-Cλ4 gene segments and mouse λ enhancer 2.4.

[0270] Described is an isolated DNA construct, comprising a contiguous region of the human λ light chain locus from hVλ3-12 downstream to the end of hJλ1.

[0271] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the direction of transcription, a targeting arm for targeting the mouse λ locus 5' with respect to Vλ2, a selection cassette flanked 5' and 3' with recombinase recognition sites and a human genomic fragment comprising a contiguous region of the human λ light chain locus from hVλ3-27 downstream to the end of hVλ2-8. The selection cassette may be a Frt'ed hygromycin cassette. The human genomic fragment may comprise a 3' targeting arm. The 3' targeting arm may comprise about 53 kb of the human λ light chain locus from hVλ3-12 downstream to the end of hVλ2-8.

[0272] Described is an isolated DNA construct, comprising a contiguous region of the human λ light chain locus from hVλ3-27 downstream to the end of hVλ3-12.

[0273] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the direction of transcription, a targeting arm for targeting the mouse λ locus 5' with respect to Vλ2, a selection cassette flanked 5' and 3' with recombinase recognition sites, a first human genomic fragment comprising a contiguous region of the human λ light chain locus from hVλ5-52 downstream to the end of hVλ1-40, a restriction enzyme site, and a second human genomic fragment comprising a contiguous region of the human λ light chain locus from hVλ3-29 downstream to the end of hVλ82K. The selection cassette may be a Frt'ed neomycin cassette. The restriction enzyme site may be a site for a homing endonuclease. The homing endonuclease may be PI-SceI. The second human genomic fragment may be a 3' targeting arm. The 3' targeting arm may comprise about 27 kb of the human λ light chain locus from hVλ3-29 downstream to the end of hVλ82K.

[0274] Described is an isolated DNA construct, comprising a contiguous region of the human λ light chain locus from hVλ5-52 downstream to the end of hVλ1-40.

[0275] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the

direction of transcription, a targeting arm for targeting the mouse κ locus 5' with respect to the endogenous Vκ gene segments, two juxtaposed recombinase recognition sites, a selection cassette 3' to the juxtaposed recombinase recognition sites, and a 3' targeting arm for targeting a mouse κ sequence 5' with respect to the κ light chain variable gene segments. The juxtaposed recombinase recognition sites may be in opposite orientation with respect to one another. The recombinase recognition sites may be different. The recombinase recognition sites may be a *lox*P site and a *lox*511 site. The selection cassette may be a neomycin cassette.

[0276] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the direction of transcription, a targeting arm for targeting the mouse κ locus 5' with respect to the mouse Jκ gene segments, a selection cassette, a recombinase recognition site 3' to the selection cassette, and a 3' targeting arm for targeting a mouse κ sequence 3' with respect to the mouse Jκ gene segments and 5' to the mouse κ intronic enhancer. The selection cassette may be a hygromycin-TK cassette. The recombinase recognition site may be in the same direction with respect to transcription as the selection cassette. The recombinase recognition site may be a *lox*P site.

[0277] Described is an isolated DNA construct, comprising, from 5' to 3' with respect to the direction of transcription, a first mouse genomic fragment comprising sequence 5' of the endogenous mouse Vκ gene segments, a first recombinase recognition site, a second recombinase recognition site, and a second mouse genomic fragment comprising sequence 3' of the endogenous mouse Jκ gene segments and 5' of the mouse κ intronic enhancer.

[0278] Described is a genetically modified mouse, wherein the genetic modification comprises a modification with one or more of the DNA constructs described above or herein.

[0279] Described is use of an isolated DNA construct to make a mouse as described herein. Described is use of an isolated DNA construct as described herein in a method for making an antigen-binding protein.

[0280] Described is a non-human stem cell that comprises a targeting vector that comprises a DNA construct as described above and herein. In one aspect, a non-human stem cell is provided, wherein the non-human stem cell is derived from a mouse described herein.

[0281] In one embodiment, the non-human stem cell is an embryonic stem (ES) cell. In a specific embodiment, the ES cell is a mouse ES cell.

[0282] Described is use of a non-human stem cell as described herein to make a mouse as described herein. Described is use of a non-human stem cell as described herein to make an antigen-binding protein.

[0283] In one aspect, a mouse embryo is provided, wherein the mouse embryo comprises a genetic modification as provided herein. In one embodiment, a host mouse embryo that comprises a donor ES cell is provided, wherein the donor ES cell comprises a genetic

modification as described herein. In one embodiment, the mouse embryo is a pre-morula stage embryo. In a specific embodiment, the pre-morula stage embryo is a 4-cell stage embryo or an 8-cell stage embryo. In another specific embodiment, the mouse embryo is a blastocyst.

[0284] Described is use of a mouse embryo as described herein to make a mouse as described herein. Described is use of a mouse embryo as described herein to make an antigen-binding protein.

[0285] Described is a non-human cell, wherein the non-human cell comprises a rearranged immunoglobulin light chain gene sequence derived from a genetically modified mouse as described herein. The cell may be a B cell. The cell may be a hybridoma. The cell may encode an immunoglobulin light chain variable domain and/or an immunoglobulin heavy chain variable domain that is somatically mutated.

[0286] Described is a non-human cell, wherein the non-human cell comprises a rearranged immunoglobulin light chain gene sequence derived from a genetically modified mouse as described herein. The cell may be a B cell. The cell may be a hybridoma. The cell may encode an immunoglobulin light chain variable domain and/or an immunoglobulin heavy chain variable domain that is somatically mutated.

[0287] Described is use of a non-human cell as described herein to make a non-human animal as described herein. Described is use of a non-human cell as described herein to make an antigen-binding protein. The non-human animal is a mouse.

[0288] Described is a mouse B cell that expresses an immunoglobulin light chain that comprises (a) a variable region derived from a hVλ gene segment and a hJλ gene segment; and, (b) a mouse Cκ gene. The mouse B cell may further express a cognate heavy chain that comprises (c) a variable region derived from a hV_H, a hD_H, and (d) a hJ_H segment. In one embodiment, the B cell does not comprise a rearranged λ gene. The B cell may not comprise a rearranged κ gene.

[0289] Described is a method for making an antibody in a genetically modified non-human animal, comprising: (a) exposing a genetically modified non-human animal to an antigen, wherein the animal has a genome comprising at least one hVλ and at least one hJλ at an endogenous mouse κ light chain locus, wherein the endogenous κ light chain locus comprises a mouse Cκ gene; (b) allowing the genetically modified animal to develop an immune response to the antigen; and, (c) isolating from the animal of (b) an antibody that specifically recognizes the antigen, or isolating from the animal of (b) a cell comprising an immunoglobulin domain that specifically recognizes the antigen, wherein the antibody comprises a light chain derived from a hVλ a hJλ and a mouse Cκ gene. The non-human animal is a mouse.

[0290] Described is a method for making an antibody in a genetically modified non-human animal, comprising: (a) exposing a genetically modified animal to an antigen, wherein the animal has a genome comprising at least one hVλ at an endogenous κ locus and at least one

hJλ at the κ locus, wherein the κ locus comprises a mouse Cκ gene; (b) allowing the genetically modified animal to develop an immune response to the antigen; and, (c) isolating from the animal of (b) an antibody that specifically recognizes the antigen, or isolating from the mouse of (b) a cell comprising an immunoglobulin domain that specifically recognizes the antigen, wherein the antibody comprises a light chain derived from a hVλ a hJλ and a mouse CK gene.

[0291] [Deleted].

[0292] Described is, a method for making an antibody in a genetically modified non-human animal, comprising: (a) exposing a genetically modified non-human animal to an antigen, wherein the animal has a genome comprising at least one hVλ at a λ light chain locus and at least one Jλ at the λ light chain locus, wherein the λ light chain locus comprises a non-human CA gene; (b) allowing the genetically modified animal to develop an immune response to the antigen; and, (c) isolating from the animal of (b) an antibody that specifically recognizes the antigen, or isolating from the animal of (b) a cell comprising an immunoglobulin domain that specifically recognizes the antigen, or identifying in the animal of B a nucleic acid sequence encoding a heavy and/or light chain variable domain that binds the antigen, wherein the antibody comprises a light chain derived from a hVλ, a hJλ and a non-human Cλ gene. The non-human animal is a mouse.

[0293] The λ light chain constant gene may be selected from a human Cλ gene and a non-human Cλ gene. The λ light chain constant gene may be a human Cλ gene. The human Cλ gene may be selected from Cλ1, Cλ2, Cλ3 and Cλ7. The λ light chain constant gene may be a mouse or rat Cλ gene. The mouse Cλ gene may be selected from Cλ1, Cλ2 and Cλ3. The mouse Cλ gene may be Cλ2. The mouse Cλ gene may be derived from a Cλ gene that is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2.

[0294] Described is a method for making a rearranged antibody gene in a genetically modified mouse, comprising: (a) exposing a genetically modified mouse to an antigen, wherein the genetic modification comprises a hVλ and a hJλ at an endogenous κ light chain locus, wherein the endogenous κ light chain locus comprises a mouse Cκ gene or functional fragment thereof; and, (b) identifying a rearranged immunoglobulin gene in said mouse, wherein the rearranged immunoglobulin gene comprises a λ light chain variable region gene segment and a mouse Cκ gene or functional fragment thereof.

[0295] The method may further comprise cloning a nucleic acid sequence encoding a heavy and/or light chain variable region from the mouse, wherein the heavy and/or light chain variable region is from an antibody that comprises a human Vλ and a mouse Cκ.

[0296] [Deleted].

[0297] Described is a method for making a rearranged antibody gene in a genetically modified mouse, comprising: (a) exposing a genetically mouse to an antigen, wherein the genetic

modification comprises a hVλ and a hJλ at a κ light chain locus, wherein the κ light chain locus comprises a mouse Cκ gene or functional fragment thereof; and, (b) identifying a rearranged immunoglobulin gene in said mouse, wherein the rearranged immunoglobulin gene comprises a λ light chain variable region gene segment and a mouse Cκ gene or functional fragment thereof.

[0298] [Deleted].

[0299] The method may further comprise cloning a nucleic acid sequence encoding a heavy and/or light chain variable region from the mouse, wherein the heavy and/or light chain variable region is from an antibody that comprises a human Vλ and a mouse Cκ.

[0300] Described is a method for making a rearranged antibody gene in a genetically modified mouse, comprising: (a) exposing a genetically modified mouse to an antigen, wherein the genetic modification comprises a hVλ and a hJλ at a non-human λ light chain locus, wherein the λ light chain locus comprises a non-human Cλ gene or functional fragment thereof; and, (b) identifying a rearranged immunoglobulin gene in said mouse, wherein the rearranged immunoglobulin gene comprises a λ light chain variable region gene segment and a Cλ gene or functional fragment thereof.

[0301] The λ light chain constant gene or functional fragment thereof may be selected from a human Cλ gene and a mouse or rat Cλ gene, or a functional fragment thereof. The λ light chain constant gene may be a mouse or rat Cλ gene, or a functional fragment thereof.

[0302] The method may further comprise cloning a nucleic acid sequence encoding a heavy and/or light chain variable region from the mouse, wherein the heavy and/or light chain variable region is from an antibody that comprises a human Vλ and a non-human (e.g., mouse or rat) Cλ.

[0303] In one aspect, a method for making an antibody is provided, comprising exposing a mouse as described herein to an antigen, allowing the mouse to mount an immune response that comprises making an antibody that specifically binds the antigen, identifying a rearranged nucleic acid sequence in the mouse that encodes heavy chain and a rearranged nucleic acid sequence in the mouse that encodes a cognate light chain variable domain sequence of an antibody, wherein the antibody specifically binds the antigen, and employing the nucleic acid sequences of the heavy and light chain variable domains fused to human constant domains to make a desired antibody, wherein the desired antibody comprises a light chain that comprises a Vλ domain fused to a mouse Cκ domain..

[0304] In one embodiment, a method for making an antibody is provided, comprising exposing a mouse as described herein to an antigen, allowing the mouse to mount an immune response that comprises making an antibody that specifically binds the antigen, identifying a rearranged nucleic acid sequence in the mouse that encodes a heavy chain and a rearranged nucleic acid sequence in the mouse that encodes a cognate light chain variable domain sequence of an

antibody, wherein the antibody specifically binds the antigen, and employing the nucleic acid sequences of the heavy and light chain variable domains fused to nucleic acid sequences of human constant domains to make a desired antibody, wherein the desired antibody comprises a light chain that comprises a Vλ domain fused to a Cκ domain.

[0305] In one embodiment, a method for making an antibody is provided, comprising exposing a mouse as described herein to an antigen, allowing the mouse to mount an immune response that comprises making an antibody that specifically binds the antigen, identifying a rearranged nucleic acid sequence in the mouse that encodes a heavy chain variable domain and a rearranged nucleic acid sequence that encodes a cognate light chain variable domain sequence of an antibody, wherein the antibody specifically binds the antigen, and employing the nucleic acid sequences fused to nucleic acid sequences that encode a human heavy chain constant domain and a human light chain constant domain to make an antibody derived from human sequences, wherein the antibody that specifically binds the antigen comprises a light chain that comprises a human Vλ domain fused to a non-human (e.g., mouse or rat) Cλ region.

[0306] In one embodiment, the Cλ region is mouse, and in one embodiment is selected from Cλ1, Cλ2 and Cλ3. In a specific embodiment, the mouse Cλ region is Cλ2.

[0307] In one aspect, a method for making a rearranged antibody light chain variable region gene sequence is provided, comprising (a) exposing a mouse as described herein to an antigen; (b) allowing the mouse to mount an immune response; (c) identifying a cell in the mouse that comprises a nucleic acid sequence that encodes a rearranged human Vλ domain sequence fused with a mouse Cκ domain, wherein the cell also encodes a cognate heavy chain comprising a human V_H domain and a non-human C_H domain, and wherein the cell expresses an antibody that binds the antigen; (d) cloning from the cell a nucleic acid sequence encoding the human Vλ domain and a nucleic acid sequence encoding the cognate human V_H domain; and, (e) using the cloned nucleic acid sequence encoding the human Vλ domain and the cloned nucleic acid sequence encoding the cognate human V_H domain to make a fully human antibody.

[0308] In one embodiment, a method for making a rearranged antibody light chain variable region gene sequence is provided, comprising (a) exposing a mouse as described in this disclosure to an antigen; (b) allowing the mouse to mount an immune response; (c) identifying a cell in the mouse that comprises a nucleic acid sequence that encodes a rearranged human Vλ domain sequence contiguous on the same nucleic acid molecule with a nucleic acid sequence encoding a Cκ domain of the mouse, wherein the cell also encodes a cognate heavy chain comprising a human V_H domain and a C_H domain of the mouse, and wherein the cell expresses an antibody that binds the antigen; (d) cloning from the cell a nucleic acids sequence encoding the human Vλ domain and a nucleic acid sequence encoding the cognate human V_H domain; and, (e) using the cloned nucleic acid sequence encoding the human Vλ domain and the cloned nucleic acid sequence encoding the cognate human V_H domain to

make a fully human antibody.

[0309] In one embodiment, a method for making a rearranged antibody light chain variable region gene sequence is provided, comprising (a) exposing a mouse as described herein to an antigen; (b) allowing the mouse to mount an immune response to the antigen; (c) identifying a cell in the mouse that comprises DNA that encodes a rearranged human Vλ domain sequence fused with a non-human Cλ domain of the mouse, wherein the cell also encodes a cognate heavy chain comprising a human V_H domain and a non-human C_H domain of the mouse, and wherein the cell expresses an antibody that binds the antigen; (d) cloning from the cell a nucleic acid sequence encoding the rearranged human Vλ domain and a nucleic acid sequence encoding the cognate human V_H domain; and, (e) using the cloned nucleic acid sequence encoding the human Vλ domain and the cloned nucleic acid sequence encoding the cognate human V_H domain to make a fully human antibody. In one embodiment, the non-human animal is mouse and the Cλ domain is mouse Cλ2. In a specific embodiment, the mouse Cλ domain is derived from a Cλ gene that is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2.

[0310] Described is a genetically modified non-human animal that expresses a human λ-derived light chain fused to an endogenous light chain constant region (C_L), wherein the animal, upon immunization with antigen, makes an antibody comprising a human Vλ domain fused to a non-human C_L domain of the animal. The non-human C_L domain may be selected from a Cκ domain and a Cλ domain. The C_L domain may be a Cκ domain. The animal is a mouse. The mouse C_L domain may be a Cλ domain. The Cλ domain may be Cλ2. The mouse Cλ domain may be derived from a Cλ gene that is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% identical to mouse Cλ2.

[0311] Described is a genetically modified non-human animal comprising a modified endogenous κ light chain locus as described herein that expresses a plurality of immunoglobulin λ light chains associated with a plurality of immunoglobulin heavy chains. The heavy chain may comprise a human sequence. The human sequence may be selected from a variable sequence, a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof. The plurality of immunoglobulin λ light chains may comprise a human sequence. The human sequence may be selected from a variable sequence, a constant sequence, and a combination thereof. The animal may comprise a disabled endogenous immunoglobulin locus and expresses the heavy chain and/or the λ light chain from a transgene or extrachromosomal episome. The animal may comprise a replacement at an endogenous (non-human) locus of some or all endogenous non-human heavy chain gene segments (*i.e.,* V, D, J), and/or some or all endogenous non-human heavy chain constant sequences (*e.g.,* C_H1, hinge, C_H2, C_H3, or a combination thereof), and/or some or all endogenous non-human light chain sequences (*e.g.,* V, J, constant, or a combination thereof), with one or more human immunoglobulin sequences. The non-human animal is a mouse.

[0312] Described is a non-human animal suitable for making antibodies that have a human λ-

derived light chain, wherein all or substantially all antibodies made in the non-human animal are expressed with a human λ-derived light chain. The human λ-derived light chain may be expressed from an endogenous light chain locus. The endogenous light chain locus may be a κ light chain locus. The animal is a mouse and the κ light chain locus may be a mouse κ light chain locus.

[0313] In one aspect, a method for making a λ-derived light chain for a human antibody is provided, comprising obtaining from a mouse as described herein a light chain sequence and a heavy chain sequence, and employing the light chain sequence and the heavy chain sequence in making a human antibody.

[0314] In one aspect, a method for making an antigen-binding protein is provided, comprising exposing a mouse as described herein to an antigen; allowing the mouse to mount an immune response; and obtaining from the mouse an antigen-binding protein that binds the antigen, or obtaining from the mouse a sequence to be employed in making an antigen-binding protein that binds the antigen.

[0315] In one aspect, a cell derived from a mouse as described herein is provided. In one embodiment, the cell is selected from an embryonic stem cell, a pluripotent cell, an induced pluripotent cell, a B cell, and a hybridoma.

[0316] In one aspect, a cell is provided that comprises a genetic modification as described herein. In one embodiment, the cell is a mouse cell. In one embodiment, the cell is selected from a hybridoma and a quadroma. In one embodiment, the cell expresses an immunoglobulin light chain that comprises a human λ variable sequence fused with a mouse κ constant sequence. In a specific embodiment, the mouse constant sequence is a mouse κ constant sequence.

[0317] In one aspect, a tissue derived from a mouse as described herein is provided.

[0318] In one aspect, use of a mouse or a cell as described herein to make an antigen-binding protein is provided. In one embodiment, the antigen-binding protein is a human protein. In one embodiment, the human protein is a human antibody.

[0319] Described is an antigen-binding protein made by a mouse animal, cell, tissue, or method as described herein. The antigen-binding protein may be a human protein. The human protein may be a human antibody.

[0320] Any of the embodiments and aspects described herein can be used in conjunction with one another, unless otherwise indicated or apparent from the context. Other embodiments will become apparent to those skilled in the art from a review of the ensuing description.

BRIEF DESCRIPTION OF THE FIGURES

[0321]

FIG. 1A shows a general illustration, not to scale, of direct genomic replacement of about three megabases (Mb) of a mouse immunoglobulin heavy chain variable gene locus (closed symbols) with about one megabase (Mb) of the human immunoglobulin heavy chain variable gene locus (open symbols).

FIG. 1B shows a general illustration, not to scale, of direct genomic replacement of about three megabases (Mb) of a mouse immunoglobulin κ light chain variable gene locus (closed symbols) with about 0.5 megabases (Mb) of the first, or proximal, of two nearly identical repeats of the human immunoglobulin κ light chain variable gene locus (open symbols).

FIG. 2A shows a detailed illustration, not to scale, of three initial steps (A-C) for direct genomic replacement of a mouse immunoglobulin heavy chain variable gene locus that results in deletion of all mouse V_H, D_H and J_H gene segments and replacement with three human V_H, all human D_H and J_H gene segments. A targeting vector for a first insertion of human immunoglobulin heavy chain gene segments is shown (3hV_H BACvec) with a 67 kb 5' mouse homology arm, a selection cassette (open rectangle), a site-specific recombination site (open triangle), a 145 kb human genomic fragment and an 8 kb 3' mouse homology arm. Human (open symbols) and mouse (closed symbols) immunoglobulin gene segments, additional selection cassettes (open rectangles) and site-specific recombination sites (open triangles) inserted from subsequent targeting vectors are shown.

FIG. 2B shows a detailed illustration, not to scale, of six additional steps (D-I) for direct genomic replacement of a mouse immunoglobulin heavy chain variable gene locus that results in the insertion of 77 additional human V_H gene segments and removal of a final selection cassette. A targeting vector for insertion of additional human V_H gene segments (18hV_H BACvec) to the initial insertion of human heavy chain gene segments (3hV_H-CRE Hybrid Allele) is shown with a 20 kb 5' mouse homology arm, a selection cassette (open rectangle), a 196 kb human genomic fragment and a 62 kb human homology arm that overlaps with the 5' end of the initial insertion of human heavy chain gene segments which is shown with a site-specific recombination site (open triangle) located 5' to the human gene segments. Human (open symbols) and mouse (closed symbols) immunoglobulin gene segments and additional selection cassettes (open rectangles) inserted by subsequent targeting vectors are shown.

FIG. 2C shows a detailed illustration, not to scale, of three initial steps (A-C) for direct genomic replacement of a mouse immunoglobulin κ light chain variable gene locus that results in deletion of all mouse Vκ, and Jκ gene segments (Igκ-CRE Hybrid Allele). Selection cassettes (open rectangles) and site-specific recombination sites (open triangles) inserted from the targeting vectors are shown.

FIG. 2D shows a detailed illustration, not to scale, of five additional steps (D-H) for direct genomic replacement of a mouse immunoglobulin κ light chain variable gene locus that results in the insertion of all human Vκ and Jκ gene segments in the proximal repeat and deletion of

the final selection cassette (40hVκdHyg Hybrid Allele). Human (open symbols) and mouse (closed symbols) immunoglobulin gene segments and additional selection cassettes (open rectangles) inserted by subsequent targeting vectors are shown.

FIG. 3A shows a general illustration, not to scale, of a screening strategy including the locations of quantitative PCR (qPCR) primer/probe to detect insertion of human heavy chain gene sequences and loss of mouse heavy chain gene sequences in targeted embryonic stem (ES) cells. The screening strategy in ES cells and mice for a first human heavy gene insertion is shown with qPCR primer/probe sets for the deleted region ("loss" probes C and D), the region inserted ("hIgH" probes G and H) and flanking regions ("retention" probes A, B, E and F) on an unmodified mouse chromosome (top) and a correctly targeted chromosome (bottom).

FIG. 3B shows a representative calculation of observed probe copy number in parental and modified ES cells for a first insertion of human immunoglobulin heavy chain gene segments. Observed probe copy number for probes A through F were calculated as $2/2^{\Delta\Delta Ct}$. ΔΔCt is calculated as ave[ΔCt(sample) - medΔCt(control)] where ΔCt is the difference in Ct between test and reference probes (between 4 and 6 reference probes depending on the assay). The term medΔCt(control) is the median ΔCt of multiple (>60) non-targeted DNA samples from parental ES cells. Each modified ES cell clone was assayed in sextuplicate. To calculate copy numbers of IgH probes G and H in parental ES cells, these probes were assumed to have copy number of 1 in modified ES cells and a maximum Ct of 35 was used even though no amplification was observed.

FIG. 3C shows a representative calculation of copy numbers for four mice of each genotype calculated using only probes D and H. Wild-type mice: WT Mice; Mice heterozygous for a first insertion of human immunoglobulin gene segments: HET Mice; Mice homozygous for a first insertion of human immunoglobulin gene segments: Homo Mice.

FIG. 4A shows a detailed illustration, not to scale, of the three steps employed for construction of a $3hV_H$ BACvec by bacterial homologous recombination (BHR). Human (open symbols) and mouse (closed symbols) immunoglobulin gene segments, selection cassettes (open rectangles) and site-specific recombination sites (open triangles) inserted from targeting vectors are shown.

FIG. 4B shows pulse-field gel electrophoresis (PFGE) of three BAC clones (B1, B2 and B3) after NotI digestion. Markers M1, M2 and M3 are low range, mid range and lambda ladder PFG markers, respectively (New England BioLabs, Ipswich, MA).

FIG. 5A shows a schematic illustration, not to scale, of sequential modifications of the mouse immunoglobulin heavy chain locus with increasing amounts of human immunoglobulin heavy chain gene segments. Homozygous mice were made from each of the three different stages of heavy chain humanization. Open symbols indicate human sequence; closed symbols indicate mouse sequence.

FIG. 5B shows a schematic illustration, not to scale, of sequential modifications of the mouse immunoglobulin κ light chain locus with increasing amounts of human immunoglobulin κ light

chain gene segments. Homozygous mice were made from each of the three different stages of κ light chain humanization. Open symbols indicate human sequence; closed symbols indicate mouse sequence.

FIG. 6 shows FACS dot plots of B cell populations in wild type and VELOCIMMUNE® humanized mice. Cells from spleen (top row, third row from top and bottom row) or inguinal lymph node (second row from top) of wild type (wt), VELOCIMMUNE® 1 (V1), VELOCIMMUNE® 2 (V2) or VELOCIMMUNE® 3 (V3) mice were stained for surface IgM expressing B cells (top row, and second row from top), surface immunoglobulin containing either κ or λ light chains (third row from top) or surface IgM of specific haplotypes (bottom row), and populations separated by FACS.

FIG. 7A shows representative heavy chain CDR3 sequences of randomly selected VELOCIMMUNE® antibodies around the V_H-D_H-J_H (CDR3) junction, demonstrating junctional diversity and nucleotide additions. Heavy chain CDR3 sequences are grouped according to D_H gene segment usage, the germline of which is provided above each group in bold. V_H gene segments for each heavy chain CDR3 sequence are noted within parenthesis at the 5' end of each sequence (e.g., 3-72 is human V_H3-72). J_H gene segments for each heavy chain CDR3 are noted within parenthesis at the 3' end of each sequence (e.g., 3 is human J_H3). SEQ ID NOs for each sequence shown are as follows proceeding from top to bottom: SEQ ID NO:21; SEQ ID NO:22; SEQ ID NO:23; SEQ ID NO:24; SEQ ID NO:25; SEQ ID NO:26; SEQ ID NO:27; SEQ ID NO:28; SEQ ID NO:29; SEQ ID NO:30; SEQ ID NO:31; SEQ ID NO:32; SEQ ID NO:33; SEQ ID NO:34; SEQ ID NO:35; SEQ ID NO:36; SEQ ID NO:37; SEQ ID NO:38; SEQ ID NO:39.

FIG. 7B shows representative light chain CDR3 sequences of randomly selected VELOCIMMUNE® antibodies around the Vκ-Jκ (CDR3) junction, demonstrating junctional diversity and nucleotide additions. Vκ gene segments for each light chain CDR3 sequence are noted within parenthesis at the 5' end of each sequence (e.g., 1-6 is human Vκ1-6). Jκ gene segments for each light chain CDR3 are noted within parenthesis at the 3' end of each sequence (e.g., 1 is human Jκ1). SEQ ID NOs for each sequence shown are as follows proceeding from top to bottom: SEQ ID NO:40; SEQ ID NO:41; SEQ ID NO:42; SEQ ID NO:43; SEQ ID NO:44; SEQ ID NO:45; SEQ ID NO:46; SEQ ID NO:47; SEQ ID NO:48; SEQ ID NO:49; SEQ ID NO:50; SEQ ID NO:51; SEQ ID NO:52; SEQ ID NO:53; SEQ ID NO:54; SEQ ID NO:55; SEQ ID NO:56; SEQ ID NO:57; SEQ ID NO:58.

FIG. 8 shows somatic hypermutation frequencies of heavy and light chains of VELOCIMMUNE® antibodies scored (after alignment to matching germline sequences) as percent of sequences changed at each nucleotide (NT; left column) or amino acid (AA; right column) position among sets of 38 (unimmunized IgM), 28 (unimmunized IgG), 32 (unimmunized Igκ from IgG), 36 (immunized IgG) or 36 (immunized Igκ from IgG) sequences. Shaded bars indicate the locations of CDRs.

FIG. 9A shows levels of serum immunoglobulin for IgM and IgG isotypes in wild type (open bars) or VELOCIMMUNE® mice (closed bars).

FIG. 9B shows levels of serum immunoglobulin for IgA isotype in wild type (open bars) or VELOCIMMUNE® mice (closed bars).

FIG. 9C shows levels of serum immunoglobulin for IgE isotype in wild type (open bars) or VELOCIMMUNE® mice (closed bars).

FIG. 10A shows antigen-specific IgG titers against interleukin-6 receptor (IL-6R) of serum from seven VELOCIMMUNE® (VI) and five wild type (WT) mice after two (bleed 1) or three (bleed 2) rounds of immunization with ectodomain of IL-6R.

FIG. 10B shows IL-6R-specific IgG isotype-specific titers from seven VELOCIMMUNE® (VI) and five wild type (WT) mice.

FIG. 11A shows the affinity distribution of anti-interleukin-6 receptor monoclonal antibodies generated in VELOCIMMUNE® mice.

FIG. 11B shows the antigen-specific blocking of anti-interleukin-6 receptor monoclonal antibodies generated in VELOCIMMUNE® (VI) and wild type (WT) mice.

FIG. 12 shows a schematic illustration, not to scale, of mouse ADAM6a and ADAM6b genes in a mouse immunoglobulin heavy chain locus. A targeting vector (mADAM6 Targeting Vector) used for insertion of mouse ADAM6a and ADAM6b into a humanized endogenous heavy chain locus is shown with a selection cassette (HYG: hygromycin) flanked by site-specific recombination sites (Frt) including engineered restriction sites on the 5' and 3' ends.

FIG. 13 shows a schematic illustration, not to scale, of a human ADAM6 pseudogene (hADAM6Ψ) located between human heavy chain variable gene segments 1-2 (V_H1-2) and 6-1 (V_H6-1). A targeting vector for bacterial homologous recombination (hADAM6Ψ Targeting Vector) to delete a human ADAM6 pseudogene and insert unique restriction sites into a human heavy chain locus is shown with a selection cassette (NEO: neomycin) flanked by site-specific recombination sites (loxP) including engineered restriction sites on the 5' and 3' ends. An illustration, not to scale, of the resulting targeted humanized heavy chain locus containing a genomic fragment that encodes for the mouse ADAM6a and ADAM6b genes including a selection cassette flanked by site-specific recombination sites is shown.

FIG. 14A shows FACS contour plots of lymphocytes gated on singlets for surface expression of IgM and B220 in the bone marrow for mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an inserted mouse genomic fragment comprising mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$). Percentage of immature ($B220^{int}IgM^{+}$) and mature ($B220^{high}IgM^{+}$) B cells is noted in each contour plot.

FIG. 14B shows the total number of immature ($B220^{int}IgM^{+}$) and mature ($B220^{high}IgM^{+}$) B cells in the bone marrow isolated from femurs of mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse

ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$).

FIG. 15A shows FACS contour plots of $CD19^+$-gated B cells for surface expression of c-kit and CD43 in the bone marrow for mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($W^{+/+}A6^{res}\kappa^{+/+}$). Percentage of pro-B ($CD19^+CD43^+ckit^+$) and pre-B ($CD19^+CD43^-ckit^-$) cells is noted in the upper right and lower left quadrants, respectively, of each contour plot.

FIG. 15B shows the total number of pro-B cells ($CD19^+CD43^+ckit^+$) and pre-B cells ($CD19^+CD43^-ckit^-$) in the bone marrow isolated from femurs of mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment comprising mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$).

FIG. 16A shows FACS contour plots of lymphocytes gated on singlets for surface expression of CD19 and CD43 in the bone marrow for mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$). Percentage of immature B ($CD19^+CD43^-$), pre-B ($CD19^+CD43^{int}$) and pro-B ($CD19^+CD43^+$) cells is noted in each contour plot.

FIG. 16B shows histograms of immature B ($CD19^+CD43^-$) and pre-B ($CD19^+CD43^{int}$) cells in the bone marrow of mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$).

FIG. 17A shows FACS contour plots of lymphocytes gated on singlets for surface expression of CD19 and CD3 in splenocytes for mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$). Percentage of B ($CD19^+CD3^-$) and T ($CD19^-CD3^+$) cells is noted in each contour plot.

FIG. 17B shows FACs contour plots for $CD19^+$-gated B cells for surface expression of Igλ and Igκ light chain in the spleen of mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$). Percentage of $Ig\lambda^+$ (upper left quadrant) and
$Ig\kappa^{\sqcup}$

(lower right quadrant) B cells is noted in each contour plot.

FIG. 17C shows the total number of $CD19^+$ B cells in the spleen of mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$).

FIG. 18A shows FACs contour plots of $CD19^+$-gated B cells for surface expression of IgD and IgM in the spleen of mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$). Percentage of mature B cells ($CD19^+IgD^{high}IgM^{int}$) is noted for each contour plot. The arrow on the right contour plot illustrates the process of maturation for B cells in relation to IgM and IgD surface expression.

FIG. 18B shows the total number of B cells in the spleen of mice homozygous for human heavy and human κ light chain variable gene loci ($H^{+/+}\kappa^{+/+}$) and mice homozygous for human heavy and human κ light chain variable gene loci having an ectopic mouse genomic fragment encoding mouse ADAM6 genes ($H^{+/+}A6^{res}\kappa^{+/+}$) during maturation from $CD19^+IgM^{high}IgD^{int}$ to $CD19^+IgM^{int}IgD^{high}$.

FIG. 19 shows a detailed illustration, not to scale, of the human λ light chain locus including the clusters of Vλ gene segments (A, B and C) and the Jλ and Cλ region pairs (J-C pairs)

FIG. 20 shows a general illustration, not to scale, of a targeting strategy used to inactivate the endogenous mouse λ light chain locus.

FIG. 21 shows a general illustration, not to scale, of a targeting strategy used to inactivate the endogenous mouse κ light chain locus.

FIG. 22A shows a general illustration, not to scale of an initial targeting vector for targeting the endogenous mouse λ light chain locus with human λ light chain sequences including 12 hVλ gene segments and hJλ1 gene segment (12/1-λ Targeting Vector).

FIG. 22B shows a general illustration, not to scale, of four initial targeting vectors for targeting the endogenous mouse κ light chain locus with human λ light chain sequences including 12 hVλ gene segments and hJλ1 gene segment (12/1-κ Targeting Vector), 12 hVλ gene segments and hJλ1, 2, 3 and 7 gene segments (12/4-κ Targeting Vector), 12 hVλ gene segments, a human Vκ-Jκ genomic sequence and hJλ1 gene segment (12(κ)1-κ Targeting Vector) and 12 hVλ gene segments, a human Vκ-Jκ genomic sequence and hJλ1, 2, 3 and 7 gene segments (12(κ)4-κ Targeting Vector).

FIG. 23A shows a general illustration, not to scale, of a targeting strategy for progressive insertion of 40 hVλ gene segments and a single hJλ gene segment into the mouse λ light chain locus.

FIG. 23B shows a general illustration, not to scale, of a targeting strategy for progressive insertion of 40 hVλ gene segments and a single hJλ gene segment into the mouse κ locus.

FIG. 24 show a general illustration, not to scale, of the targeting and molecular engineering steps employed to make unique human λ-κ hybrid targeting vectors for construction of a hybrid light chain locus containing a human κ intergenic sequence, multiple hJλ gene segments or both.

FIG. 25A shows a general illustration, not to scale, of the locus structure for a modified mouse λ light chain locus containing 40 hVλ gene segments and a single hJλ gene segment operably linked to the endogenous Cλ2 gene.

FIG. 25B shows a general illustration, not to scale, of the locus structure for four independent, modified mouse κ light chain loci containing 40 hVλ gene segments and either one or four hJλ gene segments with or without a contiguous human Vκ-Jκ genomic sequence operably linked to the endogenous Cκ gene.

FIG. 26A shows contour plots of $Ig\lambda^+$ and $Ig\kappa^+$ splenocytes gated on $CD19^+$ from a wild type mouse (WT), a mouse homozygous for 12 hVλ and four hJλ gene segments including a human Vκ-Jκ genomic sequence (12hVλ-VκJκ-4hJλ) and a mouse homozygous for 40 hVλ and one hJλ gene segment (40hVλ-1hJλ).

FIG. 26B shows the total number of $CD19^+$ B cells in harvested spleens from wild type (WT), mice homozygous for 12 hVλ and four hJλ gene segments including a human Vκ-Jκ genomic sequence (12hVλ-VκJκ-4hJλ) and mice homozygous for 40 hVλ, and one hJλ gene segment (40hVλ-1hJλ).

FIG. 27A, in the top panel, shows contour plots of splenocytes gated on singlets and stained for B and T cells ($CD19^+$ and $CD3^+$, respectively) from a wild type mouse (WT) and a mouse homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ). The bottom panel shows contour plots of splenocytes gated on $CD19^+$ and stained for $Ig\lambda^+$ and $Ig\kappa^+$ expression from a wild type mouse (WT) and a mouse homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ).

FIG. 27B shows the total number of $CD19^+$, $CD19^+Ig\kappa^+$ and $CD19+Ig\lambda^+$ B cells in harvested spleens from wild type mice (WT) and mice homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ).

FIG. 27C shows contour plots of splenocytes gated on $CD19^+$ and stained for immunoglobulin D (IgD) and immunoglobulin M (IgM) from a wild type mouse (WT) and a mouse homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ). Mature (72 for WT, 51 for 40hVλ-VκJκ-4hJλ) and transitional (13 for WT, 22 for 40hVλ-VκJκ-4hJλ) B cells are noted on each of the contour plots.

FIG. 27D shows the total number of $CD19^+$ B cells, transitional B cells ($CD19^+IgM^{hi}IgD^{lo}$) and mature B cells ($CD19^+IgM^{lo}IgD^{hi}$) in harvested spleens from wild type mice (WT) and mice homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ).

FIG. 28A, in the top panel, shows contour plots of bone marrow stained for B and T cells ($CD19^+$ and $CD3^+$, respectively) from a wild type mouse (WT) and a mouse homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ). The bottom panel shows contour plots of bone marrow gated on $CD19^+$ and stained for $ckit^+$ and $CD43^+$ from a wild type mouse (WT) and a mouse homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ). Pro and Pre B cells are noted on the contour plots of the bottom panel.

FIG. 28B shows the number of Pro ($CD19^+CD43^+ckit^+$) and Pre ($CD19^+CD43^-ckit^-$) B cells in bone marrow harvested from the femurs of wild type mice (WT) and mice homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ).

FIG. 28C shows contour plots of bone marrow gated on singlets stained for immunoglobulin M (IgM) and B220 from a wild type mouse (WT) and a mouse homozygous for 40 hVλ, and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ). Immature, mature and pro/pre B cells are noted on each of the contour plots.

FIG. 28D shows the total number of immature ($B220^{int}IgM^+$) and mature ($B220^{hi}IgM^+$) B cells in bone marrow isolated from the femurs of wild type mice (WT) and mice homozygous for 40 hVλ and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ).

FIG. 28E shows contour plots of bone marrow gated on immature ($B220^{int}IgM^+$) and mature ($B220^{hi}IgM^+$) B cells stained for Igλ and Igκ expression isolated from the femurs of a wild type mouse (WT) and a mouse homozygous for 40 hVλ and four Jλ gene segments including a human Vκ-Jκ genomic sequence (40hVλ-VκJκ-4hJλ).

FIG. 29 shows a nucleotide sequence alignment of the Vλ-Jλ-Cκ junction of eighteen independent RT-PCR clones amplified from splenocyte RNA of mice bearing human λ light chain gene sequences at an endogenous mouse κ light chain locus. A6 = SEQ ID NO:115; B6 = SEQ ID NO:116; F6 = SEQ ID NO:117; B7 = SEQ ID NO:118; E7 = SEQ ID NO:119; F7 = SEQ ID NO:120; C8 = SEQ ID NO:121; E12 = SEQ ID NO:122; 1-4 = SEQ ID NO:123; 1-20 = SEQ ID NO:124; 3B43 = SEQ ID NO:125; 5-8 = SEQ ID NO:126; 5-19 = SEQ ID NO:127; 1010 = SEQ ID NO:128; 11A1 = SEQ ID NO:129; 7A8 = SEQ ID NO:130; 3A3 = SEQ ID NO:131; 2-7 = SEQ ID NO:132. Lower case bases indicate non-germline bases resulting from either mutation and/or N addition during recombination. Consensus amino acids within the Framework 4 region (FWR4) encoded by the nucleotide sequence of hJλ1 and mouse Cκ are noted at the bottom of the sequence alignment.

FIG. 30 shows a nucleotide sequence alignment of the Vλ-Jλ-Cκ junction of twelve independent RT-PCR clones amplified from splenocyte RNA of mice bearing human λ light chain gene sequences including a contiguous human Vκ-Jκ genomic sequence at an endogenous mouse κ light chain locus. 5-2 = SEQ ID NO:145; 2-5 = SEQ ID NO:146; 1-3 = SEQ ID NO:147; 4B-1 = SEQ ID NO:148; 3B-5 = SEQ ID NO:149; 7A-1 = SEQ ID NO:150; 5-1 = SEQ ID NO:151; 4A-1 = SEQ ID NO:152; 11A-1 = SEQ ID NO:153; 5-7 = SEQ ID NO:154; 5-4 = SEQ ID NO:155; 2-3 = SEQ ID NO:156. Lower case bases indicate non-germline bases resulting from either mutation and/or N addition during recombination. Consensus amino acids within the Framework 4 region (FWR4) encoded by the nucleotide sequence of each human Jλ and mouse Cκ are noted at the bottom of the sequence alignment.

FIG. 31 shows a nucleotide sequence alignment of the Vλ-Jλ-Cλ junction of three independent RT-PCR clones amplified from splenocyte RNA of mice bearing human λ light chain gene sequences at an endogenous mouse λ light chain locus. 2D1 = SEQ ID NO:159; 2D9 = SEQ ID NO:160; 3E15 = SEQ ID NO:161. Lower case bases indicate non-germline bases resulting from either mutation and/or N addition during recombination. Consensus amino acids within the Framework 4 region (FWR4) encoded by the nucleotide sequence of hJλ1 and mouse Cλ2 are noted at the bottom of the sequence alignment.

DETAILED DESCRIPTION

[0322] This disclosure is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

[0323] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described.

[0324] The phrase "substantial" or "substantially" when used to refer to an amount of gene segments (*e.g.,* "substantially all" V gene segments) includes both functional and non functional gene segments and include, in various embodiments, *e.g.,* 80% or more, 85% or more, 90% or more, 95% or more 96% or more, 97% or more, 98% or more, or 99% or more of all gene segments; in various embodiments, "substantially all" gene segments includes, *e.g.,* at least 95%, 96%, 97%, 98%, or 99% of functional (*i.e.,* non-pseudogene) gene segments.

[0325] The term "replacement" includes wherein a DNA sequence is placed into a genome of a cell in such a way as to replace a sequence within the genome with a heterologous sequence

(*e.g.,* a human sequence in a mouse), at the locus of the genomic sequence,. The DNA sequence so placed may include one or more regulatory sequences that are part of source DNA used to obtain the sequence so placed (*e.g.,* promoters, enhancers, 5'- or 3'-untranslated regions, appropriate recombination signal sequences, *etc.*). For example, in various embodiments, the replacement is a substitution of an endogenous sequence for a heterologous sequence that results in the production of a gene product from the DNA sequence so placed (comprising the heterologous sequence), but not expression of the endogenous sequence; the replacement is of an endogenous genomic sequence with a DNA sequence that encodes a protein that has a similar function as a protein encoded by the endogenous genomic sequence (*e.g.,* the endogenous genomic sequence encodes an immunoglobulin gene or domain, and the DNA fragment encodes one or more human immunoglobulin genes or domains). In various embodiments, an endogenous gene or fragment thereof is replaced with a corresponding human gene or fragment thereof. A corresponding human gene or fragment thereof is a human gene or fragment that is an ortholog of, a homolog of, or is substantially identical or the same in structure and/or function, as the endogenous gene or fragment thereof that is replaced.

[0326] The term "contiguous" includes reference to occurrence on the same nucleic acid molecule, *e.g.,* two nucleic acid sequences are "contiguous" if they occur on the same nucleic molecule but are interrupted by another nucleic acid sequence. For example, a rearranged V(D)J sequence is "contiguous" with a constant region gene sequence, although the final codon of the V(D)J sequence is not followed immediately by the first codon of the constant region sequence. In another example, two V gene segment sequences are "contiguous" if they occur on the same genomic fragment, although they may be separated by sequence that does not encode a codon of the V region, *e.g.,* they may be separated by a regulatory sequence, *e.g.,* a promoter or other noncoding sequence. In one embodiment, a contiguous sequence includes a genomic fragment that contains genomic sequences arranged as found in a wild-type genome.

[0327] The phrase "derived from" when used concerning a variable region "derived from" a cited gene or gene segment includes the ability to trace the sequence back to a particular unrearranged gene segment or gene segments that were rearranged to form a gene that expresses the variable domain (accounting for, where applicable, splice differences and somatic mutations).

[0328] The phrase "functional" when used concerning a variable region gene segment or joining gene segment refers to usage in an expressed antibody repertoire; *e.g.,* in humans Vλ gene segments 3-1, 4-3, 2-8, *etc.* are functional, whereas Vλ gene segments 3-2, 3-4, 2-5, *etc.* are nonfunctional.

[0329] A "heavy chain locus" includes a location on a chromosome, *e.g.,* a mouse chromosome, wherein in a wild-type mouse heavy chain variable (V_H), heavy chain diversity (D_H), heavy chain joining (J_H), and heavy chain constant (C_H) region DNA sequences are found.

[0330] A "κ locus" includes a location on a chromosome, *e.g.,* a mouse chromosome, wherein in a wild-type mouse κvariable (Vκ), κ joining (JK), and κ constant (CK) region DNA sequences are found.

[0331] A "λ locus" includes a location on a chromosome, *e.g.,* a mouse chromosome, wherein in a wild-type mouse λ variable (Vλ), λ joining (Jλ), and λ constant (Cλ) region DNA sequences are found.

[0332] The term "cell," when used in connection with expressing a sequence includes any cell that is suitable for expressing a recombinant nucleic acid sequence. Cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (*e.g.,* strains of *E. coli, Bacillus spp., Streptomyces spp., etc.*), mycobacteria cells, fungal cells, yeast cells (*e.g., S. cerevisiae, S. pombe, P. pastoris, P. methanolica, etc.*), plant cells, insect cells (*e.g., SF-9, SF-21, baculovirus-infected insect cells, Trichoplusia ni, etc.*), non-human animal cells, human cells, B cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (*e.g.,* CHO K1, DXB-11 CHO, Veggie-CHO), COS (*e.g.,* COS-7), retinal cell, Vero, CV1, kidney (*e.g.,* HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, *e.g.* a retinal cell that expresses a viral gene (*e.g.,* a PER.C6™ cell).

[0333] The phrase "complementarity determining region," or the term "CDR," includes an amino acid sequence encoded by a nucleic acid sequence of an organism's immunoglobulin genes that normally (*i.e.,* in a wild-type animal) appears between two framework regions in a variable region of a light or a heavy chain of an immunoglobulin molecule (*e.g.,* an antibody or a T cell receptor). A CDR can be encoded by, for example, a germline sequence or a rearranged or unrearranged sequence, and, for example, by a naive or a mature B cell or a T cell. In some circumstances (*e.g.,* for a CDR3), CDRs can be encoded by two or more sequences (*e.g.,* germline sequences) that are not contiguous (*e.g.,* in an unrearranged nucleic acid sequence) but are contiguous in a B cell nucleic acid sequence, *e.g.,* as the result of splicing or connecting the sequences (*e.g.,* V-D-J recombination to form a heavy chain CDR3).

[0334] The phrase "gene segment," or "segment" includes reference to a V (light or heavy) or D or J (light or heavy) immunoglobulin gene segment, which includes unrearranged sequences at immunoglobulin loci (in e.g., humans and mice) that can participate in a rearrangement (mediated by, e.g., endogenous recombinases) to form a rearranged V/J or V/D/J sequence. Unless indicated otherwise, the V, D, and J segments comprise recombination signal sequences (RSS) that allow for V/J recombination or V/D/J recombination according to the

12/23 rule. Unless indicated otherwise, the segments further comprise sequences with which they are associated in nature or functional equivalents thereof (e.g., for V segments promoter(s) and leader(s)).

[0335] The term "unrearranged" includes the state of an immunoglobulin locus wherein V gene segments and J gene segments (for heavy chains, D gene segments as well) are maintained separately but are capable of being joined to form a rearranged V(D)J gene that comprises a single V,(D),J of the V(D)J repertoire.

[0336] The phrase "micromolar range" is intended to mean 1-999 micromolar; the phrase "nanomolar range" is intended to mean 1-999 nanomolar; the phrase "picomolar range" is intended to mean 1-999 picomolar.

[0337] The term "non-human animals" is intended to include any non-human animals such as cyclostomes, bony fish, cartilaginous fish such as sharks and rays, amphibians, reptiles, mammals, and birds. Suitable non-human animals include mammals. Suitable mammals include non-human primates, goats, sheep, pigs, dogs, cows, and rodents. Suitable non-human animals are selected from the rodent family including rat and mouse. The non-human animals provided by the invention are mice.

[0338] The mouse as a genetic model has been greatly enhanced by transgenic and knockout technologies, which have allowed for the study of the effects of the directed over-expression or deletion of specific genes. Despite all of its advantages, the mouse still presents genetic obstacles that render it an imperfect model for human diseases and an imperfect platform to test human therapeutics or make them. First, although about 99% of human genes have a mouse homolog (Waterston, R.H. et al. (2002) Initial sequencing and comparative analysis of the mouse genome. Nature 420, 520-562.), potential therapeutics often fail to cross-react, or cross-react inadequately, with mouse orthologs of the intended human targets. To obviate this problem, selected target genes can be "humanized," that is, the mouse gene can be eliminated and replaced by the corresponding human orthologous gene sequence (*e.g.,* US 6,586,251, US 6,596,541 and US 7,105,348). Initially, efforts to humanize mouse genes by a "knockout-plus-transgenic humanization" strategy entailed crossing a mouse carrying a deletion (*i.e.,* knockout) of the endogenous gene with a mouse carrying a randomly integrated human transgene (see, *e.g.,* Bril, W.S. et al. (2006) Tolerance to factor VIII in a transgenic mouse expressing human factor VIII cDNA carrying an Arg(593) to Cys substitution. Thromb Haemost 95, 341-347; Homanics, G.E. et al. (2006) Production and characterization of murine models of classic and intermediate maple syrup urine disease. BMC Med Genet 7, 33; Jamsai, D. et al. (2006) A humanized BAC transgenic/knockout mouse model for HbE/beta-thalassemia. Genomics 88(3):309-15; Pan, Q. et al. (2006) Different role for mouse and human CD3delta/epsilon heterodimer in preT cell receptor (preTCR) function: human CD3delta/epsilon heterodimer restores the defective preTCR function in CD3gamma- and CD3gammadelta-deficient mice. Mol Immunol 43, 1741-1750). But those efforts were hampered by size limitations; conventional knockout technologies were not sufficient to directly replace large mouse genes with their large human genomic counterparts. A straightforward

approach of direct homologous replacement, in which an endogenous mouse gene is directly replaced by the human counterpart gene at the same precise genetic location of the mouse gene (*i.e.,* at the endogenous mouse locus), is rarely attempted because of technical difficulties. Until now, efforts at direct replacement involved elaborate and burdensome procedures, thus limiting the length of genetic material that could be handled and the precision with which it could be manipulated.

[0339] Exogenously introduced human immunoglobulin transgenes rearrange in precursor B-cells in mice (Alt, F.W., Blackwell, T.K., and Yancopoulos, G.D. (1985). Immunoglobulin genes in transgenic mice. Trends Genet 1, 231-236). This finding was exploited by engineering mice using the knockout-plus-transgenic approach to express human antibodies (Green, L.L. et al. (1994) Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs. Nat Genet 7, 13-21; Lonberg, N. (2005). Human antibodies from transgenic animals. Nat Biotechnol 23, 1117-1125; Lonberg, N. et al. (1994) Antigen-specific human antibodies from mice comprising four distinct genetic modifications. Nature 368, 856-859; Jakobovits, A. et al. (2007) From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice. Nat Biotechnol 25, 1134-1143). The endogenous mouse immunoglobulin heavy chain and κ light chain loci were inactivated in these mice by targeted deletion of small but critical portions of each endogenous locus, followed by introducing human immunoglobulin gene loci as randomly integrated large transgenes, as described above, or minichromosomes (Tomizuka, K. et al. (2000) Double trans-chromosomic mice: maintenance of two individual human chromosome fragments containing Ig heavy and kappa loci and expression of fully human antibodies. Proc Natl Acad Sci U S A 97, 722-727). Such mice represented an important advance in genetic engineering; fully human monoclonal antibodies isolated from them yielded promising therapeutic potential for treating a variety of human diseases (Gibson, T.B. et al. (2006) Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. Clin Colorectal Cancer 6, 29-31; Jakobovits *et al.,* 2007; Kim, Y.H. et al. (2007) Clinical efficacy of zanolimumab (HuMax-CD4): two Phase II studies in refractory cutaneous T-cell lymphoma. Blood 109(11):4655-62; Lonberg, 2005; Maker, A.V. et al. (2005) Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. Ann Surg Oncol 12, 1005-1016; McClung, M.R., Lewiecki, E.M. et al. (2006) Denosumab in postmenopausal women with low bone mineral density. N Engl J Med 354, 821-831). But, as discussed above, these mice exhibit compromised B cell development and immune deficiencies when compared to wild type mice. Such problems potentially limit the ability of the mice to support a vigorous humoral response and, consequently, generate fully human antibodies against some antigens. The deficiencies may be due to: (1) inefficient functionality due to the random introduction of the human immunoglobulin transgenes and resulting incorrect expression due to a lack of upstream and downstream control elements (Garrett, F.E. et al. (2005) Chromatin architecture near a potential 3' end of the igh locus involves modular regulation of histone modifications during B-Cell development and in vivo occupancy at CTCF sites. Mol Cell Biol 25, 1511-1525; Manis, J.P. et al. (2003) Elucidation of a downstream boundary of the 3' IgH regulatory region. Mol Immunol 39, 753-760; Pawlitzky, I. et al. (2006)

Identification of a candidate regulatory element within the 5' flanking region of the mouse Igh locus defined by pro-B cell-specific hypersensitivity associated with binding of PU.1, Pax5, and E2A. J Immunol 176, 6839-6851); (2) inefficient interspecies interactions between human constant domains and mouse components of the B-cell receptor signaling complex on the cell surface, which may impair signaling processes required for normal maturation, proliferation, and survival of B cells (Hombach, J. et al. (1990) Molecular components of the B-cell antigen receptor complex of the IgM class. Nature 343, 760-762); and (3) inefficient interspecies interactions between soluble human immunoglobulins and mouse Fc receptors that might reduce affinity selection (Rao, S.P. et al. (2002) Differential expression of the inhibitory IgG Fc receptor FcgammaRIIB on germinal center cells: implications for selection of high-affinity B cells. J Immunol 169, 1859-1868) and immunoglobulin serum concentrations (Brambell, F.W. et al. (1964). A Theoretical Model of Gamma-Globulin Catabolism. Nature 203, 1352-1354; Junghans, R.P., and Anderson, C.L. (1996). The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. Proc Natl Acad Sci U S A 93, 5512-5516; Rao *et al.,* 2002; Hjelm, F. et al. (2006) Antibody-mediated regulation of the immune response. Scand J Immunol 64, 177-184; Nimmerjahn, F., and Ravetch, J.V. (2007). Fc-receptors as regulators of immunity. Adv Immunol 96, 179-204). These deficiencies can be corrected by *in situ* humanization of only the variable regions of the mouse immunoglobulin loci within their natural locations at the endogenous heavy and light chain loci. This would effectively result in mice that make "reverse chimeric" (*i.e.,* human V: mouse C) antibodies which would be capable of normal interactions and selection with the mouse environment based on retaining mouse constant regions. Further such reverse chimeric antibodies may be readily reformatted into fully human antibodies for therapeutic purposes.

[0340] Genetically modified animals that comprise a replacement at the endogenous immunoglobulin heavy chain locus with heterologous (*e.g.,* from another species) immunoglobulin sequences can be made in conjunction with replacements at endogenous immunoglobulin light chain loci or in conjunction with immunoglobulin light chain transgenes (*e.g.,* chimeric immunoglobulin light chain transgenes or fully human fully mouse, etc.). The species from which the heterologous immunoglobulin heavy chain sequences are derived can vary widely; as with immunoglobulin light chain sequences employed in immunoglobulin light chain sequence replacements or immunoglobulin light chain transgenes.

[0341] Immunoglobulin variable region nucleic acid sequences, *e.g.,* V, D, and/or J segments, are obtained from a human or a non-human animal. Non-human animals suitable for providing V, D, and/or J segments include, for example bony fish, cartilaginous fish such as sharks and rays, amphibians, reptiles, mammals, birds (e.g., chickens). Non-human animals include, for example, mammals. Mammals include, for example, non-human primates, goats, sheep, pigs, dogs, bovine (*e.g.,* cow, bull, buffalo), deer, camels, ferrets and rodents and non-human primates (*e.g.,* chimpanzees, orangutans, gorillas, marmosets, rhesus monkeys baboons). Suitable non-human animals are selected from the rodent family including rats, mice, and hamsters. In one embodiment, the non-human animals are mice. As clear from the context, various non-human animals can be used as sources of variable domains or variable region gene segments (*e.g.,* sharks, rays, mammals (e.g., camels, rodents such as mice and rats).

[0342] According to the context, non-human animals are also used as sources of constant region sequences to be used in connection with variable sequences or segments, for example, rodent constant sequences can be used in transgenes operably linked to human or non-human variable sequences (*e.g.,* human or non-human primate variable sequences operably linked to, *e.g.,* rodent, *e.g.,* mouse or rat or hamster, constant sequences). Thus, in various embodiments, human V, D, and/or J segments are operably linked to rodent (*e.g.,* mouse or rat or hamster) constant region gene sequences. In some embodiments, the human V, D, and/or J segments (or one or more rearranged VDJ or VJ genes) are operably linked or fused to a mouse, rat, or hamster constant region gene sequence in, *e.g.,* a transgene integrated at a locus that is not an endogenous immunoglobulin locus.

[0343] Also disclosed is a mouse that comprises a replacement of V_H, D_H, and J_H gene segments at an endogenous immunoglobulin heavy chain locus with one or more human V_H, D_H, and J_H segments, wherein the one or more human V_H, D_H, and J_H segments are operably linked to an endogenous immunoglobulin heavy chain constant gene; wherein the mouse comprises a transgene at a locus other than an endogenous immunoglobulin locus, wherein the transgene comprises an unrearranged or rearranged human V_L and human J_L segment operably linked to a mouse or rat or human constant region.

[0344] In a specific embodiment, a mouse is provided that comprises an insertion of on or more human V_H, D_H and J_H gene segments at an endogenous immunoglobulin heavy chain locus. In one embodiment, the insertion is upstream of an endogenous immunoglobulin heavy chain constant gene; in one embodiment, the insertion is downstream of an endogenous variable (V) gene segment; in one embodiment, the insertion is downstream of an endogenous diversity (D) gene segment; in one embodiment, the insertion is downstream of an endogenous joining (J) gene segment. In various embodiments, the insertion is such that the one or more human V_H, D_H and J_H gene segments are positioned in operable linkage with one or more endogenous heavy chain constant genes.

[0345] A method for a large *in situ* genetic replacement of the mouse germline immunoglobulin variable gene loci with human germline immunoglobulin variable gene loci while maintaining the ability of the mice to generate offspring is described. Specifically, the precise replacement of six megabases of both the mouse heavy chain and κ light chain immunoglobulin variable gene loci with their human counterparts while leaving the mouse constant regions intact is described. As a result, mice have been created that have a precise replacement of their entire germline immunoglobulin variable repertoire with equivalent human germline immunoglobulin variable sequences, while maintaining mouse constant regions. The human variable regions are linked to mouse constant regions to form chimeric human-mouse immunoglobulin loci that rearrange and express at physiologically appropriate levels. The antibodies expressed are "reverse chimeras," *i.e.,* they comprise human variable region sequences and mouse constant region sequences. These mice having humanized immunoglobulin variable regions that express antibodies having human variable regions and mouse constant regions are called

VELCOIMMUNE® mice.

[0346] VELOCIMMUNE® humanized mice exhibit a fully functional humoral immune system that is essentially indistinguishable from that of wild-type mice. They display normal cell populations at all stages of B cell development. They exhibit normal lymphoid organ morphology. Antibody sequences of VELOCIMMUNE® mice exhibit normal V(D)J rearrangement and normal somatic hypermutation frequencies. Antibody populations in these mice reflect isotype distributions that result from normal class switching (*e.g.,* normal isotype cis-switching). Immunizing VELOCIMMUNE® mice results in robust humoral immune responses that generate a large, diverse antibody repertoires having human immunoglobulin variable domains suitable as therapeutic candidates. This platform provides a plentiful source of naturally affinity-matured human immunoglobulin variable region sequences for making pharmaceutically acceptable antibodies and other antigen-binding proteins.

[0347] It is the precise replacement of mouse immunoglobulin variable sequences with human immunoglobulin variable sequences that allows for making VELOCIMMUNE® mice. Yet even a precise replacement of endogenous mouse immunoglobulin sequences at heavy and light chain loci with equivalent human immunoglobulin sequences, by sequential recombineering of very large spans of human immunoglobulin sequences, may present certain challenges due to divergent evolution of the immunoglobulin loci between mouse and man. For example, intergenic sequences interspersed within the immunoglobulin loci are not identical between mice and humans and, in some circumstances, may not be functionally equivalent. Differences between mice and humans in their immunoglobulin loci can still result in abnormalities in humanized mice, particularly when humanizing or manipulating certain portions of endogenous mouse immunoglobulin heavy chain loci. Some modifications at mouse immunoglobulin heavy chain loci are deleterious. Deleterious modifications can include, for example, loss of the ability of the modified mice to mate and produce offspring. In various embodiments, engineering human immunoglobulin sequences in the genome of a mouse includes methods that maintain endogenous sequences that when absent in modified mouse strains are deleterious. Exemplary deleterious effects may include inability to propagate modified strains, loss of function of essential genes, inability to express polypeptides, etc. Such deleterious effects may be directly or indirectly related to the modification engineered into the genome of the mouse.

[0348] A precise, large-scale, *in situ* replacement of six megabases of the variable regions of the mouse heavy and light chain immunoglobulin loci (V_H-D_H-J_H and Vκ-Jκ) with the corresponding 1.4 megabases human genomic sequences was performed, while leaving the flanking mouse sequences intact and functional within the hybrid loci, including all mouse constant chain genes and locus transcriptional control regions (FIG. 1A and FIG. 1B). Specifically, the human V_H, D_H, J_H, Vκ and Jκ gene sequences were introduced through stepwise insertion of 13 chimeric BAC targeting vectors bearing overlapping fragments of the human germline variable loci into mouse ES cells using VELOCIGENE® genetic engineering technology (see, *e.g.,* US Pat. No. 6,586,251 and Valenzuela, D.M. et al. (2003). High-throughput engineering of the mouse genome coupled with high-resolution expression analysis. Nat Biotechnol 21, 652-659).

[0349] Humanization of the mouse immunoglobulin genes represents the largest genetic modification to the mouse genome to date. While previous efforts with randomly integrated human immunoglobulin transgenes have met with some success (discussed above), direct replacement of the mouse immunoglobulin genes with their human counterparts dramatically increases the efficiency with which fully-human antibodies can be efficiently generated in otherwise normal mice. Further, such mice exhibit a dramatically increased diversity of fully-human antibodies that can be obtained after immunization with virtually any antigen, as compared with mice bearing disabled endogenous loci and fully human antibody transgenes. Multiple versions of replaced, humanized loci exhibit completely normal levels of mature and immature B cells, in contrast to mice with randomly integrated human transgenes, which exhibit significantly reduced B cell populations at various stages of differentiation. While efforts to increase the number of human gene segments in human transgenic mice have reduced such defects, the expanded immunoglobulin repertoires have not altogether corrected reductions in B cell populations as compared to wild-type mice.

[0350] Notwithstanding the near wild-type humoral immune function observed in mice with replaced immunoglobulin loci (i.e., VELOCIMMUNE® mice), there are other challenges encountered when employing a direct replacement of the immunoglobulin that is not encountered in some approaches that employ randomly integrated transgenes. Differences in the genetic composition of the immunoglobulin loci between mice and humans has lead to the discovery of sequences beneficial for the propagation of mice with replaced immunoglobulin gene segments. Specifically, mouse ADAM genes located within the endogenous immunoglobulin locus are optimally present in mice with replaced immunoglobulin loci, due to their role in fertility.

Genomic Location and Function of Mouse ADAM6

[0351] Male mice that lack the ability to express any functional ADAM6 protein surprisingly exhibit a defect in the ability of the mice to mate and to generate offspring. The mice lack the ability to express a functional ADAM6 protein by virtue of a replacement of all or substantially all mouse immunoglobulin variable region gene segments with human variable region gene segments. The loss of ADAM6 function results because the ADAM6 locus is located within a region of the endogenous mouse immunoglobulin heavy chain variable region gene locus, proximal to the 3' end of the V_H gene segment locus that is upstream of the D_H gene segments. In order to breed mice that are homozygous for a replacement of all or substantially all endogenous mouse heavy chain variable gene segments with human heavy chain variable gene segments, it is generally a cumbersome approach to set up males and females that are each homozygous for the replacement and await a productive mating. Successful litters are low in frequency and size. Instead, males heterozygous for the replacement have been employed to mate with females homozygous for the replacement to generate progeny that are heterozygous for the replacement, then breed a homozygous mouse therefrom. The inventors have determined that the likely cause of the loss in fertility in the male mice is the absence in

homozygous male mice of a functional ADAM6 protein.

[0352] In various aspects, male mice that comprise a damaged (*i.e.,* nonfunctional or marginally functional) ADAM6 gene exhibit a reduction or elimination of fertility. Because in mice (and other rodents) the ADAM6 gene is located in the immunoglobulin heavy chain locus, the inventors have determined that in order to propagate mice, or create and maintain a strain of mice, that comprise a replaced immunoglobulin heavy chain locus, various modified breeding or propagation schemes are employed. The low fertility, or infertility, of male mice homozygous for a replacement of the endogenous immunoglobulin heavy chain variable gene locus renders maintaining such a modification in a mouse strain difficult. Maintaining the strain comprises avoiding infertility problems exhibited by male mice homozygous for the replacement.

[0353] Disclosed is a method for maintaining a strain of mouse as described herein. The strain of mouse need not comprise an ectopic ADAM6 sequence, and in various embodiments the strain of mouse is homozygous or heterozygous for a knockout (*e.g.,* a functional knockout) of ADAM6.

[0354] The mouse strain may comprise a modification of an endogenous immunoglobulin heavy chain locus that results in a reduction or loss in fertility in a male mouse. The modification may comprise a deletion of a regulatory region and/or a coding region of an ADAM6 gene. The modification may comprise a modification of an endogenous ADAM6 gene (regulatory and/or coding region) that reduces or eliminates fertility of a male mouse that comprises the modification; the modification may reduce or eliminate fertility of a male mouse that is homozygous for the modification.

[0355] The mouse strain may be homozygous or heterozygous for a knockout (*e.g.,* a functional knockout) or a deletion of an ADAM6 gene.

[0356] The mouse strain may be maintained by isolating from a mouse that is homozygous or heterozygous for the modification a cell, and employing the donor cell in host embryo, and gestating the host embryo and donor cell in a surrogate mother, and obtaining from the surrogate mother a progeny that comprises the genetic modification. In one embodiment, the donor cell is an ES cell. The donor cell may be a pluripotent cell, *e.g.,* an induced pluripotent cell.

[0357] The mouse strain may be maintained by isolating from a mouse that is homozygous or heterozygous for the modification a nucleic acid sequence comprising the modification, and introducing the nucleic acid sequence into a host nucleus, and gestating a cell comprising the nucleic acid sequence and the host nucleus in a suitable animal. The nucleic acid sequence may be introduced into a host oocyte embryo.

[0358] The mouse strain may be maintained by isolating from a mouse that is homozygous or heterozygous for the modification a nucleus, and introducing the nucleus into a host cell, and

gestating the nucleus and host cell in a suitable animal to obtain a progeny that is homozygous or heterozygous for the modification.

[0359] The mouse strain may be maintained by employing *in vitro* fertilization (IVF) of a female mouse (wild-type, homozygous for the modification, or heterozygous for the modification) employing a sperm from a male mouse comprising the genetic modification. The male mouse may be heterozygous for the genetic modification. The male mouse may be homozygous for the genetic modification.

[0360] The mouse strain may be maintained by breeding a male mouse that is heterozygous for the genetic modification with a female mouse to obtain progeny that comprises the genetic modification, identifying a male and a female progeny comprising the genetic modification, and employing a male that is heterozygous for the genetic modification in a breeding with a female that is wild-type, homozygous, or heterozygous for the genetic modification to obtain progeny comprising the genetic modification. The step of breeding a male heterozygous for the genetic modification with a wild-type female, a female heterozygous for the genetic modification, or a female homozygous for the genetic modification may be repeated in order to maintain the genetic modification in the mouse strain.

[0361] Disclosed is a method for maintaining a mouse strain that comprises a replacement of an endogenous immunoglobulin heavy chain variable gene locus with one or more human immunoglobulin heavy chain sequences, comprising breeding the mouse strain so as to generate heterozygous male mice, wherein the heterozygous male mice are bred to maintain the genetic modification in the strain. The strain need not be maintained by any breeding of a homozygous male with a wild-type female, or a female homozygous or heterozygous for the genetic modification.

[0362] The ADAM6 protein is a member of the ADAM family of proteins, where ADAM is an acronym for A Disintegrin And Metalloprotease. The ADAM family of proteins is large and diverse, with diverse functions including cell adhesion. Some members of the ADAM family are implicated in spermatogenesis and fertilization. For example, ADAM2 encodes a subunit of the protein fertilin, which is implicated in sperm-egg interactions. ADAM3, or cyritestin, appears necessary for sperm binding to the zona pellucida. The absence of either ADAM2 or ADAM3 results in infertility. It has been postulated that ADAM2, ADAM3, and ADAM6 form a complex on the surface of mouse sperm cells.

[0363] The human ADAM6 gene, normally found between human V_H gene segments V_H1-2 and V_H6-1, appears to be a pseudogene (Figure 12). In mice, there are two ADAM6 genes-ADAM6a and ADAM6b-that are found in an intergenic region between mouse V_H and D_H gene segments, and in the mouse the ADAM6a and ADAM6b genes are oriented in opposite transcriptional orientation to that of the surrounding immunoglobulin gene segments (FIG. 12). In mice, a functional ADAM6 locus is apparently required for normal fertilization. A functional ADAM6 locus or sequence, then, refers to an ADAM6 locus or sequence that can complement, or rescue, the drastically reduced fertilization exhibited in male mice with missing or

nonfunctional endogenous ADAM6 loci.

[0364] The position of the intergenic sequence in mice that encodes ADAM6a and ADAM6b renders the intergenic sequence susceptible to modification when modifying an endogenous mouse heavy chain. When V_H gene segments are deleted or replaced, or when D_H gene segments are deleted or replaced, there is a high probability that a resulting mouse will exhibit a severe deficit in fertility. In order to compensate for the deficit, the mouse is modified to include a nucleotide sequence that encodes a protein that will complement the loss in ADAM6 activity due to a modification of the endogenous mouse ADAM6 locus. In various embodiments, the complementing nucleotide sequence is one that encodes a mouse ADAM6a, a mouse ADAM6b, or a homolog or ortholog or functional fragment thereof that rescues the fertility deficit. Also described are methods to preserve the endogenous ADAM6 locus, while rendering the endogenous immunoglobulin heavy chain sequences flanking the mouse ADAM6 locus incapable of rearranging to encode a functional endogenous heavy chain variable region. Exemplary alternative methods include manipulation of large portions of mouse chromosomes that position the endogenous immunoglobulin heavy chain variable region loci in such a way that they are incapable of rearranging to encode a functional heavy chain variable region that is operably linked to an endogenous heavy chain constant gene. The methods may include inversions and/or translocations of mouse chromosomal fragments containing endogenous immunoglobulin heavy chain gene segments.

[0365] [Deleted]

[0366] The nucleotide sequence that rescues fertility can be coupled with an inducible promoter so as to facilitate optimal expression in the appropriate cells and/or tissues, *e.g.,* reproductive tissues. Exemplary inducible promoters include promoters activated by physical (*e.g.,* heat shock promoter) and/or chemical means (*e.g.,* IPTG or Tetracycline). Further, expression of the nucleotide sequence can be linked to other genes so as to achieve expression at specific stages of development or within specific tissues. Such expression can be achieved by placing the nucleotide sequence in operable linkage with the promoter of a gene expressed at a specific stage of development. For example, immunoglobulin sequences from one species engineered into the genome of a host species are place in operable linkage with a promoter sequence of a CD19 gene (a B cell specific gene) from the host species. B cell-specific expression at precise developmental stages when immunoglobulins are expressed is achieved.

[0367] Further, expression of the nucleotide sequence can be linked to other genes so as to achieve expression at specific stages of development or within specific tissues. Such expression can be achieved by placing the nucleotide sequence in operable linkage with the promoter of a gene expressed at a specific stage of development. For example, immunoglobulin sequences from one species engineered into the genome of a host species are place in operable linkage with a promoter sequence of a CD19 gene (a B cell specific gene) from the host species. B cell-specific expression at precise developmental stages when immunoglobulins are expressed is achieved.

[0368] Yet another method to achieve robust expression of an inserted nucleotide sequence is to employ a constitutive promoter. Exemplary constitutive promoters include SV40, CMV, UBC, EF1A, PGK and CAGG. In a similar fashion, the desired nucleotide sequence is placed in operable linkage with a selected constitutive promoter, which provides high level of expression of the protein(s) encoded by the nucleotide sequence.

[0369] The term "ectopic" is intended to include a displacement, or a placement at a position that is not normally encountered in nature (*e.g.,* placement of a nucleic acid sequence at a position that is not the same position as the nucleic acid sequence is found in a wild-type mouse). The term is used in the sense of its object being out of its normal, or proper, position. For example, the phrase "an ectopic nucleotide sequence encoding ..." refers to a nucleotide sequence that appears at a position at which it is not normally encountered in the mouse. For example, in the case of an ectopic nucleotide sequence encoding a mouse ADAM6 protein (or an ortholog or homolog or fragment thereof that provides the same or similar fertility benefit on male mice), the sequence can be placed at a different position in the mouse's genome than is normally found in a wild-type mouse. In such cases, novel sequence junctions of mouse sequence will be created by placing the sequence at a different position in the mouse's genome than in a wild-type mouse. A functional homolog or ortholog of mouse ADAM6 is a sequence that confers a rescue of fertility loss (*e.g.,* loss of the ability of a male mouse to generate offspring by mating) that is observed in an ADAM6$^{-/-}$ mouse. Functional homologs or orthologs include proteins that have at least about 89% identity or more, *e.g.,* up to 99% identity, to the amino acid sequence of ADAM6a and/or to the amino acid sequence of ADAM6b, and that can complement, or rescue ability to successfully mate, of a mouse that has a genotype that includes a deletion or knockout of ADAM6a and/or ADAM6b.

[0370] The ectopic position can be anywhere (*e.g.,* as with random insertion of a transgene containing a mouse ADAM6 sequence), or can be, *e.g.,* at a position that approximates (but is not precisely the same as) its location in a wild-type mouse (*e.g.,* in a modified endogenous mouse immunoglobulin locus, but either upstream or downstream of its natural position, *e.g.,* within a modified immunoglobulin locus but between different gene segments, or at a different position in a mouse V-D intergenic sequence). One example of an ectopic placement is maintaining the position normally found in wild-type mice within the endogenous immunoglobulin heavy chain locus while rendering the surrounding endogenous heavy chain gene segments in capable of rearranging to encode a functional heavy chain containing an endogenous heavy chain constant region. In this example, this may be accomplished by inversion of the chromosomal fragment containing the endogenous immunoglobulin heavy chain variable loci, *e.g.* using engineered site-specific recombination sites placed at positions flanking the variable region locus. Thus, upon recombination the endogenous heavy chain variable region loci are placed at a great distance away from the endogenous heavy chain constant region genes thereby preventing rearrangement to encode a functional heavy chain containing an endogenous heavy chain constant region. Other exemplary methods to achieve functional silencing of the endogenous immunoglobulin heavy chain variable gene locus while maintaining a functional ADAM6 locus will apparent to persons of skill upon reading this

disclosure and/or in combination with methods known in the art. With such a placement of the endogenous heavy chain locus, the endogenous ADAM6 genes are maintained and the endogenous immunoglobulin heavy chain locus is functionally silenced.

[0371] Another example of an ectopic placement is placement within a humanized immunoglobulin heavy chain locus. For example, a mouse comprising a replacement of one or more endogenous V_H gene segments with human V_H gene segments, wherein the replacement removes an endogenous ADAM6 sequence, can be engineered to have a mouse ADAM6 sequence located within sequence that contains the human V_H gene segments. The resulting modification would generate an (ectopic) mouse ADAM6 sequence within a human gene sequence, and the (ectopic) placement of the mouse ADAM6 sequence within the human gene sequence can approximate the position of the human ADAM6 pseudogene (*i.e.,* between two V segments) or can approximate the position of the mouse ADAM6 sequence (*i.e.,* within the V-D intergenic region). The resulting sequence junctions created by the joining of a (ectopic) mouse ADAM6 sequence within or adjacent to a human gene sequence (*e.g.,* an immunoglobulin gene sequence) within the germline of the mouse would be novel as compared to the same or similar position in the genome of a wild-type mouse.

[0372] Described are mice that lack an ADAM6 or ortholog or homolog thereof, wherein the lack renders the mice infertile, or substantially reduces fertility of the mice. The lack of ADAM6 or ortholog or homolog thereof may be due to a modification of an endogenous immunoglobulin heavy chain locus. A substantial reduction in fertility is, *e.g.,* a reduction in fertility (*e.g.,* breeding frequency, pups per litter, litters per year, etc.) of about 50%, 60%, 70%, 80%, 90%, or 95% or more. A rescue of fertility in substantial part is, *e.g.,* a restoration of fertility such that the mouse exhibits a fertility that is at least 70%, 80%, or 90% or more as compared with an unmodified (*i.e.,* an animal without a modification to the ADAM6 gene or ortholog or homolog thereof) heavy chain locus.

[0373] In a mouse the loss of ADAM6 function may be rescued by adding a mouse ADAM6 gene. In one embodiment, the loss of ADAM6 function in the mouse is rescued by adding an ortholog or homolog of a closely related specie with respect to the mouse, *e.g.,* a rodent, *e.g.,* a mouse of a different strain or species, a rat of any species, a rodent; wherein the addition of the ortholog or homolog to the mouse rescues the loss of fertility due to loss of ADAM6 function or loss of an ADAM6 gene. Orthologs and homologs from other species, in various embodiments, are selected from a phylogenetically related species and, in various embodiments, exhibit a percent identity with the endogenous ADAM6 (or ortholog) that is about 80% or more, 85% or more, 90% or more, 95% or more, 96% or more, or 97% or more; and that rescue ADAM6-related or (in a non-mouse) ADAM6 ortholog-related loss of fertility. For example, in a genetically modified male rat that lacks ADAM6 function (*e.g.,* a rat with an endogenous immunoglobulin heavy chain variable region replaced with a human immunoglobulin heavy chain variable region, or a knockout in the rat immunoglobulin heavy chain region), loss of fertility in the rat is rescued by addition of a rat ADAM6 or, in some embodiments, an ortholog of a rat ADAM6 (*e.g.,* an ADAM6 ortholog from another rat strain or species, or, in one embodiment, from a mouse).

[0374] Described are genetically modified mice that exhibit no fertility or a reduction in fertility due to modification of a nucleic acid sequence encoding an ADAM6 protein (or ortholog or homolog thereof) or a regulatory region operably linked with the nucleic acid sequence, comprise a nucleic acid sequence that complements, or restores, the loss in fertility where the nucleic acid sequence that complements or restores the loss in fertility is from a different strain of the same species or from a phylogenetically related species. The complementing nucleic acid sequence may be an ADAM6 ortholog or homolog or functional fragment thereof. The complementing ADAM6 ortholog or homolog or functional fragment thereof may be from a non-human animal that is closely related to the genetically modified mouse having the fertility defect. For example, where the genetically modified animal is a mouse of a particular strain, an ADAM6 ortholog or homolog or functional fragment thereof can be obtained from a mouse of another strain, or a mouse of a related species. In one embodiment, as the genetically modified animal comprising the fertility defect is a mouse and hence of the order Rodentia, the ADAM6 ortholog or homolog or functional fragment thereof is from another animal of the order Rodentia.

[0375] [Deleted]

[0376] In one embodiment, the genetically modified mouse is from a member of the family Muridae, and the ADAM6 ortholog or homolog is from a different species of the family Muridae. In a specific embodiment, the ADAM6 ortholog or homolog is from a rat, gerbil, spiny mouse, or crested rat of the family Muridae.

[0377] Described are one or more rodent ADAM6 orthologs or homologs or functional fragments thereof of a rodent in a family restores fertility to a genetically modified rodent of the same family that lacks an ADAM6 ortholog or homolog (*e.g.,* Cricetidae (*e.g.,* hamsters, New World rats and mice, voles); Muridae (*e.g.,* true mice and rats, gerbils, spiny mice, crested rats)).

[0378] ADAM6 orthologs, homologs, and fragments thereof may be assessed for functionality by ascertaining whether the ortholog, homolog, or fragment restores fertility to a genetically modified male mouse that lacks ADAM6 activity (that comprises a knockout of ADAM6 or its ortholog). In various embodiments, functionality is defined as the ability of a sperm of a genetically modified mouse lacking an endogenous ADAM6 or ortholog or homolog thereof to migrate an oviduct and fertilize an ovum of the same species of genetically modified animal.

[0379] In various aspects, mice that comprise deletions or replacements of the endogenous heavy chain variable region locus or portions thereof can be made that contain an ectopic nucleotide sequence that encodes a protein that confers similar fertility benefits to mouse ADAM6 (*e.g.,* an ortholog or a homolog or a fragment thereof that is functional in a male mouse). The ectopic nucleotide sequence can include a nucleotide sequence that encodes a protein that is an ADAM6 homolog or ortholog (or fragment thereof) of a different mouse strain or a different species, *e.g.,* a different rodent species, and that confers a benefit in fertility, *e.g.,*

increased number of litters over a specified time period, and/or increased number of pups per litter, and/or the ability of a sperm cell of a male mouse to traverse through a mouse oviduct to fertilize a mouse egg.

[0380] In one embodiment, the ADAM6 is a homolog or ortholog that is at least 89% to 99% identical to a mouse ADAM6 protein (e.g., at least 89% to 99% identical to mouse ADAM6a or mouse ADAM6b). In one embodiment, the ectopic nucleotide sequence encodes one or more proteins independently selected from a protein at least 89% identical to mouse ADAM6a, a protein at least 89% identical to mouse ADAM6b, and a combination thereof. In one embodiment, the homolog or ortholog is a rat, hamster, mouse, or guinea pig protein that is or is modified to be about 89% or more identical to mouse ADAM6a and/or mouse ADAM6b. In one embodiment, the homolog or ortholog is or is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a mouse ADAM6a and/or mouse ADAM6b.

[0381] In one aspect, mice are provided, wherein the mice comprise (a) an insertion of one or more human Vλ and Jλ gene segments upstream of a mouse immunoglobulin κ light chain constant region, (b) an insertion of one or more human V_H, one or more human D_H and one or more human J_H gene segments upstream of an non-human immunoglobulin heavy chain constant region, and (c) a nucleotide sequence that encodes an ADAM6 protein or a functional fragment thereof. In one embodiment, the non-human heavy and/or light chain constant regions are rodent constant regions (*e.g.,* selected from mouse, rat or hamster constant regions).

[0382] In one embodiment, the mouse comprises at least 12 to at least 40 human Vλ gene segments and at least one human Jλ gene segment. In a specific embodiment, the mouse comprises 12 human Vλ gene segments and at least one human Jλ gene segment. In a specific embodiment, the mouse comprises 28 human Vλ gene segments and at least one human Jλ gene segment. In one embodiment, the mouse comprises 40 human Vλ gene segments and at least one human Jλ gene segment. In various embodiments, the at least one human Jλ gene segment is selected from Jλ1, Jλ2, Jλ3 and Jλ7. In a specific embodiment, the mouse comprises at least four human Jλ gene segments. In one embodiment, the at least four human Jλ gene segments comprise at least Jλ1, Jλ2, Jλ3 and Jλ7.

[0383] In one embodiment, the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof is ectopic in the mouse. In one embodiment, the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof (that is functional in the mouse) is present the same location as compared to a wild-type type non-human ADAM6 locus. In one embodiment, the nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is present at an ectopic location in the genome of the mouse. The nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is present within immunoglobulin gene segments which are human heavy chain gene segments.

[0384] In one embodiment, the mouse lacks an endogenous immunoglobulin V_L and/or a J_L

gene segment at an endogenous immunoglobulin light chain locus. In one embodiment, the mouse comprises endogenous immunoglobulin V_L and/or J_L gene segments that are incapable of rearranging to form an immunoglobulin V_L domain in the non-human animal. In one embodiment, all or substantially all endogenous immunoglobulin Vκ and Jκ gene segments are replaced with one or more human Vλ and Jλ gene segments. In one embodiment, all or substantially all endogenous immunoglobulin Vλ and Jλ gene segments are replaced with one or more human Vλ and Jλ gene segments. In one embodiment, all or substantially all endogenous immunoglobulin V_L and J_L gene segments are intact in the non-human animal and the non-human animal comprises one or more human Vλ gene segments and one or more human Jλ gene segments inserted between endogenous immunoglobulin V_L and/or J_L gene segments and an endogenous immunoglobulin light chain constant region. In a specific embodiment, the intact endogenous immunoglobulin V_L and J_L gene segments are rendered incapable of rearranging to form a V_L domain of an antibody in the mouse. In various embodiments, the endogenous immunoglobulin light chain locus of the mouse is an immunoglobulin κ light chain locus. In various embodiments, the endogenous immunoglobulin light chain locus of the mouse is an immunoglobulin λ light chain locus. In various embodiments, the endogenous immunoglobulin V_L and J_L gene segments are Vκ and Jκ gene segments. In various embodiments, the endogenous immunoglobulin V_L and J_L gene segments are Vλ and Jλ gene segments.

[0385] In one embodiment, the mouse further comprises a human Vκ-Jκ intergenic region from a human κ light chain locus, wherein the human Vκ-Jκ intergenic region is contiguous with the one or more human Vλ and Jλ gene segments. In a specific embodiment, the human Vκ-Jκ intergenic region is placed between a human Vλ gene segment and a human Jλ gene segment.

[0386] Described are cells and/or tissues derived from mice as described herein, wherein the cells and/or tissues comprise (a) an insertion of one or more human Vλ and Jλ gene segments upstream of an non-human immunoglobulin light chain constant region, (b) an insertion of one or more human V_H, one or more human D_H and one or more human J_H gene segments upstream of an non-human immunoglobulin heavy chain constant region, and (c) a nucleotide sequence that encodes an ADAM6 protein or a functional fragment thereof. The non-human heavy and/or light chain constant regions may be mouse constant regions. The non-human heavy and/or light chain constant regions may be rat constant regions. The non-human heavy and/or light chain constant regions may be hamster constant regions.

[0387] In one embodiment, the nucleotide sequence that encodes an ADAM6 protein or functional fragment thereof is ectopic in the cell and/or tissue. In one embodiment the mouse cell and/or tissue is derived from a mouse and the nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is present at an ectopic location. In the mouse cell and/or tissue is derived from a mouse and the nucleotide sequence encodes a mouse ADAM6 protein or functional fragment thereof and is present within immunoglobulin gene segments. The immunoglobulin gene segments are heavy chain gene segments.

[0388] Described is the use of a mouse as described herein to make an antigen-binding protein, wherein the mouse expresses (a) an antibody that comprises (i) an immunoglobulin light chain that comprises a human Vλ domain and a mouse κ light chain constant region and (ii) an immunoglobulin heavy chain that comprises a human V_H domain and a non-human constant region; and (b) an ADAM6 protein or functional fragment thereof. The antigen binding protein may be human. The non-human constant regions are rodent constant regions.

[0389] In one aspect, a mouse cell or tissue derived from a mouse as described herein is provided. In one embodiment, the mouse cell or tissue comprises one or more human immunoglobulin Vλ gene segments and at least one human immunoglobulin Jλ gene segments contiguous with a mouse immunoglobulin κ light chain constant region gene and one or more human V_H, one or more human D_H and one or more human J_H gene segments contiguous with a non-human immunoglobulin heavy chain constant region gene, wherein the cell or tissue expresses an ADAM6 protein or functional fragment thereof. In one embodiment, the non-human light chain constant region gene is a mouse Cκ or mouse Cλ.

[0390] In one embodiment, the nucleotide sequence that encodes the ADAM6 protein or functional fragment thereof is ectopic. In various embodiments, the mouse cell is a mouse B cell. In various embodiments, the non-human cell is an embryonic stem cell.

[0391] In one embodiment, the tissue is derived from spleen, bone marrow or lymph node of the mouse.

[0392] In one aspect, use of a cell or tissue derived from a mouse as described herein to make a hybridoma or quadroma is provided.

[0393] In one aspect, a mouse cell comprising a modified genome as described herein is provided, wherein the mouse cell is an oocyte, a host embryo, or a fusion of a cell from a mouse as described herein and a cell from a different mouse.

[0394] In one aspect, use of a cell or tissue derived from a mouse as described herein to make a fully human antibody is provided. In one embodiment, the fully human antibody comprises a human V_H domain and a human Vλ domain isolated from a non-human animal as described herein.

[0395] In one aspect, a method for making an antibody that binds to an antigen of interest is provided, wherein the method comprises (a) exposing a mouse as described herein to an antigen of interest, (b) isolating one or more B lymphocytes of the mouse, wherein the one or more B lymphocytes express an antibody that binds the antigen of interest, and (c) identifying a nucleic acid sequence that encodes an immunoglobulin light chain of the antibody that binds that antigen of interest, wherein the immunoglobulin light chain comprises a human Vλ domain and a non-human light chain constant domain, and (d) employing the nucleic acid sequence of

(c) with a human immunoglobulin light chain constant region nucleic acid sequence to make a human antibody that binds the antigen of interest.

[0396] In one embodiment, the non-human light chain constant domain is a mouse Cκ. In one embodiment, the non-human light chain constant domain is a mouse Cλ.

[0397] In one aspect, a fertile male mouse comprising a modification at an immunoglobulin heavy chain locus is provided, wherein the fertile male mouse comprises an ectopic ADAM6 sequence that is functional in the male mouse.

Ectopic ADAM6 in Humanized Heavy Chain Mice

[0398] Developments in gene targeting, *e.g.,* the development of bacterial artificial chromosomes (BACs), now enable the recombination of relatively large genomic fragments. BAC engineering has allowed for the ability to make large deletions, and large insertions, into mouse ES cells.

[0399] Mice that make human antibodies have been available for some time now. Although they represent an important advance in the development of human therapeutic antibodies, these mice display a number of significant abnormalities that limit their usefulness. For example, they display compromised B cell development. The compromised development may be due to a variety of differences between the transgenic mice and wild-type mice.

[0400] Human antibodies might not optimally interact with mouse pre B cell or B cell receptors on the surface of mouse cells that signal for maturation, proliferation, or survival during clonal selection. Fully human antibodies might not optimally interact with a mouse Fc receptor system; mice express Fc receptors that do not display a one-to-one correspondence with human Fc receptors. Finally, various mice that make fully human antibodies do not include all genuine mouse sequences, *e.g.,* downstream enhancer elements and other locus control elements, which may be required for wild-type B cell development.

[0401] Mice that make fully human antibodies generally comprise endogenous immunoglobulin loci that are disabled in some way, and human transgenes that comprise variable and constant immunoglobulin gene segments are introduced into a random location in the mouse genome. As long as the endogenous locus is sufficiently disabled so as not to rearrange gene segments to form a functional immunoglobulin gene, the goal of making fully human antibodies in such a mouse can be achieved-albeit with compromised B cell development.

[0402] Although compelled to make fully human antibodies from the human transgene locus, generating human antibodies in a mouse is apparently an unfavored process. In some mice, the process is so unfavored as to result in formation of chimeric human variable/mouse constant heavy chains (but not light chains) through the mechanism of *trans*-switching. By this mechanism, transcripts that encode fully human antibodies undergo isotype switching in *trans*

from the human isotype to a mouse isotype. The process is in *trans,* because the fully human transgene is located apart from the endogenous locus that retains an undamaged copy of a mouse heavy chain constant region gene. Although in such mice *trans*-switching is readily apparent the phenomenon is still insufficient to rescue B cell development, which remains frankly impaired. In any event, *trans*-switched antibodies made in such mice retain fully human light chains, since the phenomenon of *trans*-switching apparently does not occur with respect to light chains; *trans*-switching presumably relies on switch sequences in endogenous loci used (albeit differently) in normal isotype switching in cis. Thus, even when mice engineered to make fully human antibodies select a *trans*-switching mechanism to make antibodies with mouse constant regions, the strategy is still insufficient to rescue normal B cell development.

[0403] A primary concern in making antibody-based human therapeutics is making a sufficiently large diversity of human immunoglobulin variable region sequences to identify useful variable domains that specifically recognize particular epitopes and bind them with a desirable affinity, usually-but not always-with high affinity. Prior to the development of VELOCIMMUNE® mice (described herein), there was no indication that mice expressing human variable regions with mouse constant regions would exhibit any significant differences from mice that made human antibodies from a transgene. That supposition, however, was incorrect.

[0404] VELOCIMMUNE® mice, which contain a precise replacement of mouse immunoglobulin variable regions with human immunoglobulin variable regions at the endogenous mouse loci, display a surprising and remarkable similarity to wild-type mice with respect to B cell development. In a surprising and stunning development, VELOCIMMUNE® mice displayed an essentially normal, wild-type response to immunization that differed only in one significant respect from wild-type mice-the variable regions generated in response to immunization are fully human.

[0405] VELOCIMMUNE® mice contain a precise, large-scale replacement of germline variable regions of mouse immunoglobulin heavy chain (IgH) and immunoglobulin light chain (e.g., κ light chain, Igκ) with corresponding human immunoglobulin variable regions, at the endogenous loci. In total, about six megabases of mouse loci are replaced with about 1.5 megabases of human genomic sequence. This precise replacement results in a mouse with hybrid immunoglobulin loci that make heavy and light chains that have a human variable regions and a mouse constant region. The precise replacement of mouse V_H-D_H-J_H and VK-JK segments leave flanking mouse sequences intact and functional at the hybrid immunoglobulin loci. The humoral immune system of the mouse functions like that of a wild-type mouse. B cell development is unhindered in any significant respect and a rich diversity of human variable regions is generated in the mouse upon antigen challenge.

[0406] VELOCIMMUNE® mice are possible because immunoglobulin gene segments for heavy and κ light chains rearrange similarly in humans and mice, which is not to say that their loci are the same or even nearly so-clearly they are not. However, the loci are similar enough that humanization of the heavy chain variable gene locus can be accomplished by replacing

about three million base pairs of contiguous mouse sequence that contains all the V_H, D_H, and J_H gene segments with about one million bases of contiguous human genomic sequence covering basically the equivalent sequence from a human immunoglobulin locus.

[0407] In some embodiments, further replacement of certain mouse constant region gene sequences with human gene sequences (*e.g.,* replacement of mouse C_H1 sequence with human C_H1 sequence, and replacement of mouse C_L sequence with human C_L sequence) results in mice with hybrid immunoglobulin loci that make antibodies that have human variable regions and partly human constant regions, suitable for, *e.g.,* making fully human antibody fragments, *e.g.,* fully human Fab's. Mice with hybrid immunoglobulin loci exhibit normal variable gene segment rearrangement, normal somatic hypermutation, and normal class switching. These mice exhibit a humoral immune system that is indistinguishable from wild type mice, and display normal cell populations at all stages of B cell development and normal lymphoid organ structures-even where the mice lack a full repertoire of human variable region gene segments. Immunizing these mice results in robust humoral responses that display a wide diversity of variable gene segment usage.

[0408] The precise replacement of mouse germline variable region gene segments allows for making mice that have partly human immunoglobulin loci. Because the partly human immunoglobulin loci rearrange, hypermutate, and class switch normally, the partly human immunoglobulin loci generate antibodies in a mouse that comprise human variable regions. Nucleotide sequences that encode the variable regions can be identified and cloned, then fused (*e.g.,* in an *in vitro* system) with any sequences of choice, *e.g.,* any immunoglobulin isotype suitable for a particular use, resulting in an antibody or antigen-binding protein derived wholly from human sequences.

[0409] Large-scale humanization by recombineering methods were used to modify mouse embryonic stem (ES) cells to precisely replace up to three megabases of the mouse heavy chain immunoglobulin locus that included essentially all of the mouse V_H, D_H, and J_H gene segments with equivalent human gene segments with up to a one megabase human genomic sequence containing some or essentially all human V_H, D_H, and J_H gene segments. Up to a one-half megabase segment of the human genome comprising one of two repeats encoding essentially all human Vκ and Jκ gene segments was used to replace a three megabase segment of the mouse immunoglobulin κ light chain locus containing essentially all of the mouse VK and JK gene segments.

[0410] Mice with such replaced immunoglobulin loci can comprise a disruption or deletion of the endogenous mouse ADAM6 locus, which is normally found between the 3'-most V_H gene segment and the 5'-most D_H gene segment at the mouse immunoglobulin heavy chain locus. Disruption in this region can lead to reduction or elimination of functionality of the endogenous mouse ADAM6 locus. If the 3'-most V_H gene segments of the human heavy chain repertoire are used in a replacement, an intergenic region containing a pseudogene that appears to be a human ADAM6 pseudogene is present between these V_H gene segments, i.e., between

human V_H1-2 and V_H1-6. However, male mice that comprise this human intergenic sequence exhibit a reduction in fertility.

[0411] Mice are described that comprise the replaced loci as described above, and that also comprise an ectopic nucleic acid sequence encoding a mouse ADAM6, where the mice exhibit essentially normal fertility. The ectopic nucleic acid sequence may comprise a mouse ADAM6a and/or a mouse ADAM6b sequence or functional fragments thereof placed between a human V_H1-2 gene segment and a human V_H6-1 gene segment at a modified endogenous heavy chain locus. The ectopic nucleic acid sequence may be SEQ ID NO:3, placed between human V_H1-2 and V_H1-6 at the modified endogenous heavy chain locus. The direction of transcription of the ADAM6 genes of SEQ ID NO:3 are opposite with respect to the direction of transcription of the surrounding human V_H gene segments. Although examples herein show rescue of fertility by placing the ectopic sequence between the indicated human V_H gene segments, skilled persons will recognize that placement of the ectopic sequence at any suitable transcriptionally-permissive locus in the mouse genome (or even extrachromosomally) will be expected to similarly rescue fertility in a male mouse.

[0412] The phenomenon of complementing a mouse that lacks a functional ADAM6 locus with an ectopic sequence that comprises a mouse ADAM6 gene or ortholog or homolog or functional fragment thereof is a general method that is applicable to rescuing any mice with nonfunctional or minimally functional endogenous ADAM6 loci. Thus, a great many mice that comprise an ADAM6-disrupting modification of the immunoglobulin heavy chain locus can be rescued with the compositions and methods of the invention. Accordingly, the invention comprises mice with a wide variety of modifications of immunoglobulin heavy chain loci that compromise endogenous ADAM6 function. Some (non-limiting) examples are provided in this description. In addition to the VELOCIMMUNE® mice described, the compositions and methods related to ADAM6 can be used in a great many applications, *e.g.,* when modifying a heavy chain locus in a wide variety of ways.

[0413] Described is a mouse that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein (or ortholog or homolog or functional fragment thereof), a replacement of all or substantially all mouse V_H gene segments with one or more human V_H gene segments, a replacement of all or substantially all mouse D_H gene segments and J_H gene segments with human D_H and human J_H gene segments; wherein the mouse lacks a C_H1 and/or hinge region. In one embodiment, the mouse makes a single variable domain binding protein that is a dimer of immunoglobulin chains selected from: (a) human V_H - mouse C_H1 - mouse C_H2 - mouse C_H3; (b) human V_H -mouse hinge - mouse C_H2 - mouse C_H3; and, (c) human V_H - mouse C_H2 - mouse C_H3.

[0414] In one aspect, the nucleotide sequence that rescues fertility is placed within a human immunoglobulin heavy chain variable region sequence (*e.g.,* between human V_H1-2 and V_H1-6 gene segments) in a mouse that has a replacement of one or more mouse immunoglobulin

heavy chain variable gene segments (mV_H's, mD_H's, and/or mJ_H's) with one or more human immunoglobulin heavy chain variable gene segments (hV_H's, hD_H's, and/or hJ_H's), and the mouse further comprises a replacement of one or more mouse immunoglobulin κ light chain variable gene segments (mVκ's and/or mJκ's) with one or more human immunoglobulin κ light chain variable gene segments (hVκ's and/or hJκ's). In one embodiment, the nucleotide sequence is placed between a human V_H1-2 gene segment and a human V_H1-6 gene segment in a VELOCIMMUNE® mouse (US 6,596,541 and US 7,105,348). In one embodiment, the VELOCIMMUNE® mouse so modified comprises a replacement with all or substantially all human immunoglobulin heavy chain variable gene segments (all hV_H's, hD_H's, and hJ_H's) and all or substantially all human immunoglobulin κ light chain variable gene segments (hVκ's and hJK's).

[0415] In one embodiment, the one or more mouse immunoglobulin heavy chain variable gene segments comprises about three megabases of the mouse immunoglobulin heavy chain locus. In one embodiment, the one or more mouse immunoglobulin heavy chain variable gene segments comprises at least 89 V_H gene segments, at least 13 D_H gene segments, at least four J_H gene segments or a combination thereof of the mouse immunoglobulin heavy chain locus. In one embodiment, the one or more human immunoglobulin heavy chain variable gene segments comprises about one megabase of a human immunoglobulin heavy chain locus. In one embodiment, the one or more human immunoglobulin heavy chain variable gene segments comprises at least 80 V_H gene segments, at least 27 D_H gene segments, at least six J_H gene segments or a combination thereof of a human immunoglobulin heavy chain locus.

[0416] In one embodiment, the one or more mouse immunoglobulin κ light chain variable gene segments comprises about three megabases of the mouse immunoglobulin κ light chain locus. In one embodiment, the one or more mouse immunoglobulin κ light chain variable gene segments comprises at least 137 Vκ gene segments, at least five Jκ gene segments or a combination thereof of the mouse immunoglobulin κ light chain locus. In one embodiment, the one or more human immunoglobulin κ light chain variable gene segments comprises about one-half megabase of a human immunoglobulin κ light chain locus. In a specific embodiment, the one or more human immunoglobulin κ light chain variable gene segments comprises the proximal repeat (with respect to the immunoglobulin κ constant region) of a human immunoglobulin κ light chain locus. In one embodiment, the one or more human immunoglobulin κ light chain variable gene segments comprises at least 40Vκ gene segments, at least five Jκ gene segments or a combination thereof of a human immunoglobulin κ light chain locus.

[0417] The nucleotide sequence is placed between two human immunoglobulin gene segments which are human heavy chain gene segments.

[0418] Described is that a functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) present in the midst of mouse gene segments that are present at the endogenous mouse heavy chain variable region locus, said locus incapable of rearranging to

encode a functional heavy chain containing an endogenous heavy chain constant region. The endogenous mouse heavy chain locus may comprise at least one and up to 89 V_H gene segments, at least one and up to 13 D_H gene segments, at least one and up to four J_H gene segments and a combination thereof. A functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) may encode one or more ADAM6 proteins that are functional in the mouse, wherein the one or more ADAM6 proteins comprise SEQ ID NO: 1, SEQ ID NO: 2 and/or a combination thereof.

[0419] In one aspect, a functional mouse ADAM6 locus (or ortholog or homolog or functional fragment thereof) is present in the midst of human V_H gene segments that replace endogenous mouse V_H gene segments. In one embodiment, at least 89 mouse V_H gene segments are removed and replaced with one or more human V_H gene segments, and the mouse ADAM6 locus is present immediately adjacent to the 3' end of the human V_H gene segments, or between two human V_H gene segments. In a specific embodiment, the mouse ADAM6 locus is present between two V_H gene segments within about 20 kilo bases (kb) to about 40 kilo bases (kb) of the 3' terminus of the inserted human V_H gene segments. In a specific embodiment, the mouse ADAM6 locus is present between two V_H gene segments within about 29 kb to about 31 kb of the 3' terminus of the inserted human V_H gene segments. In a specific embodiment, the mouse ADAM6 locus is present within about 30 kb of the 3' terminus of the inserted human V_H gene segments. In a specific embodiment, the mouse ADAM6 locus is present within about 30,184 bp of the 3' terminus of the inserted human V_H gene segments. In a specific embodiment, the replacement includes human V_H gene segments V_H1-2 and V_H6-1, and the mouse ADAM6 locus is present downstream of the V_H1-2 gene segment and upstream of the V_H6-1 gene segment. In a specific embodiment, the mouse ADAM6 locus is present between a human V_H1-2 gene segment and a human V_H6-1 gene segment, wherein the 5' end of the mouse ADAM6 locus is about 13,848 bp from the 3' terminus of the human V_H1-2 gene segment and the 3' end of the ADAM6 locus is about 29,737 bp 5' of the human V_H6-1 gene segment. In a specific embodiment, the mouse ADAM6 locus comprises SEQ ID NO:3 or a fragment thereof that confers ADAM6 function within cells of the mouse. In a specific embodiment, the arrangement of human V_H gene segments is then the following (from upstream to downstream with respect to direction of transcription of the human V_H gene segments): human V_H1-2 - mouse ADAM6 locus - human V_H6-1. In a specific embodiment, the ADAM6 pseudogene between human V_H1-2 and human V_H6-1 is replaced with the mouse ADAM6 locus. In one embodiment, the orientation of one or more of mouse ADAM6a and mouse ADAM6b of the mouse ADAM6 locus is opposite with respect to direction of transcription as compared with the orientation of the human V_H gene segments. Alternatively, the mouse ADAM6 locus is present in the intergenic region between the 3'-most human V_H gene segment and the 5'-most D_H gene segment. This can be the case whether the 5'-most D_H segment is mouse or human.

[0420] Similarly, a mouse modified with one or more human V_L gene segments (*e.g.,* Vκ or Vλ segments) replacing all or substantially all endogenous mouse V_H gene segments can be modified so as to either maintain the endogenous mouse ADAM6 locus, as described above, *e.g.,* by employing a targeting vector having a downstream homology arm that includes a mouse ADAM6 locus or functional fragment thereof, or to replace a damaged mouse ADAM6 locus with an ectopic sequence positioned between two human V_L gene segments or between the human V_L gene segments and a D_H gene segment or a J gene segment. The replacement includes two or more human V_L gene segments, and the mouse ADAM6 locus or functional fragment thereof is present between the two 3'-most V_L gene segments. The arrangement of human V_L gene segments may then be the following (from upstream to downstream with respect to direction of transcription of the human gene segments): human V_L3'-1 - mouse ADAM6 locus - human V_L3'. The orientation of one or more of mouse ADAM6a and mouse ADAM6b of the mouse ADAM6 locus may be opposite with respect to direction of transcription as compared with the orientation of the human V_L gene segments. Alternatively, the mouse ADAM6 locus is present in the intergenic region between the 3'-most human V_L gene segment and the 5'-most D_H gene segment. This can be the case whether the 5'-most D_H segment is mouse or human.

[0421] Described is a mouse with a replacement of one or more endogenous mouse V_H gene segments, and that comprises at least one endogenous mouse D_H gene segment. In such a mouse, the modification of the endogenous mouse V_H gene segments can comprise a modification of one or more of the 3'-most V_H gene segments, but not the 5'-most D_H gene segment, where care is taken so that the modification of the one or more 3'-most V_H gene segments does not disrupt or render the endogenous mouse ADAM6 locus nonfunctional. For example, the mouse may comprise a replacement of all or substantially all endogenous mouse V_H gene segments with one or more human V_H gene segments, and the mouse comprises one or more endogenous D_H gene segments and a functional endogenous mouse ADAM6 locus.

[0422] [Deleted]

[0423] Employing mice that contain an ectopic sequence that encodes a mouse ADAM6 protein or an ortholog or homolog or functional homolog thereof are useful where modifications disrupt the function of endogenous mouse ADAM6. The probability of disrupting endogenous mouse ADAM6 function is high when making modifications to mouse immunoglobulin loci, in particular when modifying mouse immunoglobulin heavy chain variable regions and surrounding sequences. Therefore, such mice provide particular benefit when making mice with immunoglobulin heavy chain loci that are deleted in whole or in part, are humanized in whole or in part, or are replaced (*e.g.,* with Vκ or Vλ sequences) in whole or in part. Methods for making the genetic modifications described for the mice described below are known to those skilled in the art.

[0424] Mice containing an ectopic sequence encoding a mouse ADAM6 protein, or a substantially identical or similar protein that confers the fertility benefits of a mouse ADAM6 protein, are particularly useful in conjunction with modifications to a mouse immunoglobulin heavy chain variable gene locus that disrupt or delete the endogenous mouse ADAM6 sequence. Although primarily described in connection with mice that express antibodies with human variable regions and mouse constant regions, such mice are useful in connection with any genetic modifications that disrupt endogenous mouse ADAM6 genes. Persons of skill will recognize that this encompasses a wide variety of genetically modified mice that contain modifications of mouse immunoglobulin heavy chain variable gene loci. These include, for example, mice with a deletion or a replacement of all or a portion of mouse immunoglobulin heavy chain gene segments, regardless of other modifications.

[0425] Described are genetically modified mice that comprise an ectopic mouse, rodent, or other ADAM6 gene (or ortholog or homolog or fragment) functional in a mouse, and one or more human immunoglobulin variable and/or constant region gene segments. Other ADAM6 gene orthologs or homologs or fragments functional in a mouse may include sequences from bovine, canine, primate, rabbit or other non-human sequences.

[0426] Disclosed is a mouse that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein, a replacement of all or substantially all mouse V_H gene segments with one or more human V_H gene segments; a replacement of all or substantially all mouse D_H gene segments with one or more human D_H gene segments; and a replacement of all or substantially all mouse J_H gene segments with one or more human J_H gene segments.

[0427] In one embodiment, the mouse further comprises a replacement of a mouse C_H1 nucleotide sequence with a human C_H1 nucleotide sequence. In one embodiment, the mouse further comprises a replacement of a mouse hinge nucleotide sequence with a human hinge nucleotide sequence. In one embodiment, the mouse further comprises a replacement of an immunoglobulin light chain variable locus (V_L and J_L) with a human immunoglobulin light chain variable locus. In one embodiment, the mouse further comprises a replacement of a mouse immunoglobulin light chain constant region nucleotide sequence with a human immunoglobulin light chain constant region nucleotide sequence. In a specific embodiment, the V_L, J_L, and C_L are immunoglobulin κ light chain sequences. In a specific embodiment, the mouse comprises a mouse C_H2 and a mouse C_H3 immunoglobulin constant region sequence fused with a human hinge and a human C_H1 sequence, such that the mouse immunoglobulin loci rearrange to form a gene that encodes a binding protein comprising (a) a heavy chain that has a human variable region, a human C_H1 region, a human hinge region, and a mouse C_H2 and a mouse C_H3 region; and (b) a gene that encodes an immunoglobulin light chain that comprises a human variable domain and a human constant region.

[0428] In one aspect, the mouse provided comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein, a replacement of all or substantially all mouse V_H gene

segments with one or more human V_L gene segments, and optionally a replacement of all or substantially all D_H gene segments and/or J_H gene segments with one or more human D_H gene segments and/or human J_H gene segments, or optionally a replacement of all or substantially all D_H gene segments and J_H gene segments with one or more human J_L gene segments.

[0429] The mouse may comprise a replacement of all or substantially all mouse V_H, D_H, and J_H gene segments with one or more V_L, one or more D_H, and one or more J gene segments (*e.g.,* Jκ or Jλ), wherein the gene segments are operably linked to an endogenous mouse hinge region, wherein the mouse forms a rearranged immunoglobulin chain gene that contains, from 5' to 3' in the direction of transcription, human V_L - human or mouse D_H - human or mouse J - mouse hinge - mouse C_H2 - mouse C_H3. In one embodiment, the J region is a human Jκ region. The J region may be a human J_H region. The J region is a human Jλ region. The human V_L region may be selected from a human Vλ region and a human Vκ region.

[0430] The mouse may express a single variable domain antibody having a mouse or human constant region and a variable region derived from a human Vκ, a human D_H and a human Jκ; a human Vκ, a human D_H, and a human J_H; a human Vλ, a human D_H, and a human Jλ; a human Vλ, a human D_H, and a human J_H; a human Vκ, a human D_H, and a human Jλ; a human Vλ, a human D_H, and a human Jκ. Recombination recognition sequences may be modified so as to allow for productive rearrangements to occur between recited V, D, and J gene segments or between recited V and J gene segments.

[0431] Described is a mouse that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein (or ortholog or homolog or functional fragment thereof), a replacement of all or substantially all mouse V_H gene segments with one or more human V_L gene segments, a replacement of all or substantially all mouse D_H gene segment and J_H gene segments with human J_L gene segments; wherein the mouse lacks a C_H1 and/or hinge region.

[0432] In one embodiment, the mouse lacks a sequence encoding a C_H1 domain. In one embodiment, the mouse lacks a sequence encoding a hinge region. In one embodiment, the mouse lacks a sequence encoding a C_H1 domain and a hinge region.

[0433] In a specific embodiment, the mouse expresses a binding protein that comprises a human immunoglobulin light chain variable domain (λ or κ) fused to a mouse C_H2 domain that is attached to a mouse C_H3 domain.

[0434] Described is a mouse that comprises an ectopic ADAM6 sequence that encodes a functional ADAM6 protein (or ortholog or homolog or functional fragment thereof), a replacement of all or substantially all mouse V_H gene segments with one or more human V_L

gene segments, a replacement of all or substantially all mouse D_H and J_H gene segments with human J_L gene segments.

[0435] In one embodiment, the mouse comprises a deletion of an immunoglobulin heavy chain constant region gene sequence encoding a C_H1 region, a hinge region, a C_H1 and a hinge region, or a C_H1 region and a hinge region and a C_H2 region.

[0436] In one embodiment, the mouse makes a single variable domain binding protein comprising a homodimer selected from the following: (a) human V_L - mouse C_H1 - mouse C_H2 - mouse C_H3; (b) human V_L - mouse hinge - mouse C_H2 - mouse C_H3; (c) human V_L - mouse C_H2 - mouse C_H3.

[0437] Described is a mouse with a disabled endogenous heavy chain immunoglobulin locus, comprising a disabled or deleted endogenous mouse ADAM6 locus, wherein the mouse comprises a nucleic acid sequence that expresses a human or mouse or human/mouse or other chimeric antibody.

[0438] In one embodiment, the mouse further comprises a disabled endogenous immunoglobulin light chain locus. In a specific embodiment, the endogenous immunoglobulin light chain locus is selected from a kappa (κ) and a lambda (λ) light chain locus. In a specific embodiment, the mouse comprises a disabled endogenous κ light chain locus and a disabled λ light chain locus, wherein the mouse expresses an antibody that comprises a human immunoglobulin heavy chain variable domain and a human immunoglobulin light chain domain. In one embodiment, the human immunoglobulin light chain domain is selected from a human κ light chain domain and a human λ light chain domain. In a specific embodiment, the mouse comprises a disabled endogenous κ light chain locus, wherein the mouse expresses an antibody that comprises a human/mouse (i.e., human variable/mouse constant) immunoglobulin heavy chain and a human/mouse immunoglobulin light chain comprising a human Vλ domain. In one embodiment, the human/mouse immunoglobulin light chain comprises a mouse Cκ. In one embodiment, the human/mouse immunoglobulin light chain comprises a mouse Cλ. In a specific embodiment, the mouse Cλ is a Cλ2.

[0439] Described is a genetically modified animal that expresses a chimeric antibody and expresses an ADAM6 protein or ortholog or homolog thereof that is functional in the genetically modified animal.

[0440] The genetically modified animal is a mouse. In one embodiment, the genetically modified animal is a mouse, and the ADAM6 protein or ortholog or homolog thereof is from a mouse strain that is a different strain than the genetically modified animal.

[0441] A chimeric antibody may comprise a human variable domain and a constant region sequence of a rodent. The rodent may be selected from a rodent of the family Cricetidae and a rodent of family Muridae. The rodent may be of the family Cricetidae and of the family Muridae

is a mouse. The rodent may be of the family Cricetidae and of the family Muridae is a rat. The chimeric antibody may comprise a human variable domain and a constant domain from an animal selected from a mouse or rat; the mouse or rat may be selected from the family Cricetidae and the family Muridae. The chimeric antibody may comprise a human heavy chain variable domain, a human light chain variable domain and a constant region sequence derived from a rodent selected from mouse and rat, wherein the human heavy chain variable domain and the human light chain are cognate. Cognate includes that the human heavy chain and the human light chain variable domains are from a single B cell that expresses the human light chain variable domain and the human heavy chain variable domain together and present the variable domains together on the surface of an individual B cell.

[0442] The chimeric antibody may be expressed from an immunoglobulin locus. The heavy chain variable domain of the chimeric antibody is expressed from a rearranged endogenous immunoglobulin heavy chain locus. The light chain variable domain of the chimeric antibody may be expressed from a rearranged endogenous immunoglobulin light chain locus.

[0443] [Deleted]

[0444] Described is a mouse, comprising a humanized immunoglobulin heavy chain locus, wherein the humanized immunoglobulin heavy chain locus comprises a non-human ADAM6 sequence or ortholog or homolog thereof.

[0445] The non-human animal may be a rodent selected from a mouse, a rat, and a hamster.

[0446] The non-human ADAM6 ortholog or homolog may be a sequence that is orthologous and/or homologous to a mouse ADAM6 sequence, wherein the ortholog or homolog is functional in the mouse.

[0447] In a specific embodiment, the ADAM6 ortholog or homolog is from an animal that is selected from a different mouse species, a rat, and a hamster.

[0448] In a specific embodiment, the ADAM6 sequence is from an animal selected from a rodent of superfamily Dipodoidea and a rodent of the superfamily Muroidea. In a specific embodiment, the mouse is of superfamily Muroidea, and the ADAM6 ortholog or homolog is from a mouse or a rat or a hamster of superfamily Muroidea.

[0449] The humanized heavy chain locus comprises one or more human V_H gene segments, one or more human D_H gene segments and one or more human J_H gene segments. In a specific embodiment, the one or more human V_H gene segments, one or more human D_H gene segments and one or more human J_H gene segments are operably linked to one or more human, chimeric and/or rodent (e.g., mouse or rat) constant region genes. In one embodiment, the constant region genes are mouse. In one embodiment, the constant region genes are rat. In one embodiment, the constant region genes are hamster. In one

embodiment, the constant region genes comprise a sequence selected from a hinge, a C_H2, a C_H3, and a combination thereof. In specific embodiment, the constant region genes comprise a hinge, a C_H2, and a CH3 sequence.

[0450] In one embodiment, the non-human ADAM6 sequence is contiguous with a human immunoglobulin heavy chain sequence. The non-human ADAM6 sequence is positioned within a human immunoglobulin heavy chain sequence. The human immunoglobulin heavy chain sequence comprises a V, D and J gene segment.

[0451] In one embodiment, the non-human ADAM6 sequence is juxtaposed with a V gene segment. In one embodiment, the non-human ADAM6 sequence is positioned between two V gene segments. In one embodiment, the non-human ADAM6 sequence is juxtaposed between a V and a D gene segment. In one embodiment, the mouse ADAM6 sequence is positioned between a V and a J gene segment. In one embodiment, the mouse ADAM6 sequence is juxtaposed between a D and a J gene segment.

[0452] Described is a genetically modified non-human animal, comprising a B cell that expresses a human V_H domain cognate with a human V_L domain from an immunoglobulin locus, wherein the non-human animal expresses a non-immunoglobulin non-human protein from the immunoglobulin locus.

[0453] [Deleted]

[0454] The non-immunoglobulin non-human protein may be a rodent protein, for example of family Muridae.

[0455] [Deleted]

[0456] [Deleted]

[0457] [Deleted]

[0458] The human immunoglobulin sequence comprises one or more V_H gene segments, one or more D_H gene segments and one or more JH gene segments.

[0459] In one embodiment, the immunoglobulin sequence comprises one or more V_H gene segments have a high frequency in natural human repertoires. In a specific embodiment, the one or more V_H gene segments comprise no more than two V_H gene segments, no more than three V_H gene segments, no more than four V_H gene segments, no more than five V_H gene segments, no more than six V_H gene segments, no more than seven V_H gene segments, no more than eight V_H gene segments, no more than nine V_H gene segments, no more than 10 V_H gene segments, no more than 11 V_H gene segments, no more than 12 V_H gene segments,

no more than 13 V_H gene segments, no more than 14 V_H gene segments, no more than 15 V_H gene segments, no more than 16, V_H gene segments, no more than 17 V_H gene segments, no more than 18 V_H gene segments, no more than 19 V_H gene segments, no more than 20 V_H gene segments, no more than 21 V_H gene segments, no more than 22 V_H gene segments or no more than 23 V_H gene segments.

[0460] In a specific embodiment, the one or more V_H gene segments comprise five V_H gene segments. In a specific embodiment, the one or more V_H gene segments comprise 10 V_H gene segments. In a specific embodiment, the one or more V_H gene segments comprise 15 V_H gene segments. In a specific embodiment, the one or more V_H gene segments comprise 20 V_H gene segments.

[0461] In various embodiments, the V_H gene segments are selected from V_H6-1, V_H1-2, V_H1-3, V_H2-5, V_H3-7, V_H1-8, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H1-18, V_H3-20, V_H3-21, V_H3-23, V_H1-24, V_H2-26, V_H4-28, V_H3-30, V_H4-31, V_H3-33, V_H4-34, V_H3-35, V_H3-38, V_H4-39, V_H3-43, V_H1-45, V_H1-46, V_H3-48, V_H3-49, V_H5-51, V_H3-53, V_H1-58, V_H4-59, V_H4-61, V_H3-64, V_H3-66, V_H1-69, V_H2-70, V_H3-72, V_H3-73 and V_H3-74. In various embodiments, the V_H gene segments are selected from V_H1-2, V_H1-8, V_H1-18, V_H1-46, V_H1-69, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-21, V_H3-23, V_H3-30, V_H3-33, V_H3-43, V_H3-48, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H5-51 and V_H6-1. In various embodiments, the V_H gene segments are selected from V_H1-18, V_H1-46, V_H1-69, V_H3-7, V_H3-11, V_H3-15, V_H3-21, V_H3-23, V_H3-30, V_H3-33, V_H3-48, V_H4-34, V_H4-39, V_H4-59 and V_H5-51. In various embodiments, the V_H gene segments are selected from V_H1-18, V_H1-69, V_H3-7, V_H3-11, V_H3-15, V_H3-21, V_H3-23, V_H3-30, V_H3-43, V_H3-48, V_H4-39, V_H4-59 and V_H5-51. In various embodiments, the V_H gene segments are selected from V_H1-18, V_H3-11, V_H3-21, V_H3-23, V_H3-30, V_H4-39 and V_H4-59. In various embodiments, the V_H gene segments are selected from V_H1-18, V_H3-21, V_H3-23, V_H3-30 and V_H4-39. In various embodiments, the V_H gene segments are selected from V_H1-18, V_H3-23 and V_H4-39. In various embodiments, the V_H gene segments are selected from V_H3-21, V_H3-23 and V_H3-30. In various embodiments, the V_H gene segments are selected from V_H3-23, V_H3-30 and V_H4-39.

[0462] In a specific embodiment, human immunoglobulin sequence comprises at least 18 V_H gene segments, 27 D_H gene segments and six J_H gene segments. In a specific embodiment, the human immunoglobulin sequence comprises at least 39 V_H gene segments, 27 D_H gene segments and six J_H gene segments. In a specific embodiment, the human immunoglobulin sequence comprises at least 80 V_H gene segments, 27 D_H gene segments and six J_H gene segments.

[0463] In one embodiment, the mouse comprises a replacement of endogenous mouse V_H

gene segments with one or more human V_H gene segments, wherein the human V_H gene segments are operably linked to a mouse C_H region gene, such that the mouse rearranges the human V_H gene segments and expresses a reverse chimeric immunoglobulin heavy chain that comprises a human V_H domain and a mouse C_H. In one embodiment, 90-100% of unrearranged mouse V_H gene segments are replaced with at least one unrearranged human V_H gene segment. In a specific embodiment, all or substantially all of the endogenous mouse V_H gene segments are replaced with at least one unrearranged human V_H gene segment. In one embodiment, the replacement is with at least 19, at least 39, or at least 80 or 81 unrearranged human V_H gene segments. In one embodiment, the replacement is with at least 12 functional unrearranged human V_H gene segments, at least 25 functional unrearranged human V_H gene segments, or at least 43 functional unrearranged human V_H gene segments. In one embodiment, the mouse comprises a replacement of all mouse D_H and J_H segments with at least one unrearranged human D_H segment and at least one unrearranged human J_H segment. In one embodiment, the at least one unrearranged human D_H segment is selected from 1-1, 1-7, 1-26, 2-8, 2-15, 3-3, 3-10, 3-16, 3-22, 5-5, 5-12, 6-6, 6-13, 7-27, and a combination thereof. In one embodiment, the at least one unrearranged human J_H segment is selected from 1, 2, 3, 4, 5, 6, and a combination thereof. In a specific embodiment, the one or more human V_H gene segment is selected from a 1-2, 1-8, 1-24, 1-69, 2-5, 3-7, 3-9, 3-11, 3-13, 3-15, 3-20, 3-23, 3-30, 3-33, 3-48, 3-53, 4-31, 4-39, 4-59, 5-51, a 6-1 human V_H gene segment, and a combination thereof.

[0464] In various embodiments, the human immunoglobulin sequence is in operable linkage with a constant region in the germline of the mouse. In one embodiment, the constant region is a human, chimeric human/mouse or chimeric human/rat or chimeric human/hamster, a mouse, a rat, or a hamster constant region. In one embodiment, the constant region is a rodent (e.g., mouse or rat or hamster) constant region. In a specific embodiment, the rodent is a mouse or rat. In various embodiments, the constant region comprises at least a C_H2 domain and a C_H3 domain.

[0465] The human immunoglobulin heavy chain sequence is located at an immunoglobulin heavy chain locus in the germline of the mouse.

[0466] The mouse further comprises a human immunoglobulin light chain sequence which comprises one or more unrearranged light chain V and J sequences in the germline of the mouse. The immunoglobulin light chain sequence is an immunoglobulin λ light chain sequence. The human immunoglobulin light chain sequence comprises one or more Vλ gene segments and one or more Jλ gene segments.

[0467] In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 12 Vλ gene segments and one Jλ gene segments. In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 12 Vλ gene segments and four Jλ gene segments.

[0468] In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 28 Vλ gene segments and one Jλ gene segments. In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 28 Vλ gene segments and four Jλ gene segments.

[0469] In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 40 Vλ gene segments and one Jλ gene segments. In a specific embodiment, the human immunoglobulin light chain sequence comprises at least 40 Vλ gene segments and four Jλ gene segments.

[0470] The human immunoglobulin light chain sequence is in operable linkage with a constant region in the germline of the non-human animal (e.g., rodent, e.g., mouse or rat or hamster). The constant region is a mouse κ constant (mCκ) region.

[0471] The human immunoglobulin light chain sequence is located at an immunoglobulin light chain locus in the germline of the mouse. The immunoglobulin light chain locus in the germline of the mouse is an immunoglobulin κ light chain locus.

[0472] Described is a method of making a human antibody, wherein the human antibody comprises variable domains derived from one or more variable region nucleic acid sequences encoded in a cell of a non-human animal as described herein.

[0473] Described is a pharmaceutical composition, comprising a polypeptide that comprises antibody or antibody fragment that is derived from one or more variable region nucleic acid sequences isolated from a mouse as described herein. The polypeptide may be an antibody. The polypeptide may be a heavy chain only antibody. The polypeptide may be a single chain variable fragment (e.g., an scFv).

[0474] In one aspect, use of a mouse as described herein to make an antibody is provided. In various embodiments, the antibody comprises one or more variable domains that are derived from one or more variable region nucleic acid sequences isolated from the mouse. In a specific embodiment, the variable region nucleic acid sequences comprise immunoglobulin heavy chain gene segments. In a specific embodiment, the variable region nucleic acid sequences comprise immunoglobulin light chain gene segments.

Mice Expressing Human λ Variable Domains

[0475] Genetically modified mice comprising a modification that reduces fertility due to loss of an ADAM protein activity (e.g., ADAM6-dependent) can be bred with mice as described herein that comprise human λ variable sequences at endogenous mouse constant light genes. For example, mice that comprise a damaged ADAM6 gene (or a deleted ADAM6 gene), e.g., animals with humanized immunoglobulin heavy chain loci, are combined with mice that

comprise a light chain locus (endogenous or transgenic) that comprises human λ segments and Jλ segments linked to mouse immunoglobulin κ light chain constant region genes, wherein the non-human animals comprise an activity that restores the ADAM-dependent fertility. The genetic modification that restores the ADAM-dependent fertility can be in either mouse with a humanized heavy chain, or in a mouse with humanized λ variable segments. Progeny comprise genes that form a humanized heavy chain (*i.e.,* result in expressing a human heavy chain variable domain) and a humanized light chain locus (*i.e.,* result in expressing a human λ light chain variable domain, fused to a mouse κ region), wherein animals exhibit a fertility that is increased as compared with a mouse that lacks the ADAM6 activity or activity of an ortholog or homolog of ADAM6.

[0476] VELOCIMMUNE® genetically engineered mice comprise a replacement of unrearranged V(D)J gene segments at endogenous mouse loci with human V(D)J gene segments. VELOCIMMUNE® mice express chimeric antibodies having human variable domains and mouse constant domains (see, *e.g.,* US Pat. No. 7,605,237). Most other reports concern mice that express fully human antibodies from fully human transgenes in mice that have disabled endogenous immunoglobulin loci.

[0477] Antibody light chains are encoded by one of two separate loci: kappa (κ) and lambda (λ). Mouse antibody light chains are primarily of the κ type. Mice that make mouse antibodies, and modified mice that make fully human or chimeric human-mouse antibodies, display a bias in light chain usage. Humans also exhibit light chain bias, but not so pronounced as in mice; the ratio of κ light chains to λ light chains in mice is about 95:5, whereas in humans the ratio is about 60:40. The more pronounced bias in mice is not thought to severely affect antibody diversity, because in mice the λ variable locus is not so diverse in the first instance. This is not so in humans. The human λ light chain locus is richly diverse.

[0478] The human λ light chain locus extends over 1,000 kb and contains over 80 genes that encode variable (V) or joining (J) segments (FIG. 19). Within the human λ light chain locus, over half of all observed Vλ domains are encoded by the gene segments 1-40, 1-44, 2-8, 2-14, and 3-21. Overall, about 30 or so of the human Vλ gene segments are believed to be functional. There are seven Jλ gene segments, only four of which are regarded as generally functional Jλ gene segments Jλ1, Jλ2, Jλ3, and Jλ7.

[0479] The λ light chain locus in humans is similar in structure to the λ locus of both mice and humans in that the human λ light chain locus has several variable region gene segments that are capable of recombining to form a functional light chain protein. The human λ light chain locus contains approximately 70 V gene segments and 7 Jλ-Cλ gene segment pairs. Only four of these Jλ-Cλ gene segment pairs appear to be functional. In some alleles, a fifth Jλ-Cλ gene segment pair is reportedly a pseudo gene (Cλ6). The 70 Vλ gene segments appear to contain 38 functional gene segments. The 70 Vλ sequences are arranged in three clusters, all of which contain different members of distinct V gene family groups (clusters A, B and C; FIG. 19). This is a potentially rich source of relatively untapped diversity for generating antibodies with human V regions in non-human animals.

[0480] In stark contrast, the mouse λ light chain locus contains only two or three (depending on the strain) mouse Vλ region gene segments (FIG. 20). At least for this reason, the severe κ bias in mice is not thought to be particularly detrimental to total antibody diversity.

[0481] According published maps of the mouse λ light chain locus, the locus consists essentially of two clusters of gene segments within a span of approximately 200 kb (FIG. 20). The two clusters contain two sets of V, J, and C genes that are capable of independent rearrangement: Vλ2-Jλ2-Cλ2-Jλ4-Cλ4 and Vλ1-Jλ3-Cλ3-Jλ1-Cλ1. Although Vλ2 has been found to recombine with all Jλ gene segments, Vλ1 appears to exclusively recombine with Cλ1. Cλ4 is believed to be a pseudo gene with defective splice sites.

[0482] The mouse κ light chain locus is strikingly different. The structure and number of gene segments that participate in the recombination events leading to a functional light chain protein from the mouse κ locus is much more complex (FIG. 21). Thus, mouse λ light chains do not greatly contribute to the diversity of an antibody population in a typical mouse.

[0483] Exploiting the rich diversity of the human λ light chain locus in mice would likely result in, among other things, a source for a more complete human repertoire of light chain V domains. Previous attempts to tap this diversity used human transgenes containing chunks of the human λ light chain locus randomly incorporated into the mouse genome (see, *e.g.,* US 6,998,514 and US 7,435,871). Mice containing these randomly integrated transgenes reportedly express fully human λ light chains, however, in some cases, one or both endogenous light chain loci remain intact. This situation is not desirable as the human λ light chain sequences contend with the mouse light chain (κ or λ) in the expressed antibody repertoire of the mouse.

[0484] In contrast, the inventors describe genetically modified mice that are capable of expressing one or more λ light chain nucleic acid sequences directly from a mouse κ light chain locus, including by replacement at an endogenous mouse κ light chain locus. Genetically modified mice capable of expressing human λ light chain sequences from an endogenous locus may be further bred to mice that comprise a human heavy chain locus and thus be used to express antibodies comprising V regions (heavy and light) that are fully human. In various embodiments. The V regions express with mouse constant regions. In various embodiments, no endogenous mouse immunoglobulin gene segments are present and the V regions express with human constant regions. These antibodies would prove useful in numerous applications, both diagnostic as well as therapeutic.

[0485] Many advantages can be realized for various embodiments of expressing binding proteins derived from human Vλ and Jλ gene segments in mice. Advantages can be realized by placing human λ sequences at an endogenous light chain locus, for example, the mouse κ or λ locus. Antibodies made from such mice can have light chains that comprise human Vλ domains fused to a mouse C_L region, specifically a mouse Cκ or Cλ region. The mice will also express human Vλ domains that are suitable for identification and cloning for use with human C_L regions, specifically Cκ and/or Cλ regions. Because B cell development in such mice is

otherwise normal, it is possible to generate compatible Vλ domains (including somatically mutated Vλ domains) in the context of either Cλ or Cκ regions.

[0486] Genetically modified mice are described that comprise an unrearranged Vλ gene segment at an immunoglobulin κ light chain locus. Mice that express antibodies that comprise a light chain having a human Vλ domain fused to a mouse Cκ region are described.

[0487] A genetically modified mouse is described that comprises (1) one or more unrearranged human Vλ gene segments and one or more unrearranged human Jλ gene segments at an endogenous immunoglobulin light chain locus of the non-human animal, (2) one or more human V_H gene segments, one more human D_H gene segments, and one or more human J_H gene segments at an endogenous immunoglobulin heavy chain locus of the non-human animal, wherein the mouse is capable of expressing an ADAM6 protein or functional fragment thereof, wherein the ADAM6 protein is functional in a male of the non-human animal. A genetically modified mouse is described that express antibodies containing heavy chains that comprise human V_H domains and non-human heavy chain constant regions and light chains that comprise human Vλ domains and mouse κ light chain constant regions, wherein the mice are capable of expressing an ADAM6 protein or functional fragment thereof.

[0488] In one embodiment, the ADAM6 protein or functional fragment thereof is encoded by an ectopic sequence in the germline of the mouse. In one embodiment, the ADAM6 protein or functional fragment thereof is encoded by an endogenous sequence of the mouse.

[0489] The endogenous light chain locus of the mouse is an immunoglobulin κ light chain locus.

[0490] In one embodiment, the mouse lacks an endogenous V_L and/or J_L gene segment at the endogenous light chain locus. In a specific embodiment, the V_L and/or J_L gene segment are a Vκ and/or Jκ gene segment. In a specific embodiment, the VL and/or JL gene segment are a Vλ and/or Jλ gene segment.

[0491] In one embodiment, the V_L and J_L gene segments of the mouse are replaced by one or more human Vλ and one or more human Jλ gene segments. In a specific embodiment, the V_L and J_L gene segments of the mouse are κ gene segments. In a specific embodiment, the V_L and J_L gene segments of the mouse are λ gene segments.

[0492] In one embodiment, the one or more human Vλ gene segments are from a fragment of cluster A of the human immunoglobulin λ light chain locus. In a specific embodiment, the fragmen of cluster A extends from human Vλ3-27 through human Vλ3-1. In a specific embodiment, the fragment of cluster A extends from human Vλ3-12 through human Jλ1. In one embodiment, the one or more human Vλ gene segments are from a fragment of cluster B of the human immunoglobulin λ light chain locus. In a specific embodiment, the fragment of cluster B extends from human Vλ5-52 through human Vλ1-40. In a specific embodiment, the

one or more human Vλ gene segments are from a fragment of cluster A and from a fragment of cluster B of the human immunoglobulin λ light chain locus as described herein.

[0493] In one embodiment, the mouse comprises at least 12 human Vλ gene segments. In one embodiment, the mouse comprises at least 28 human Vλ gene segments. In one embodiment, the mouse comprises at least 40 human Vλ gene segments.

[0494] In one embodiment, the at least one human Jλ gene segment is selected from the group consisting of Jλ1, Jλ2, Jλ3, Jλ7, and a combination thereof.

[0495] Described is a fertile mouse, wherein the fertile non-human animal expresses (1) an immunoglobulin light chain comprising a human Vλ domain or a human Vκ domain, and (2) an immunoglobulin heavy chain comprising a human V_H domain, wherein the male mouse comprises a modified heavy chain variable region locus and an ectopic ADAM6 gene that is functional in the male mouse.

[0496] In one aspect, use of a mouse as described herein to make an antigen-binding protein is provided. In one embodiment, the antigen-binding protein is human. In one embodiment, the antigen-binding protein is an antibody. In one embodiment, the antigen-binding protein comprises a human V_H domain and/or a human Vλ domain derived from a mouse as described herein.

[0497] In one aspect, a cell or tissue derived from a mouse as described herein is provided. In one embodiment, the tissue is derived from a spleen, bone marrow or a lymph node. In one embodiment, the cell is a B cell. In one embodiment, the cell is an embryonic stem (ES) cell. In one embodiment, the cell is a germ cell.

[0498] Also described is an oocyte comprising a diploid genome of a genetically modified mouse as described herein.

Sterile Transcripts of the Immunoglobulin κ Light Chain Locus

[0499] Variations on the theme of expressing human immunoglobulin λ sequences in mice are reflected in various embodiments of genetically modified mice capable of such expression. Thus, in some embodiments, the genetically modified mice comprise certain non-coding sequence(s) from a human locus. In one embodiment, the genetically modified mouse comprises human Vλ and Jλ gene segments at an endogenous κ light chain locus, and further comprises a human κ light chain genomic fragment. In a specific embodiment, the human κ light chain genomic fragment is a non-coding sequence naturally found between a human Vκ gene segment and a human Jκ gene segment.

[0500] The human and mouse κ light chain loci contain sequences that encode sterile

transcripts that lack either a start codon or an open reading frame, and that are regarded as elements that regulate transcription of the κ light chain loci. These sterile transcripts arise from an intergenic sequence located downstream or 3' of the most proximal Vκ gene segment and upstream or 5' of the κ light chain intronic enhancer (Eκi) that is upstream of the κ light chain constant region gene (Cκ). The sterile transcripts arise from rearrangement of the intergenic sequence to form a VκJκ1 segment fused to a Cκ.

[0501] A replacement of the κ light chain locus upstream of the Cκ gene would remove the intergenic region encoding the sterile transcripts. Therefore, in various embodiments, a replacement of mouse κ light chain sequence upstream of the mouse Cκ gene with human λ light chain gene segments would result in a humanized mouse κ light chain locus that contains human Vλ and Jλ gene segments but not the κ light chain intergenic region that encodes the sterile transcripts.

[0502] As described herein, humanization of the endogenous mouse κ light chain locus with human λ light chain gene segments, wherein the humanization removes the intergenic region, results in a striking drop in usage of the κ light chain locus, coupled with a marked increase in λ light chain usage. Therefore, although a humanized mouse that lacks the intergenic region is useful in that it can make antibodies with human light chain variable domains (e.g., human λ or κ domains), usage from the locus decreases.

[0503] Also described is humanization of the endogenous mouse κ light chain locus with human Vλ and Jλ gene segments coupled with an insertion of a human κ intergenic region to create a Vλ locus that contains, with respect to transcription, between the final human Vλ gene segment and the first human Jλ gene segment, a κ intergenic region; which exhibits a B cell population with a higher expression than a locus that lacks the κ intergenic region. This observation is consistent with a hypothesis that the intergenic region directly through a sterile transcript, or indirectly suppresses usage from the endogenous λ light chain locus. Under such a hypothesis, including the intergenic region would result in a decrease in usage of the endogenous λ light chain locus, leaving the mouse a restricted choice but to employ the modified (λ into κ) locus to generate antibodies.

[0504] In various embodiments, a replacement of mouse κ light chain sequence upstream of the mouse Cκ gene with human λ light chain sequence further comprises a human κ light chain intergenic region disposed, with respect to transcription, between the 3' untranslated region of the 3' most Vλ gene segment and 5' to the first human Jλ gene segment. Alternatively, such an intergenic region may be omitted from a replaced endogenous κ light chain locus (upstream of the mouse Cκ gene) by making a deletion in the endogenous λ light chain locus. Likewise, under this embodiment, the mouse generates antibodies from an endogenous κ light chain locus containing human λ light chain sequences.

Approaches to Engineering Mice to Express Human Vλ Domains

[0505] Various approaches to making genetically modified mice that make antibodies that contain a light chain that has a human Vλ domain fused to an endogenous Cκ region are described. Genetic modifications are described that, in various embodiments, comprise a deletion of one or both endogenous light chain loci. For example, to eliminate mouse λ light chains from the endogenous antibody repertoire a deletion of a first Vλ-Jλ-Cλ gene cluster and replacement, in whole or in part, of the Vλ-Jλ gene segments of a second gene cluster with human Vλ-Jλ gene segments can be made. Genetically modified mouse embryos, cells, and targeting constructs for making the mice, mouse embryos, and cells are also provided.

[0506] The deletion of one endogenous Vλ-Jλ-Cλ gene cluster and replacement of the Vλ-Jλ gene segments of another endogenous Vλ-Jλ-Cλ gene cluster employs a relatively minimal disruption in natural antibody constant region association and function in the animal, in various embodiments, because endogenous Cλ genes are left intact and therefore retain normal functionality and capability to associate with the constant region of an endogenous heavy chain. Thus, in such embodiments the modification does not affect other endogenous heavy chain constant regions dependent upon functional light chain constant regions for assembly of a functional antibody molecule containing two heavy chains and two light chains. Further, in various embodiments the modification does not affect the assembly of a functional membrane-bound antibody molecule involving an endogenous heavy chain and a light chain, e.g., a hVλ domain linked to a mouse Cλ region. Because at least one functional Cλ gene is retained at the endogenous locus, animals containing a replacement of the Vλ-Jλ gene segments of an endogenous Vλ-Jλ-Cλ gene cluster with human Vλ-Jλ gene segments should be able to make normal λ light chains that are capable of binding antigen during an immune response through the human Vλ-Jλ gene segments present in the expressed antibody repertoire of the animal.

[0507] A schematic illustration (not to scale) of a deleted endogenous mouse Vλ-Jλ-Cλ gene cluster is provided in FIG. 20. As illustrated, the mouse λ light chain locus is organized into two gene clusters, both of which contain function gene segments capable of recombining to form a function mouse λ light chain. The endogenous mouse Vλ1-Jλ3-Cλ3-Jλ1-Cλ1 gene cluster is deleted by a targeting construct (Targeting Vector 1) with a neomycin cassette flanked by recombination sites. The other endogenous gene cluster (Vλ2-Vλ3-Jλ2-Cλ2-Jλ4-Cλ4) is deleted in part by a targeting construct (Targeting Vector 2) with a hygromycin-thymidine kinase cassette flanked by recombination sites. In this second targeting event, the Cλ2-Jλ4-Cλ4 endogenous gene segments are retained. The second targeting construct (Targeting Vector 2) is constructed using recombination sites that are different than those in the first targeting construct (Targeting Vector 1) thereby allowing for the selective deletion of the selection cassette after a successful targeting has been achieved. The resulting double-targeted locus is functionally silenced in that no endogenous λ light chain can be produced. This modified locus can be used for the insertion of human Vλ and Jλ gene segments to create an endogenous mouse λ locus comprising human Vλ and Jλ gene segments, whereby, upon recombination at the modified locus, the animal produces λ light chains comprising rearranged human Vλ and Jλ gene segments linked to an endogenous mouse Cλ gene segment.

[0508] Genetically modifying a mouse to render endogenous λ gene segments nonfunctional,

in various embodiments, results in a mouse that exhibits exclusively κ light chains in its antibody repertoire, making the mouse useful for evaluating the role of λ light chains in the immune response, and useful for making an antibody repertoire comprising Vκ domains but not Vλ domains.

[0509] A genetically modified mouse that expresses a hVλ linked to a mouse Cλ gene having been recombined at the endogenous mouse λ light chain locus can be made by any method known in the art. A schematic illustration (not to scale) of the replacement of the endogenous mouse Vλ2-Vλ3-Jλ2 gene segments with human Vλ and Jλ gene segments is provided in FIG. 22A. As illustrated, an endogenous mouse λ light chain locus that had been rendered nonfunctional is replaced by a targeting construct (12/1-λ Targeting Vector) that includes a neomycin cassette flanked by recombination sites. The Vλ2-Vλ3-Jλ2 gene segments are replaced with a genomic fragment containing human λ sequence that includes 12 hVλ gene segments and a single hJλ gene segment.

[0510] Thus, this first approach positions one or more hVλ gene segments at the endogenous λ light chain locus contiguous with a single hJλ gene segment (FIG. 22A).

[0511] Further modifications to the modified endogenous λ light chain locus can be achieved with using similar techniques to insert more hVλ gene segments. For example, schematic illustrations of two additional targeting constructs (+16-λ and +12-λ Targeting Vectors) used for progressive insertion of addition human hVλ gene segments are provided in FIG. 23A. As illustrated, additional genomic fragments containing specific human hVλ gene segments are inserted into the modified endogenous λ light chain locus in successive steps using homology provided by the previous insertion of human λ light chain sequences. Upon recombination with each targeting construct illustrated, in sequential fashion, 28 additional hVλ gene segments are inserted into the modified endogenous λ light chain locus. This creates a chimeric locus that produces a λ light chain protein that comprises human Vλ □ Jλ gene segments linked to a mouse Cλ gene.

[0512] The above approaches to insert human λ light chain gene segments at the mouse λ locus, maintains the enhancers positioned downstream of the Cλ2-Jλ4-Cλ4 gene segments (designated Enh 2.4, Enh and Enh 3.1 FIG. 22A and FIG. 23A). This approach results in a single modified allele at the endogenous mouse λ light chain locus (FIG. 25A).

[0513] Compositions and methods for making a mouse that expresses a light chain comprising hVλ and Jλ gene segments operably linked to a mouse Cλ gene segment, are provided, including compositions and method for making a mouse that expresses such genes from an endogenous mouse λ light chain locus. The methods include selectively rendering one endogenous mouse Vλ-Jλ-Cλ gene cluster nonfunctional (e.g., by a targeted deletion), and employing a hVλ and Jλ gene segments at the endogenous mouse λ light chain locus to express a hVλ domain in a mouse.

[0514] Alternatively, in a second approach, human λ light chain gene segments may be

positioned at the endogenous κ light chain locus. The genetic modification, in various embodiments, comprises a deletion of the endogenous κ light chain locus. For example, to eliminate mouse κ light chains from the endogenous antibody repertoire a deletion of the mouse Vκ and Jκ gene segments can be made. Genetically modified mouse embryos, cells, and targeting constructs for making the mice, mouse embryos, and cells are also provided.

[0515] For the reasons stated above, the deletion of the mouse Vκ and Jκ gene segments employs a relatively minimal disruption. A schematic illustration (not to scale) of deleted mouse Vκ and Jκ gene segments is provided in FIG. 21. The endogenous mouse Vκ and Jκ gene segments are deleted via recombinase-mediated deletion of mouse sequences position between two precisely positioned targeting vectors each employing site-specific recombination sites. A first targeting vector (Jκ Targeting Vector) is employed in a first targeting event to delete the mouse Jκ gene segments. A second targeting vector (Vκ Targeting Vector) is employed in a second, sequential targeting event to delete a sequence located 5' of the most distal mouse Vκ gene segment. Both targeting vectors contain site-specific recombination sites thereby allowing for the selective deletion of both selection cassettes and all intervening mouse κ light chain sequences after a successful targeting has been achieved. The resulting deleted locus is functionally silenced in that no endogenous κ light chain can be produced. This modified locus can be used for the insertion of hVλ and Jλ gene segments to create an endogenous mouse κ locus comprising hVλ and Jλ gene segments, whereby, upon recombination at the modified locus, the animal produces λ light chains comprising rearranged hVλ and Jλ gene segments operably linked to an endogenous mouse Cκ gene segment. Various targeting vectors comprising human λ light chain sequences can be used in conjunction with this deleted mouse κ locus to create a hybrid light chain locus containing human λ gene segments operably linked with a mouse Cκ region.

[0516] Thus, a second approach positions one or more human Vλ gene segments are positioned at the mouse κ light chain locus contiguous with a single human Jλ gene segment (12/1-κ Targeting Vector, FIG. 22B).

[0517] In various embodiments, modifications to this approach can be made to add gene segments and/or regulatory sequences to optimize the usage of the human λ light chain sequences from the mouse κ locus within the mouse antibody repertoire.

[0518] In a third approach, one or more hVλ gene segments are positioned at the mouse κ light chain locus contiguous with four hJλ gene sequences (12/4-κ Targeting Vector FIG. 22B).

[0519] In a third approach, one or more hVλ gene segments are positioned at the mouse κ light chain locus contiguous with a human κ intergenic sequence and a single hJλ gene sequence (12(κ)1-κ Targeting Vector, FIG. 22B).

[0520] In a fourth approach, one or more hVλ gene segments are positioned at the mouse κ light chain locus contiguous with a human κ intergenic sequence four hJλ gene sequences (12(κ)4-κ Targeting Vector FIG. 22B).

[0521] All of the above approaches to insert human λ light chain gene segments at the mouse κ locus, maintain the κ intronic enhancer element upstream of the Cκ gene (designated Eκi, FIG. 22B and FIG. 23B) and the 3' κ enhancer downstream of the Cκ gene (designated Eκ3', FIG. 22B and FIG. 23B). The approaches result in four separate modified alleles at the endogenous mouse κ light chain locus (FIG. 25B).

[0522] In various embodiments, genetically modified mouse comprise a knockout of the endogenous mouse λ light chain locus. In one embodiment, the λ light chain locus is knocked out by a strategy that deletes the region spanning Vλ2 to Jλ2, and the region spanning Vλ1 to Cλ1 (FIG. 20). Any strategy that reduces or eliminates the ability of the endogenous λ light chain locus to express endogenous λ domains is suitable for use with embodiments in this disclosure.

Lambda Domain Antibodies from Genetically Modified Mice

[0523] Mice comprising human λ sequences at either the mouse κ or λ light chain locus will express a light chain that comprises a hVλ region fused to a mouse C_L (Cκ or Cλ) region. These are advantageously bred to mice that (a) comprise a functionally silenced light chain locus (e.g., a knockout of the endogenous mouse κ or λ light chain locus); (b) comprise an endogenous mouse λ light chain locus that comprises hV and hJ gene segments operably linked to an endogenous mouse Cλ gene; (c) comprise an endogenous mouse κ light chain locus that comprises hVκ and hJκ gene segments operably linked to an endogenous mouse Cκ gene; and, (d) a mouse in which one κ allele comprises hVκs and hJκs; the other κ allele comprising hVλs and hJλs; one λ allele comprising hVλs and hJλs and one λ allele silenced or knocked out, or both λ alleles comprising hVλs and hJλs; and, two heavy chain alleles that each comprise hV_Hs, hD_Hs, and hJ_Hs.

[0524] The antibodies that comprise the hVλ domains expressed in the context of either Cκ or Cλ are used to make fully human antibodies by cloning the nucleic acids encoding the hVλ domains into expression constructs that bear genes encoding human Cλ. Resulting expression constructs are transfected into suitable host cells for expressing antibodies that display a fully hVλ domain fused to hCλ.

EXAMPLES

[0525] The following examples are provided so as to describe how to make and use methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Unless indicated otherwise, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1. Humanization of Mouse Immunoglobulin Genes

[0526] Human and mouse bacterial artificial chromsomes (BACs) were used to engineer 13 different BAC targeting vectors (BACvecs) for humanization of the mouse immunoglobulin heavy chain and κ light chain loci. Tables 1 and 2 set forth detailed descriptions of the steps performed for the construction of all BACvecs employed for the humanization of the mouse immunoglobulin heavy chain and κ light chain loci, respectively.

[0527] Identification of human and mouse BACs. Mouse BACs that span the 5' and 3' ends of the immunoglobulin heavy chain and κ light chain loci were identified by hybridization of filters spotted with BAC library or by PCR screening mouse BAC library DNA pools. Filters were hybridized under standard conditions using probes that corresponded to the regions of interest. Library pools were screened by PCR using unique primer pairs that flank the targeted region of interest. Additional PCR using the same primers was performed to deconvolute a given well and isolate the corresponding BAC of interest. Both BAC filters and library pools were generated from 129 SvJ mouse ES cells (Incyte Genomics/Invitrogen). Human BACs that cover the entire immunoglobulin heavy chain and κ light chain loci were identified either by hybridization of filters spotted with BAC library (Caltech B, C, or D libraries & RPCI-11 library, Research Genetics/Invitrogen) through screening human BAC library pools (Caltech library, Invitrogen) by a PCR-based method or by using a BAC end sequence database (Caltech D library, TIGR).

[0528] Construction of BACvecs by bacterial homologous recombination and ligation. Bacterial homologous recombination (BHR) was performed as described (Valenzuela *et al.,* 2003; Zhang, Y., Buchholz, F., Muyrers, J.P., and Stewart, A.F. (1998). A new logic for DNA engineering using recombination in Escherichia coli. Nat Genet 20, 123-128). In most cases, linear fragments were generated by ligating PCR-derived homology boxes to cloned cassettes followed by gel isolation of ligation products and electroporation into BHR-competent bacteria harboring the target BAC. After selection on appropriate antibiotic petri dishes, correctly recombined BACs were identified by PCR across both novel junctions followed by restriction analysis on pulsed-field gels (Schwartz, D.C., and Cantor, C.R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37, 67-75) and spot-checking by PCR using primers distributed across the human sequences.

[0529] A 3hV_H BACvec was constructed using three sequential BHR steps for the initial step of humanization of the immunoglobulin heavy chain locus (FIG. 4A and Table 1). In the first step (Step 1), a cassette was introduced into a human parental BAC upstream from the human V_H1-3 gene segment that contains a region of homology to the mouse immunoglobulin heavy chain locus (HB1), a gene that confers kanamycin resistance in bacteria and G418 resistance in animals cells (kanR) and a site-specific recombination site (*e.g.*, loxP). In the second step (Step 2), a second cassette was introduced just downstream from the last J_H segment that contains a second region of homology to the mouse immunoglobulin heavy chain locus (HB2) and a gene that confers resistance in bacteria to spectinomycin (specR). This second step

included deleting human immunoglobulin heavy chain locus sequences downstream from J_H6 and the BAC vector chloramphenicol resistance gene (cmR). In the third step (Step 3), the doubly modified human BAC (B1) was then linearized using I-CeuI sites that had been added during the first two steps and integrated into a mouse BAC (B2) by BHR through the two regions of homology (HB1 and HB2). The drug selections for first (cm/kan), second (spec/kan) and third (cm/kan) steps were designed to be specific for the desired products. Modified BAC clones were analyzed by pulse-filed gel electrophoresis (PFGE) after digestion with restriction enzymes to determine appropriate construction (FIG. 4B).

[0530] In a similar fashion, 12 additional BACvecs were engineered for humanization of the heavy chain and κ light chain loci. In some instances, BAC ligation was performed in lieu of BHR to conjoin two large BACs through introduction of rare restriction sites into both parental BACvecs by BHR along with careful placement of selectable markers. This allowed for the survival of the desired ligation product upon selection with specific drug marker combinations. Recombinant BACs obtained by ligation after digestion with rare restriction enzymes were identified and screened in a similar fashion to those obtained by BHR (as described above).

TABLE 1

BACvec	Step	Description	Process
$3hV_H$	1	Insert upstream mouse homology box into human proximal BAC CTD-2572o2	BHR
	2	Insert downstream mouse homology box into human proximal BAC CTD-2572o2	BHR
	3	Insert 3hVH/27hDH/9hJH into mouse proximal BAC CT7-302a07 to create 3hVH BACvec	BHR
DC	1	Insert cassette at distal end of mouse IgH locus using mouse BAC CT7-253i20	BHR
$18hV_H$	1	Insert specR marker at downstream end of 3hVH insertion using human BAC CTD-2572o2	BHR
	2	Insert I-CeuI and Not sites flanking puroR at upstream end of 3hVH insertion	BHR
	3	Insert Not site at downstream end of Rel2-408p02 BAC (≈10 kb downstream of VH2-5)	BHR
	4	Insert I-Ceu1 site at upstream end of Rel2-408p02 BAC (≈23 kb upstream of VH1-18)	BHR
	5	Ligate 184kb fragment from step 4 into 153kb vector from step 2	Ligation
	6	Trim human homology from CTD-2572o2 BAC deleting ≈85kb and leaving 65kb homology to 3hVH	BHR
	7	Insert cassette and Not site at distal end of mouse IgH locus in CT7-253i20 BAC	BHR
	8	Subclone mouse distal homology arm for insertion upstream from human BACs	Ligation

BACvec	Step	Description	Process
	9	Insert 20 kb mouse arm upstream of Rel2-408p02	BHR
	10	Swap selection cassette from hygR to neoR to create 18hVH BACvec	BHR
$39hV_H$	1	Insert ICeuI and PIScel sites flanking hygR into distal end of human BAC CTD-2534n10	BHR
	2	Insert CmR at proximal end of CTD-2534n10 BAC to allow for selection for ligation to RP11-72n10 BAC	BHR
	3	Insert PIScel site into RP11-72n10 BAC for ligation to CTD-2534n10 BAC	BHR
	4	Insert ICeuI and AscI sites flanking puroR at distal end of RP11-72n10 BAC	BHR
	5	Ligate 161 kb fragment from construct of step 4 into construct of step 2 replacing hygR	Ligation
	6	Insert neoR and AscI site at proximal end of mouse distal homology arm using CT7-253i20 BAC	BHR
	7	Insert specR and ICeuI site at distal end of mouse distal homology arm	BHR
	8	Ligate mouse distal homology arm onto human insert from step	Ligation
	9	Swap selection cassette from neo to hyg using UbCp and pA as homolgy boxes to create 39hVH BACvec	BHR
$53hV_H$	1	Insert specR at proximal end of human CTD-3074b5 BAC	BHR
	2	Insert AscI site at distal end of human CTD-3074b5 BAC	BHR
	3	Insert hygR and AscI site at proximal end of mouse distal homology arm using CT7-253i20 BAC	BHR
	4	Ligate mouse distal homology arm onto construct from step 2	Ligation
	5	Swap selection cassette from hyg to neo using UbCp and pA as homolgy boxes to create 53hVH BACvec	BHR
$70hV_H$	1	Insert PIScel and ICeuI sites flanking spec at distal end of human CTD-2195p5 BAC	BHR
	2	Insert ICeuI site at proximal end of RP11-926p12 BAC for ligation to CTD-2195p5 BAC	BHR
	3	Insert PIScel and AscI sites at distal end of RP11-926p12 BAC for ligation of mouse arm	BHR
	4	Ligate mouse distal homology arm onto construct from step 3	Ligation
	5	Ligate mouse distal homology arm and hlgH fragment from RP11-926p12 BAC onto CTD-2195p5 BAC to create 70 hVH BACvec	Ligation

BACvec	Step	Description	Process
80hV$_H$	1	Insert ICeuI and AscI sites flanking hygR at distal end of CTD-2313e3 BAC	BHR
	2	Ligate mouse dista homology arm onto human CTD-2313e3 BAC from step 1 to create 80hVH BACvec	Ligation

TABLE 2

BACvec	Step	Description	Process
Igκ-PC	1	Insert loxP site within mouse J-C intron using CT7-254m04 BAC	BHR
Igκ-DC	1	Insert loxP site at distal end of mouse IgK locus using CT7-302g12 BAC	BHR
6hVκ	1	Insert PISceI site ≈400 bp downstream from hJκ5 in CTD-2366j12 BAC	BHR
	2	Insert ICeuI and AscI sites flanking hygR at distal end of CTD-2366j12 BAC	BHR
	3	Insert ICeuI and PI-SceI sites flanking puroR ≈xxbp downstream from mJκx using CT7-254m04 BAC	BHR
	4	Insert hIgVκ/Jκ upstream from mouse Enhκ/Cκ using construct from step 3	Ligation
	5	Replace cmR in construct of step 4 with specR	BHR
	6	Insert Neo selection cassette at distal end of mouse Igκ locus using CT7-302g12 BAC	BHR
	7	Ligate mouse distal homology arm upstream of human insert in construct of step 6 to create 6hVκ BACvec	Ligation
16hVκ	1	Insert NeoR at distal end of RP11-1061b13 BAC	BHR
	2	Replace cmR in construct of step 1 with specR	BHR
	3	Insert Hyg selection cassette at distal end of mouse Igκ locus using CT7-302g12 BAC	BHR
	4	Ligate mouse distal homology arm upstream of human insert from construct of step 2 to create 16hVκ BACvec	Ligation
30hVκ	1	Insert HygR at distal end of RP11-99g6 BAC	BHR
	2	Replace cmR in construct of step 1 with specR	BHR
	3	Insert Neo selection cassette at distal end of mouse Igκ locus using CT7-302g12 BAC	BHR
	4	Ligate mouse distal homology arm upstream of human insert from construct of step 2 to create 30hVκ BACvec	Ligation
40hVκ	1	Insert NeoR at distal end of hIgH locus in CTD-2559d6 BAC	BHR
	2	Replace cmR in construct of step 1 with specR	BHR
	3	Ligate mouse distal homology arm upstream of hIgH locus in construct of step 2 to create 40hVκ BACvec	Ligation

BACvec	Step	Description	Process

[0531] Modification of embryonic stem (ES) cells and generation of mice. ES cell (F1H4) targeting was performed using the VELOCIGENE® genetic engineering method as described (Valenzuela *et al.,* 2003). Derivation of mice from modified ES cells by either blastocyst (Valenzuela *et al.,* 2003) or 8-cell injection (Poueymirou *et al.,* 2007) was as described. Targeted ES cells and mice were confirmed by screening DNA from ES cells or mice with unique sets of probes and primers in a PCR based assay (*e.g.,* FIG. 3A, 3B and 3C). All mouse studies were overseen and approved by Regeneron's Institutional Animal Care and Use Committee (IACUC).

[0532] Karyotype Analysis and Fluorescent *in situ* Hybridization (FISH). Karyotype Analysis was performed by Coriell Cell Repositories (Coriell Institute for Medical Research, Camden, NJ). FISH was performed on targeted ES cells as described (Valenzuela *et al.,* 2003). Probes corresponding to either mouse BAC DNA or human BAC DNA were labeled by nick translation (Invitrogen) with the fluorescently labeled dUTP nucleotides spectrum orange or spectrum green (Vysis).

[0533] Immunoglobulin Heavy Chain Variable Gene Locus. Humanization of the variable region of the heavy chain locus was achieved in nine sequential steps by the direct replacement of about three million base pairs (Mb) of contiguous mouse genomic sequence containing all V_H, D_H and J_H gene segments with about one Mb of contiguous human genomic sequence containing the equivalent human gene segments (FIG. 1A and Table 1) using VELOCIGENE® genetic engineering technology (see, *e.g.,* US Pat. No. 6,586,251 and Valenzuela *et al.,* 2003).

[0534] The intron between J_H gene segments and constant region genes (the J-C intron) contains a transcriptional enhancer (Neuberger, M.S. (1983). Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells. Embo J 2, 1373-1378) followed by a region of simple repeats required for recombination during isotype switching (Kataoka, T., Kawakami, T., Takahashi, N., and Honjo, T. (1980). Rearrangement of immunoglobulin gamma 1-chain gene and mechanism for heavy-chain class switch. Proc Natl Acad Sci U S A 77, 919-923). The junction between human V_H-D_H-J_H region and the mouse C_H region (the proximal junction) was chosen to maintain the mouse heavy chain intronic enhancer and switch domain in order preserve both efficient expression and class switching of the humanized heavy chain locus within the mouse. The exact nucleotide position of this and subsequent junctions in all the replacements was possible by use of the VELOCIGENE® genetic engineering method (*supra*), which employed bacterial homologous recombination driven by synthesized oligonucleotides. Thus, the proximal junction was placed about 200 bp downstream from the last J_H gene segment and the distal junction was placed several hundred upstream of the most 5' V_H gene segment of the human locus and about 9 kb downstream from the mouse V_H1-86 gene segment, also known as J558.55. The mouse V_H1-86 (J558.55)

gene segment is the most distal heavy chain variable gene segment, reported to be a pseudogene in C57BL/6 mice, but potentially active, albeit with a poor RSS sequence, in the targeted 129 allele. The distal end of the mouse heavy chain locus reportedly may contain control elements that regulate locus expression and/or rearrangement (Pawlitzky *et al.,* 2006).

[0535] A first insertion of human immunoglobulin DNA sequence into the mouse was achieved using 144 kb of the proximal end of the human heavy chain locus containing 3 V_H, all 27 D_H and 9 J_H human gene segments inserted into the proximal end of the mouse IgH locus, with a concomitant 16.6 kb deletion of mouse genomic sequence, using about 75 kb of mouse homology arms (Step A, FIG. 2A; Tables 1 and 3, $3hV_H$). This large 144kb insertion and accompanying 16.6 kb deletion was performed in a single step (Step A) that occurred with a frequency of 0.2% (Table 3). Correctly targeted ES cells were scored by a loss-of-native-allele (LONA) assay (Valenzuela *et al.,* 2003) using probes within and flanking the deleted mouse sequence and within the inserted human sequence, and the integrity of the large human insert was verified using multiple probes spanning the entire insertion (FIG. 3A, 3B and 3C). Because many rounds of sequential ES cell targeting were anticipated, targeted ES cell clones at this, and all subsequent, steps were subjected to karyotypic analysis (*supra*) and only those clones showing normal karyotypes in at least 17 of 20 spreads were utilized for subsequent steps.

[0536] Targeted ES cells from Step A were re-targeted with a BACvec that produced a 19 kb deletion at the distal end of the heavy chain locus (Step B, FIG. 2A). The Step B BACvec contained a hygromycin resistance gene (hyg) in contrast to the neomycin resistance gene (neo) contained on the BACvec of Step A. The resistance genes from the two BACvecs were designed such that, upon successful targeting to the same chromosome, approximately three Mb of the mouse heavy chain variable gene locus containing all of the mouse V_H gene segments other than V_H1-86 and all of the D_H gene segments other than DQ52, as well as the two resistance genes, were flanked by loxP sites; DQ52 and all of the mouse J_H chain gene segments were deleted in Step A. ES cell clones doubly targeted on the same chromosome were identified by driving the $3hV_H$ proximal cassette to homozygosity in high G418 (Mortensen, R.M. et al. (1992) Production of homozygous mutant ES cells with a single targeting construct. Mol Cell Biol 12, 2391-2395) and following the fate of the distal hyg cassette. Mouse segments up to four Mb in size, having been modified in a manner to be flanked by loxP sites, have been successfully deleted in ES cells by transient expression of CRE recombinase with high efficiencies (up to ≈11%) even in the absence of drug selection (Zheng, B. et al. (2000) Engineering mouse chromosomes with Cre-loxP: range, efficiency, and somatic applications. Mol Cell Biol 20, 648-655). In a similar manner, the inventors achieved a three Mb deletion in 8% of ES cell clones following transient CRE expression (Step C, FIG. 2A; Table 3). The deletion was scored by the LONA assay using probes at either end of the deleted mouse sequence, as well as the loss of neo and hyg and the appearance of a PCR product across the deletion point containing the sole remaining loxP site. Further, the deletion was confirmed by fluorescence *in situ* hybridization (data not shown).

[0537] The remainder of the human heavy chain variable region was added to the $3hV_H$ allele

in a series of 5 steps using the VELOCIGENE® genetic engineering method (Steps E-H, FIG. 2B), with each step involving precise insertion of up to 210 kb of human gene sequences. For each step, the proximal end of each new BACvec was designed to overlap the most distal human sequences of the previous step and the distal end of each new BACvec contained the same distal region of mouse homology as used in Step A. The BACvecs of steps D, F and H contained neo selection cassettes, whereas those of steps E and G contained hyg selection cassettes, thus selections were alternated between G418 and hygromycin. Targeting in Step D was assayed by the loss of the unique PCR product across the distal loxP site of $3hV_H$ Hybrid Allele. Targeting for Steps E through I was assayed by loss of the previous selection cassette. In the final step (Step I, FIG. 2B), the neo selection cassette, flanked by Frt sites (McLeod, M. et al. (1986) Identification of the crossover site during FLP-mediated recombination in the Saccharomyces cerevisiae plasmid 2 microns circle. Mol Cell Biol 6, 3357-3367), was removed by transient FLPe expression (Buchholz, F. et al. (1998) Improved properties of FLP recombinase evolved by cycling mutagenesis. Nat Biotechnol 16, 657-662). The human sequences of the BACvecs for Steps D, E and G were derived from two parental human BACs each, whereas those from Steps F and H were from single BACs. Retention of human sequences was confirmed at every step using multiple probes spanning the inserted human sequences (as described above, *e.g.* FIG. 3A, 3B and 3C). Only those clones with normal karyotype and germline potential were carried forward in each step. ES cells from the final step were still able to contribute to the germline after nine sequential manipulations (Table 3). Mice homozygous for each of the heavy chain alleles were viable, appeared healthy and demonstrated an essentially wild-type humoral immune system (see Example 3).

TABLE 3

Hybrid Allele	Human sequence	Targeting construct	Targeting efficiency	% usage	Total V_H	Functional V_H
$3hV_H$	144 kb	240 kb	0.2%	5	3	3
$3hV_H$/DC	144 kb	110 kb	0.1%	5	3	3
$3hV_H$-CRE	144 kb	-	8%	5	3	3
$18hV_H$	340 kb	272 kb	0.1%	25	18	12
$39hV_H$	550 kb	282 kb	0.2%	60	39	25
$53hV_H$	655 kb	186 kb	0.4%	65	53	29
$70hV_H$	850 kb	238 kb	0.5%	90	70	39
$80hV_H$	940 kb	124 kb	0.2%	100	80	43
$80hV_H$dNeo	940 kb	-	2.6%	100	80	43

[0538] Immunoglobulin κ Light Chain Variable Gene Locus. The κ light chain variable region was humanized in eight sequential steps by the direct replacement of about three Mb of mouse sequence containing all Vκ and Jκ gene segments with about 0.5 Mb of human sequence containing the proximal human Vκ and Jκ gene segments in a manner similar to that

of the heavy chain (FIG. 1B; Tables 2 and 4).

[0539] The variable region of the human κ light chain locus contains two nearly identical 400 kb repeats separated by a 800 kb spacer (Weichhold, G.M. et al. (1993) The human immunoglobulin kappa locus consists of two copies that are organized in opposite polarity. Genomics 16, 503-511). Because the repeats are so similar, nearly all of the locus diversity can be reproduced in mice by using the proximal repeat. Further, a natural human allele of the κ light chain locus missing the distal repeat has been reported (Schaible, G. et al. (1993) The immunoglobulin kappa locus: polymorphism and haplotypes of Caucasoid and non-Caucasoid individuals. Hum Genet 91, 261-267). The inventors replaced about three Mb of mouse κ light chain variable gene sequence with about 0.5 Mb of human κ light chain variable gene sequence to effectively replace all of the mouse Vκ and Jκ gene segments with the proximal human Vκ and all of the human Jκ gene segments (FIG. 2C and 2D; Tables 2 and 4). In contrast to the method described in Example 1 for the heavy chain locus, the entire mouse Vκ gene region, containing all Vκ and Jκ gene segments, was deleted in a three-step process before any human sequence was added. First, a neo cassette was introduced at the proximal end of the variable region (Step A, FIG. 2C). Next, a hyg cassette was inserted at the distal end of the κ locus (Step B, FIG. 2C). LoxP sites were again situated within each selection cassette such that CRE treatment induced deletion of the remaining 3 Mb of the mouse Vκ region along with both resistance genes (Step C, FIG. 2C).

[0540] A human genomic fragment of about 480 kb in size containing the entire immunoglobulin κ light chain variable region was inserted in four sequential steps (FIG. 2D; Tables 2 and 4), with up to 150 kb of human immunoglobulin κ light chain sequence inserted in a single step, using methods similar to those employed for the heavy chain (see Example 1). The final hygromycin resistance gene was removed by transient FLPe expression. As with the heavy chain, targeted ES cell clones were evaluated for integrity of the entire human insert, normal karyotype and germ-line potential after every step. Mice homozygous for each of the κ light chain chain alleles were generated and found to be healthy and of normal appearance.

T**ABLE 4**

Hybrid Allele	Human sequence	Targeting construct	Targeting efficiency	% usage	Total Vκ	Functional Vκ
Igκ-PC	0	132 kb	1.1%	-	-	-
Igκ-PC/DC	0	90 kb	0.4%	-	-	-
Igκ-CRE	0	-	1%	-	-	-
6hVκ	110 kb	122 kb	0.3%	14	6	4
16hVκ	240 kb	203 kb	0.4%	47	16	11
30hVκ	390 kb	193 kb	0.1%	70	30	18
40hVκ	480 kb	185 kb	0.2%	100	40	25
40hVκdHyg	480 kb	-	0.7%	100	40	25

Example 2. Generation of Fully Humanized Mice by Combination of Multiple Humanized Immunoglobulin Alleles

[0541] At several points, ES cells bearing a portion of the human immunoglobulin heavy chain or κ light chain variable repertoires as described in Example 1 were microinjected and the resulting mice bred to create multiple versions of VELOCIMMUNE® mice with progressively larger fractions of the human germline immunoglobulin repertoires (Table 5; FIG. 5A and 5B). VELOCIMMUNE® 1 (V1) mice possess 18 human V_H gene segments and all of the human D_H and J_H gene segments combined with 16 human Vκ gene segments and all the human Jκ gene segments. VELOCIMMUNE® 2 (V2) and VELOCIMMUNE® (V3) mice have increased variable repertoires bearing a total of 39 V_H and 30 Vκ, and 80 V_H and 40 Vκ, respectively. Since the genomic regions encoding the mouse V_H, D_H and J_H gene segments, and Vκ and Jκ gene segments, have been completely replaced, antibodies produced by any version of VELOCIMMUNE® mice contain human variable regions linked to mouse constant regions. The mouse λ light chain loci remain intact in all versions of the VELOCIMMUNE® mice and serve as a comparator for efficiency of expression of the various VELOCIMMUNE® κ light chain loci.

[0542] Mice doubly homozygous for both immunoglobulin heavy chain and κ light chain humanizations were generated from a subset of the alleles described in Example 1. All genotypes observed during the course of breeding to generate the doubly homozygous mice occurred in roughly Mendelian proportions. Male progeny homozygous for each of the human heavy chain alleles showed reduced fertility. Reduced fertility resulted from loss of mouse ADAM6 activity. The mouse heavy chain variable gene locus contains two embedded functional ADAM6 genes (ADAM6a and ADAM6b). During humanization of the mouse heavy chain variable gene locus, the inserted human genomic sequence contained an ADAM6 pseudogene. Mouse ADAM6 may be required for fertility, and thus lack of mouse ADAM6 genes in humanized heavy chain variable gene loci might lead to reduced fertility in these mice notwithstanding the presence of the human pseudogene. Examples 7-9 describe the precise replacement of deleted mouse ADAM6 genes back into a humanized heavy chain variable gene locus, and restoration of a wild-type level of fertility in mice with a humanized heavy chain immunoglobulin locus.

TABLE 5

Version of VELOCIMMUNE® Mouse	Heavy Chain				κ Light Chain		
	Human V_H	Allele	5' V_H gene		Human Vκ	Allele	5' Vκ gene
V1	18	18hV_H	V_H1-18		16	16hVκ	Vκ1-16
V2	39	39hV_H	V_H4-39		30	30hVκ	Vκ2-29
V3	80	80hV_H	V_H3-74		40	40hVκ	Vκ2-40

Example 3. Lymphocyte Populations in Mice with Humanized Immunoglobulin Genes

[0543] Mature B cell populations in the three different versions of VELOCIMMUNE® mice were evaluated by flow cytometry.

[0544] Briefly, cell suspensions from bone marrow, spleen and thymus were made using standard methods. Cells were resuspended at $5x10^5$ cells/mL in BD Pharmingen FACS staining buffer, blocked with anti-mouse CD16/32 (BD Pharmingen), stained with the appropriate cocktail of antibodies and fixed with BD Cytofix™ all according to the manufacturer's instructions. Final cell pellets were resuspended in 0.5 mL staining buffer and analyzed using a BD FACSCALIBUR™ and BD CELLQUEST PRO™ software. All antibodies (BD Pharmingen) were prepared in a mass dilution/cocktail and added to a final concentration of 0.5 mg/10^5 cells. Antibody cocktails for bone marrow (A-D) staining were as follows: A: anti-mouse IgM^b-FITC, anti-mouse IgM^a-PE, anti-mouse CD45R(B220)-APC; B: anti-mouse CD43(S7)-PE, anti-mouse CD45R(B220)-APC; C: anti-mouse CD24(HSA)-PE; anti-mouse CD45R(B220)-APC; D: anti-mouse BP-1-PE, anti-mouse CD45R(B220)-APC. Antibody cocktails for spleen and inguinal lymph node (E-H) staining were as follows: E: anti-mouse IgM^b-FITC, anti-mouse IgM^a-PE, anti-mouse CD45R(B220)-APC; F: anti-mouse Ig, L_1, L_2, L_3 Light Chain-FITC, anti mouse Igκ Light Chain-PE, anti-mouse CD45R(B220)-APC; G: anti-mouse Ly6G/C-FITC, anti-mouse CD49b(DX5)-PE, anti-mouse CD11b-APC; H: anti-mouse CD4(L3T4)-FITC, anti-mouse CD45R(B220)-PE, anti-mouse CD8a-APC. Results are shown in FIG. 6.

[0545] Lymphocytes isolated from spleen or lymph node of homozygous VELOCIMMUNE® mice were stained for surface expression of the markers B220 and IgM and analyzed using flow cytometry (FIG. 6). The sizes of the $B220^+$ IgM^+ mature B cell populations in all versions of VELOCIMMUNE® mice tested were virtually identical to those of wild type mice, regardless of the number of V_H gene segments they contained. In addition, mice containing homozygous hybrid humanized immunoglobulin heavy chain loci, even those with only 3 V_H gene segments but normal mouse immunoglobulin κ light chain loci or mice containing homozygous hybrid humanized κ light chain loci with normal mouse immunoglobulin heavy chain loci, also had normal numbers of $B220^+$ IgM^+ cells in their peripheral compartments (not shown). These results indicate that chimeric loci with human variable gene segments and mouse constant regions can fully populate the mature B cell compartment. Further, the number of variable gene segments at either the heavy chain or κ light chain loci, and thus the theoretical diversity of the antibody repertoire, does not correlate with the ability to generate wild type populations of mature B cells. In contrast, mice with randomly integrated fully-human immunoglobulin transgenes and inactivated mouse immunoglobulin loci have reduced numbers of B cells in these compartments, with the severity of the deficit depending on the number of variable gene segments included in the transgene (Green, L.L., and Jakobovits, A. (1998). Regulation of B cell development by variable gene complexity in mice reconstituted with human

immunoglobulin yeast artificial chromosomes. J Exp Med 188, 483-495). This demonstrates that the *"in situ* genetic humanization" strategy results in a fundamentally different functional outcome than the randomly integrated transgenes achieved in the "knockout-plus-transgenic" approach.

[0546] Allelic Exclusion and Locus Choice. The ability to maintain allelic exlusion was examined in mice heterozygous for different versions of the humanized immunoglobulin heavy chain locus.

[0547] The humanization of the immunoglobulin loci was carried out in an F1 ES line (F1H4 (Valenzuela *et al.,* 2003)), derived from 129S6/SvEvTac and C57BL/6NTac heterozygous embryos. The human heavy chain germline variable gene sequences are targeted to the 129S6 allele, which carries the IgM^a haplotype, whereas the unmodified mouse C576BL/6N allele bears the IgM^b haplotype. These allelic forms of IgM can be distinguished by flow cytometry using antibodies specific to the polymorphisms found in the IgM^a or IgM^b alleles. As shown in FIG. 6 (bottom row), the B cells identified in mice heterozygous for each version of the humanized heavy chain locus only express a single allele, either IgM^a (the humanized allele) or IgM^b (the wild type allele). This demonstrates that the mechanisms involved in allelic exclusion are intact in VELOCIMMUNE® mice. In addition, the relative number of B cells positive for the humanized allele (IgM^a) is roughly proportional to the number of V_H gene segments present. The humanized immunoglobulin locus is expressed in approximately 30% of the B cells in VELOCIMMUNE® 1 heterozygote mice, which have 18 human V_H gene segments, and in 50% of the B cells in VELOCIMMUNE® 2 and 3 (not shown) heterozygote mice, with 39 and 80 human V_H gene segments, respectively. Notably, the ratio of cells expressing the humanized versus wild type mouse allele (0.5 for VELOCIMMUNE® 1 mice and 0.9 for VELOCIMMUNE® 2 mice) is greater than the ratio of the number of variable gene segments contained in the humanized versus wild type loci (0.2 for VELOCIMMUNE® 1 mice and 0.4 for VELOCIMMUNE® 2 mice). This may indicate that the probability of allele choice is intermediate between a random choice of one or the other chromosome and a random choice of any particular V segment RSS. Further, there may be a fraction of B-cells, but not all, in which one allele becomes accessible for recombination, completes the process and shuts down recombination before the other allele becomes accessible. In addition, the even distribution of cells that have surface IgM (sIgM) derived from either the hybrid humanized heavy chain locus or the wild type mouse heavy chain locus is evidence that the hybrid locus is operating at a normal level. In contrast, randomly integrated human immunoglobulin transgenes compete poorly with wild type mouse immunoglobulin loci (Bruggemann, M. et al. (1989) A repertoire of monoclonal antibodies with human heavy chains from transgenic mice. PNAS 86, 6709-6713; Green *et al.,* 1994; Tuaillon, N. et al. (1993) Human immunoglobulin heavy-chain minilocus recombination in transgenic mice: gene-segment use in mu and gamma transcripts. Proc Natl Acad Sci U S A 90, 3720-3724). This further demonstrates the immunoglobulins produced by VELOCIMMUNE® mice are functionally different than those produced by randomly integrated transgenes in mice made by "knockout-plus-transgenic"

approaches.

[0548] Polymorphisms of the Cκ regions are not available in 129S6 or C57BL/6N to examine allelic exclusion of humanized versus non-humanized κ light chain loci. However, VELOCIMMUNE® mice all possess wild type mouse λ light chain loci, therefore, it is possible to observe whether rearrangement and expression of humanized κ light chain loci can prevent mouse λ light chain expression. The ratio of the number of cells expressing the humanized κ light chain relative to the number of cells expressing mouse λ light chain was relatively unchanged in VELOCIMMUNE® mice compared with wild type mice, regardless of the number of human Vκ gene segments inserted at the κ light chain locus (FIG. 6, third row from top). In addition there was no increase in the number of double positive (κ plus λ) cells, indicating that productive recombination at the hybrid κ light chain loci results in appropriate suppression of recombination of the mouse λ light chain loci. In contrast, mice containing randomly integrated κ light chain transgenes with inactivated mouse κ light chain loci but wild type mouse λ light chain loci exhibit dramatically increased λ/κ ratios (Jakobovits, 1998), implying that the introduced κ light chain transgenes do not function well in such mice. This further demonstrates the different functional outcome observed in immunoglobulins made by VELOCIMMUNE® mice as compared to those made by "knockout-plus-transgenic" mice.

[0549] B cell Development. Because the mature B cell populations in VELOCIMMUNE® mice resemble those of wild type mice (described above), it is possible that defects in early B cell differentiation are compensated for by the expansion of mature B cell populations. The various stages of B cell differentiation were examined by analysis of B cell populations using flow cytometry. Table 6 sets forth the ratio of the fraction of cells in each B cell lineage defined by FACs, using specific cell surface markers, in VELOCIMMUNE® mice compared to wild type littermates.

[0550] Early B cell development occurs in the bone marrow, and different stages of B cell differentiation are characterized by changes in the types and amounts of cell surface marker expression. These differences in surface expression correlate with the molecular changes occurring at the immunoglobulin loci inside the cell. The pro-B to pre-B cell transition requires the successful rearrangement and expression of functional heavy chain protein, while transition from the pre-B to mature B stage is governed by the correct rearrangement and expression of a κ or λ light chain. Thus, inefficient transition between stages of B cell differentiation can be detected by changes in the relative populations of B cells at a given stage.

TABLE 6

Version of VELOCIMMUNE® Mice	Bone Marrow					Spleen	
	pro-B $CD43^{hi}$ $B220^{lo}$	pre-B $CD24^{hi}$ $B220^{lo}$	Immature $B220^{lo}$ IgM^{+}	Mature $B220^{hi}$ IgM^{+}		Emerging $B220^{hi}$ IgM^{+} IgD^{+}	Mature B220hi IgM+
V1	1.1	1.0	0.9	1.0		1.1	1.0
V2	1.0	1.0	1.0	1.0		1.0	1.0
V3	1.0	1.0	1.1	1.0		1.0	1.1

[0551] No major defects were observed in B cell differentiation in any of the VELOCIMMUNE® mice. The introduction of human heavy chain gene segments does not appear to affect the pro-B to pre-B transition, and introduction of human κ light chain gene segments does not affect the pre-B to B transition in VELOCIMMUNE® mice. This demonstrates that "reverse chimeric" immunoglobulin molecules possessing human variable regions and mouse constants function normally in the context of B cell signaling and co-receptor molecules leading to appropriate B cell differentiation in a mouse environment. In contrast, the balance between the different populations during B cell differentiation are perturbed to varying extents in mice that contain randomly integrated immunoglobulin transgenes and inactivated endogenous heavy chain or κ light chain loci (Green and Jakobovits, 1998).

Example 4. Variable Gene Repertoire in Humanized Immunoglobulin Mice

[0552] Usage of human variable gene segments in the humanized antibody repertoire of VELOCIMMUNE® mice was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) of human variable regions from multiple sources including splenocytes and hybridoma cells. Variable region sequence, gene segment usage, somatic hypermutation, and junctional diversity of rearranged variable region gene segments were determined.

[0553] Briefly, total RNA was extracted from 1 x 10^7-2 x 10^7 splenocytes or about 10^4-10^5 hybridoma cells using TRIZOL™ (Invitrogen) or Qiagen RNEASY™ Mini Kit (Qiagen) and primed with mouse constant region specific primers using the SUPERSCRIPT™ III One-Step RT-PCR system (Invitrogen). Reactions were carried out with 2-5 µL of RNA from each sample using the aforementioned 3' constant specific primers paired with pooled leader primers for each family of human variable regions for both the heavy chain and κ light chain, separately. Volumes of reagents and primers, and RT-PCR/PCR conditions were performed according to the manufacturer's instructions. Primers sequences were based upon multiple sources (Wang, X. and Stollar, B.D. (2000) Human immunoglobulin variable region gene analysis by single cell RT-PCR. J Immunol Methods 244:217-225; Ig-primer sets, Novagen). Where appropriate, nested secondary PCR reactions were carried out with pooled family-specific framework primers and the same mouse 3' immunoglobulin constant-specific primer used in the primary reaction. Aliquots (5 µL) from each reaction were analyzed by agarose electrophoresis and reaction products were purified from agarose using a MONTAGE™ Gel Extraction Kit (Millipore). Purified products were cloned using the TOPO™ TA Cloning System (Invitrogen) and transformed into DH10β *E.coli* cells by electroporation. Individual clones were selected from each transformation reaction and grown in 2 mL LB broth cultures with antibiotic selection overnight at 37°C. Plasmid DNA was purified from bacterial cultures by a kit-based approach (Qiagen).

[0554] Immunoglobulin Variable Gene Usage. Plasmid DNA of both heavy chain and κ light

chain clones were sequenced with either T7 or M13 reverse primers on the ABI 3100 Genetic Analyzer (Applied Biosystems). Raw sequence data were imported into SEQUENCHER™ (v4.5, Gene Codes). Each sequence was assembled into contigs and aligned to human immunoglobulin sequences using IMGT V-Quest (Brochet, X., Lefranc, M.P., and Giudicelli, V. (2008). IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysis. Nucleic Acids Res 36, W503-508) search function to identify human V_H, D_H, J_H and Vκ, Jκ segment usage. Sequences were compared to germline sequences for somatic hypermutation and recombination junction analysis.

[0555] Mice were generated from ES cells containing the initial heavy chain modification ($3hV_H$-CRE Hybrid Allele, bottom of FIG. 2A) by RAG complementation (Chen, J. et al. (1993) RAG-2-deficient blastocyst complementation: an assay of gene function in lymphocyte development. Proc Natl Acad Sci U S A 90, 4528-4532), and cDNA was prepared from splenocyte RNA. The cDNA was amplified using primer sets (described above) specific for the predicted chimeric heavy chain mRNA that would arise by V(D)J recombination within the inserted human gene segments and subsequent splicing to either mouse IgM or IgG constant domains. Sequences derived from these cDNA clones (not shown) demonstrated that proper V(D)J recombination had occurred within the human variable gene sequences, that the rearranged human V(D)J gene segments were properly spliced in-frame to mouse constant domains and that class-switch recombination had occurred. Further sequence analysis of mRNA products of subsequent hybrid immunoglobulin loci was performed.

[0556] In a similar experiment, B cells from non-immunized wild type and VELOCIMMUNE® mice were separated by flow cytometry based upon surface expression of B220 and IgM or IgG. The $B220^+$ IgM^+ or surface IgG^+ ($sIgG^+$) cells were pooled and V_H and Vκ sequences were obtained following RT-PCR amplification and cloning (described above). Representative gene usage in a set of RT-PCR amplified cDNAs from unimmunized VELOCIMMUNE® 1 mice (Table 7) and VELOCIMMUNE® 3 mice (Table 8) was recorded (*defective RSS; †missing or pseudogene).

TABLE 7

V_H	Observed
1-18	3
1-17P	0
3-16*	0
3-15	13
3-13	9
3-11	6
3-9	8
1-8	6
3-7	2
2-5	2
1-3	0
1-2	11
6-1	5

J_H	Observed
1	2
2	1

D_H	Observed
1-1	1
2-2	2
3-3	4
4-4	0
5-5	0
5-18	4
6-6	5
1-7	7
2-8	0
3-9	4
3-10	2
4-11	1
5-12	1
6-13	3
1-14	0
2-15	0
3-16	1

Vκ	Observed
1-16	2
3-15	1
1-12	5
3-11	1
1-9	5
1-8	2
3-7*	0
1-6	5
1-5	8
5-2	6
4-1	8

Jκ	Observed
1	12
2	10
3	5
4	10

3	8
4	33
5	5
6	16

4-17	0
6-19	2
1-20	2
2-21	1
3-22	0
4-23	2
5-24	1
6-25	1
1-26	6
7-27	10

5	0

TABLE 8

V_H	Observed
7-81†	0
3-74†	0
3-73	1
3-72	2
2-70	2
1-69	3
3-66	1
3-64	1
4-61	1
4-59	10
1-58	0
3-53	0
5-51	5
3-49	2
3-48	7
1-46	1
1-45	0
3-43	10
4-39	4
3-38*	0
3-35*	0
4-34	8
3-33	14
4-31	4
3-30	13
4-28	0
2-26	0
1-24	3
3-23	18
3-21	0
3-20	0
1-18	4
1-17P	1
3-16*	0
3-15	13
3-13	6
3-11	5
3-9	31
1-8	7
3-7	11
2-5	1
1-3	0
1-2	6
6-1	9

D_H	Observed
1-1	7
2-2	8
3-3	9
4-4	4
5-5	6
5-18	6
6-6	29
1-7	30
2-8	4
3-9	8
3-10	10
4-11	4
5-12	5
6-13	17
1-14	2
2-15	3
3-16	4
4-17	3
6-19	8
1-20	3
2-21	1
3-22	5
4-23	2
5-24	2
6-25	2
1-26	17
7-27	7

J_H	Observed
1	2
2	8
3	26
4	95
5	11
6	58

V_K	Observed
2-40	1
1-39	34
1-37	2
1-33	35
2-30	8
2-29	2
2-28	7
1-27	5
2-24	7
6-21*	3
3-20	10
1-17	13
1-16	10
3-15	13
1-12	13
3-11	13
1-9	11
1-8	1
3-7*	0
1-6	6
1-5	7
5-2	0
4-1	21

J_K	Observed
1	50
2	37
3	28
4	64
5	22

[0557] As shown in Tables 7 and 8, nearly all of the functional human V_H, D_H, J_H, Vκ and Jκ gene segments are utilized. Of the functional variable gene segments described but not detected in the VELOCIMMUNE® mice of this experiment, several have been reported to possess defective recombination signal sequences (RSS) and, thus, would not be expected to be expressed (Feeney, A.J. (2000) Factors that influence formation of B cell repertoire. Immunol Res 21, 195-202). Analysis of several other sets of immunoglobulin sequences from various VELOCIMMUNE® mice, isolated from both naive and immunized repertoires, has shown usage of these gene segments, albeit at lower frequencies (data not shown). Aggregate gene usage data has shown that all functional human V_H, D_H, J_H, Vκ, and Jκ gene segments contained in VELOCIMMUNE® mice have been observed in various naive and immunized repertoires (data not shown). Although the human V_H7-81 gene segment has been identified in the analysis of human heavy chain locus sequences (Matsuda, F. et al. (1998) The complete nucleotide sequence of the human immunoglobulin heavy chain variable region locus. J Exp Med 188, 2151-2162), it is not present in the VELOCIMMUNE® mice as confirmed by re-sequencing of the entire VELOCIMMUNE® 3 mouse genome.

[0558] Sequences of heavy and light chains of antibodies are known to show exceptional variability, especially in short polypeptide segments within the rearranged variable domain. These regions, known as hypervariable regions or complementary determining regions (CDRs) create the binding site for antigen in the structure of the antibody molecule. The intervening polypeptide sequences are called framework regions (FRs). There are three CDRs (CDR1, CDR2, CDR3) and 4 FRs (FR1, FR2, FR3, FR4) in both heavy and light chains. One CDR, CDR3, is unique in that this CDR is created by recombination of both the V_H, D_H and J_H and Vκ and Jκ gene segments and generates a significant amount of repertoire diversity before antigen is encountered. This joining is imprecise due to both nucleotide deletions via exonuclease activity and non-template encoded additions via terminal deoxynucleotidyl transferase (TdT) and, thus, allows for novel sequences to result from the recombination process. Although FRs can show substantial somatic mutation due to the high mutability of the variable region as a whole, variability is not, however, distributed evenly across the variable region. CDRs are concentrated and localized regions of high variability in the surface of the antibody molecule that allow for antigen binding. Heavy chain and light chain sequences of selected antibodies from VELOCIMMUNE® mice around the CDR3 junction demonstrating junctional diversity are shown in FIG. 7A and 7B, respectively.

[0559] As shown in FIG. 7A, non-template encoded nucleotide additions (N-additions) are observed at both the V_H-D_H and D_H-J_H joint in antibodies from VELOCIMMUNE® mice, indicating proper function of TdT with the human segments. The endpoints of the V_H, D_H and J_H segments relative to their germline counterparts indicate that exonuclease activity has also occurred. Unlike the heavy chain locus, the human κ light chain rearrangements exhibit little or no TdT additions at CDR3, which is formed by the recombination of the Vκ and Jκ segments (FIG. 7B). This is expected due to the lack of TdT expression in mice during light chain

rearrangements at the pre-B to B cell transition. The diversity observed in the CDR3 of rearranged human Vκ regions is introduced predominantly through exonuclease activity during the recombination event.

[0560] Somatic hypermutation. Additional diversity is added to the variable regions of rearranged immunoglobulin genes during the germinal center reaction by a process termed somatic hypermutation. B cells expressing somatically mutated variable regions compete with other B cells for access to antigen presented by the follicular dendritic cells. Those B cells with higher affinity for the antigen will further expand and undergo class switching before exiting to the periphery. Thus, B cells expressing switched isotypes typically have encountered antigen and undergone germinal center reactions and will have increased numbers of mutations relative to naive B cells. Further, variable region sequences from predominantly naive $sIgM^+$ B cells would be expected to have relatively fewer mutations than variable sequences from $sIgG^+$ B cells which have undergone antigen selection.

[0561] Sequences from random V_H or Vκ clones from $sIgM^+$ or $sIgG^+$ B cells from non-immunized VELOCIMMUNE® mice or $sIgG^+$ B cells from immunized mice were compared with their germline variable gene segments and changes relative to the germline sequence annotated. The resulting nucleotide sequences were translated *in silico* and mutations leading to amino acid changes also annotated. The data were collated from all the variable regions and the percent change at a given position was calculated (FIG. 8).

[0562] As shown in FIG. 8, human heavy chain variable regions derived from $sIgG^+$ B cells from non-immunized VELOCIMMUNE® mice exhibit many more nucleotides relative to $sIgM^+$ B cells from the same splenocyte pools, and heavy chain variable regions derived from immunized mice exhibit even more changes. The number of changes is increased in the complementarity-determining regions (CDRs) relative to the framework regions, indicating antigen selection. The corresponding amino acid sequences from the human heavy chain variable regions also exhibit significantly higher numbers of mutations in IgG vs IgM and even more in immunized IgG. These mutations again appear to be more frequent in the CDRs compared with the framework sequences, suggesting that the antibodies were antigen-selected *in vivo*. A similar increase in the number the nucleotide and amino acid mutations are seen in the Vκ sequences derived from IgG^+ B cells from immunized mice.

[0563] The gene usage and somatic hypermutation observed in VELOCIMMUNE® mice demonstrate that essentially all gene segments present are capable of rearrangement to form fully functionally reverse chimeric antibodies in these mice. Further, VELOCIMMUNE® antibodies fully participate within the mouse immune system to undergo affinity selection and maturation to create fully mature human antibodies that can effectively neutralize their target antigen. VELOCIMMUNE® mice are able to mount robust immune responses to multiple classes of antigens that result in usage of a wide range of human antibodies that are both high affinity and suitable for therapeutic use (data not shown).

Example 5. Analysis of Lymphoid Structure and Serum Isotypes

[0564] The gross structures of spleen, inguinal lymph nodes, Peyer's patches and thymus of tissue samples from wild type or VELOCIMMUNE® mice stained with H&E were examined by light microscopy. The levels of immunoglobulin isotypes in serum collected from wild-type and VELOCIMMUNE® mice were analyzed using LUMINEX™ technology.

[0565] Lymphoid Organ Structure. The structure and function of the lymphoid tissues are in part dependent upon the proper development of hematopoietic cells. A defect in B cell development or function may be exhibited as an alteration in the structure of the lymphoid tissues. Upon analysis of stained tissue sections, no significant difference in appearance of secondary lymphoid organs between wild type and VELOCIMMUNE® mice was identified (data not shown).

[0566] Serum Immunoglobulin Levels. The level of expression of each isotype is similar in wild type and VELOCIMMUNE® mice (FIG. 9A, 9B and 9C). This demonstrates that humanization of the variable gene segments had no apparent adverse effect upon class switching or immunoglobulin expression and secretion and therefore apparently maintain all the endogenous mouse sequences necessary for these functions.

Example 6. Immunization and Antibody Production in Humanized Immunoglobulin Mice

[0567] Different versions of VELOCIMMUNE® mice were immunized with antigen to examine the humoral response to foreign antigen challenge.

[0568] Immunization and Hybridoma Development. VELOCIMMUNE® and wild-type mice can be immunized with an antigen in the form of protein, DNA, a combination of DNA and protein, or cells expressing the antigen. Animals are typically boosted every three weeks for a total of two to three times. Following each antigen boost, serum samples from each animal are collected and analyzed for antigen-specific antibody responses by serum titer determination. Prior to fusion, mice received a final pre-fusion boost of 5 µg protein or DNA, as desired, via intra-peritoneal and/or intravenous injections. Splenocytes are harvested and fused to Ag8.653 myeloma cells in an electrofusion chamber according to the manufacture's suggested protocol (Cyto Pulse Sciences Inc., Glen Burnie, MD). Ten days after culture, hybridomas are screened for antigen specificity using an ELISA assay (Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual. Cold Spring Harbor Press, New York). Alternatively, antigen specific B cells are isolated directly from immunized VELOCIMMUNE® mice and screened using standard techniques, including those described here, to obtain human antibodies specific for an antigen of interest.

[0569] Serum Titer Determination. To monitor animal anti-antigen serum response, serum

samples are collected about 10 days after each boost and the titers are determined using antigen specific ELISA. Briefly, Nunc MAXISORP™ 96 well plates are coated with 2 µg/mL antigen overnight at 4° C and blocked with bovine serum albumin (Sigma, St. Louis, MO). Serum samples in a serial 3 fold dilutions are allowed to bind to the plates for one hour at room temperature. The plates are then washed with PBS containing 0.05% Tween-20 and the bound IgG are detected using HRP-conjugated goat anti-mouse Fc (Jackson Immuno Research Laboratories, Inc., West Grove, PA) for total IgG titer, or biotin-labeled isotype specific or light chain specific polyclonal antibodies (SouthernBiotech Inc.) for isotype specific titers, respectively. For biotin-labeled antibodies, following plate wash, HRP-conjugated streptavidin (Pierce, Rockford, IL) is added. All plates are developed using colorimetric substrates such as BD OPTEIA™ (BD Biosciences Pharmingen, San Diego, CA). After the reaction is stopped with 1 M phosphoric acid, optical absorptions at 450 nm are recorded and the data are analyzed using PRISM™ software from Graph Pad. Dilutions required to obtain two-fold of background signal are defined as titer.

[0570] In one experiment, VELOCIMMUNE® mice were immunized with human interleukin-6 receptor (hIL-6R). A representative set of serum titers for VELOCIMMUNE® and wild type mice immunized with hIL-6R is shown in FIG. 10A and 10B.

[0571] VELOCIMMUNE® and wild-type mice mounted strong responses towards the IL-6R with similar titer ranges (FIG. 10A). Several mice from the VELOCIMMUNE® and wild-type cohorts reached a maximal response after a single antigen boost. These results indicate that the immune response strength and kinetics to this antigen were similar in the VELOCIMMUNE® and wild type mice. These antigen-specific antibody responses were further analyzed to examine the particular isotypes of the antigen-specific antibodies found in the sera. Both VELOCIMMUNE® and wild type groups predominantly elicited an IgG1 response (FIG. 10B), suggesting that class switching during the humoral response is similar in mice of each type.

[0572] Affinity Determination of Antibody Binding to Antigen in Solution. An ELISA-based solution competition assay is typically designed to determine antibody-binding affinity to the antigen.

[0573] Briefly, antibodies in conditioned medium are premixed with serial dilutions of antigen protein ranging from 0 to 10 mg/mL. The solutions of the antibody and antigen mixture are then incubated for two to four hours at room temperature to reach binding equilibria. The amounts of free antibody in the mixtures are then measured using a quantitative sandwich ELISA. Ninety-six well MAXISORB™ plates (VWR, West Chester, PA) are coated with 1 µg/mL antigen protein in PBS solution overnight at 4°C followed by BSA nonspecific blocking. The antibody-antigen mixture solutions are then transferred to these plates followed by one-hour incubation. The plates are then washed with washing buffer and the plate-bound antibodies were detected with an HRP-conjugated goat anti-mouse IgG polyclonal antibody reagent (Jackson Immuno Research Lab) and developed using colorimetric substrates such as BD OPTEIA™ (BD Biosciences Pharmingen, San Diego, CA). After the reaction is stopped with 1

M phosphoric acid, optical absorptions at 450 nm are recorded and the data are analyzed using PRISM™ software from Graph Pad. The dependency of the signals on the concentrations of antigen in solution are analyzed with a 4 parameter fit analysis and reported as IC_{50}, the antigen concentration required to achieve 50% reduction of the signal from the antibody samples without the presence of antigen in solution.

[0574] In one experiment, VELOCIMMUNE® mice were immunized with hIL-6R (as described above). FIG. 11A and 11B show a representative set of affinity measurements for anti-hIL6R antibodies from VELOCIMMUNE® and wild-type mice.

[0575] After immunized mice receive a third antigen boost, serum titers are determined by ELISA. Splenocytes are isolated from selected wild type and VELOCIMMUNE® mouse cohorts and fused with Ag8.653 myeloma cells to form hybridomas and grown under selection (as described above). Out of a total of 671 anti-IL-6R hybridomas produced, 236 were found to express antigen-specific antibodies. Media harvested from antigen positive wells was used to determine the antibody affinity of binding to antigen using a solution competition ELISA. Antibodies derived from VELOCIMMUNE® mice exhibit a wide range of affinity in binding to antigen in solution (FIG. 11A). Furthermore, 49 out of 236 anti-IL-6R hybridomas were found to block IL-6 from binding to the receptor in an in vitro bioassay (data not shown). Further, these 49 anti-IL-6R blocking antibodies exhibited a range of high solution affinities similar to that of blocking antibodies derived from the parallel immunization of wild type mice (FIG. 11B).

Example 7. Construction of a Mouse ADAM6 Targeting Vector

[0576] A targeting vector for insertion of mouse ADAM6a and ADAM6b genes into a humanized heavy chain locus was constructed using VELOCIGENE® genetic engineering technology (*supra*) to modify a Bacterial Artificial Chromosome (BAC) 929d24 obtained from Dr. Fred Alt (Havard University). 929d24 BAC DNA was engineered to contain genomic fragments containing the mouse ADAM6a and ADAM6b genes and a hygromycin cassette for targeted deletion of a human ADAM6 pseudogene (hADAM6Ψ) located between human V_H1-2 and V_H6-1 gene segments of a humanized heavy chain locus (FIG. 12).

[0577] First, a genomic fragment containing the mouse ADAM6b gene, -800 bp of upstream (5') sequence and -4800 bp of downstream (3') sequence was subcloned from the 929d24 BAC clone. A second genomic fragment containing the mouse ADAM6a gene, -300 bp of upstream (5') sequence and -3400 bp of downstream (3') sequence, was separately subcloned from the 929d24 BAC clone. The two genomic fragments containing the mouse ADAM6b and ADAM6a genes were ligated to a hygromycin cassette flanked by Frt recombination sites to create the targeting vector (Mouse ADAM6 Targeting Vector, Figure 20; SEQ ID NO:3). Different restriction enzyme sites were engineered onto the 5' end of the targeting vector following the mouse ADAM6b gene and onto the 3' end following the mouse ADAM6a gene (bottom of FIG. 12) for ligation into the humanized heavy chain locus.

[0578] A separate modification was made to a BAC clone containing a replacement of the mouse heavy chain locus with the human heavy chain locus, including the human ADAM6 pseudogene located between the human V_H1-2 and V_H6-1 gene segments of the humanized locus for the subsequent ligation of the mouse ADAM6 targeting vector (FIG. 13).

[0579] Briefly, a neomycin cassette flanked by *lox*P recombination sites was engineered to contain homology arms containing human genomic sequence at positions 3' of the human V_H1-2 gene segment (5' with respect to hADAM64Ψ) and 5' of human V_H6-1 gene segment (3' with respect to hADAM6Ψ; see middle of FIG. 13). The location of the insertion site of this targeting construct was about 1.3 kb 5' and ~350 bp 3' of the human ADAM6 pseudogene. The targeting construct also included the same restriction sites as the mouse ADAM6 targeting vector to allow for subsequent BAC ligation between the modified BAC clone containing the deletion of the human ADAM6 pseudogene and the mouse ADAM6 targeting vector.

[0580] Following digestion of BAC DNA derived from both constructs, the genomic fragments were ligated together to construct an engineered BAC clone containing a humanized heavy chain locus containing an ectopically placed genomic sequence comprising mouse ADAM6a and ADAM6b nucleotide sequences. The final targeting construct for the deletion of a human ADAM6 gene within a humanized heavy chain locus and insertion of mouse ADAM6a and ADAM6b sequences in ES cells contained, from 5' to 3', a 5' genomic fragment containing ~13 kb of human genomic sequence 3' of the human V_H1-2 gene segment, -800 bp of mouse genomic sequence downstream of the mouse ADAM6b gene, the mouse ADAM6b gene, ~4800 bp of genomic sequence upstream of the mouse ADAM6b gene, a 5' Frt site, a hygromycin cassette, a 3' Frt site, -300 bp of mouse genomic sequence downstream of the mouse ADAM6a gene, the mouse ADAM6a gene, ~3400 bp of mouse genomic sequence upstream of the mouse ADAM6a gene, and a 3' genomic fragment containing -30 kb of human genomic sequence 5' of the human V_H6-1 gene segment (bottom of FIG. 13).

[0581] The engineered BAC clone (described above) was used to electroporate mouse ES cells that contained a humanized heavy chain locus to created modified ES cells comprising a mouse genomic sequence ectopically placed that comprises mouse ADAM6a and ADAM6b sequences within a humanized heavy chain locus. Positive ES cells containing the ectopic mouse genomic fragment within the humanized heavy chain locus were identified by a quantitative PCR assay using TAQMAN™ probes (Lie, Y.S. and Petropoulos, C.J. (1998) Advances in quantitative PCR technology: 5'nuclease assays. Curr Opin Biotechnol 9(1):43-48). The upstream and downstream regions outside of the modified portion of the humanized heavy chain locus were confirmed by PCR using primers and probes located within the modified region to confirm the presence of the ectopic mouse genomic sequence within the humanized heavy chain locus as well as the hygromycin cassette. The nucleotide sequence across the upstream insertion point included the following, which indicates human heavy chain genomic sequence upstream of the insertion point and an I-Ceu I restriction site (contained within the parentheses below) linked contiguously to mouse genomic sequence present at the insertion point: (CCAGCTTCAT TAGTAATCGT TCATCTGTGG TAAAAAGGCA GGATTTGAAG

CGATGGAAGA TGGGAGTACG GGGCGTTGGA AGACAAAGTG CCACACAGCG CAGCCTTCGT CTAGACCCCC GGGCTAACTA TAACGGTCCT AAGGTAGCGA G) GGGATGACAG ATTCTCTGTT CAGTGCACTC AGGGTCTGCC TCCACGAGAA TCACCATGCC CTTTCTCAAG ACTGTGTTCT GTGCAGTGCC CTGTCAGTGG (SEQ ID NO:4). The nucleotide sequence across the downstream insertion point at the 3' end of the targeted region included the following, which indicates mouse genomic sequence and a PI-Sce I restriction site (contained within the parentheses below) linked contiguously with human heavy chain genomic sequence downstream of the insertion point:

(AGGGGTCGAG GGGGAATTTT ACAAAGAACA AAGAAGCGGG CATCTGCTGA CATGAGGGCC GAAGTCAGGC TCCAGGCAGC GGGAGCTCCA CCGCGGTGGC GCCATTTCAT TACCTCTTTC TCCGCACCCG ACATAGATAAAGCTT) ATCCCCCACC AAGCAAATCC CCCTACCTGG GGCCGAGCTT CCCGTATGTG GGAAAATGAA TCCCTGAGGT CGATTGCTGC ATGCAATGAA ATTCAACTAG (SEQ ID NO:5).

[0582] Targeted ES cells described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® mouse engineering method (see, *e.g.,* US Pat. Nos. 7,6598,442, 7,576,259, 7,294,754). Mice bearing a humanized heavy chain locus containing an ectopic mouse genomic sequence comprising mouse ADAM6a and ADAM6b sequences were identified by genotyping using a modification of allele assay (Valenzuela *et al.,* 2003) that detected the presence of the mouse ADAM6a and ADAM6b genes within the humanized heavy chain locus.

[0583] Mice bearing a humanized heavy chain locus that contains mouse ADAM6a and ADAM6b genes are bred to a FLPe deletor mouse strain (see, *e.g.,* Rodriguez, C.I. et al. (2000) High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. Nature Genetics 25:139-140) in order to remove any Frt'ed hygromycin cassette introduced by the targeting vector that is not removed, *e.g.,* at the ES cell stage or in the embryo. Optionally, the hygromycin cassette is retained in the mice.

[0584] Pups are genotyped and a pup heterozygous for a humanized heavy chain locus containing an ectopic mouse genomic fragment that comprises mouse ADAM6a and ADAM6b sequences is selected for characterizing mouse ADAM6 gene expression and fertility.

Example 8. Characterization of ADAM6 Rescue Mice

[0585] Flow Cytometry. Three mice at age 25 weeks homozygous for human heavy and human κ light chain variable gene loci (H/κ) and three mice at age 18-20 weeks homozygous for human heavy and human κ light chain having the ectopic mouse genomic fragment encoding the mouse ADAM6a and ADAM6b genes within both alleles of the human heavy chain locus (H/κ-A6) were sacrificed for identification and analysis of lymphocyte cell populations by FACs on the BD LSR II System (BD Bioscience). Lymphocytes were gated for specific cell lineages and analyzed for progression through various stages of B cell

development. Tissues collected from the animals included blood, spleen and bone marrow. Blood was collected into BD microtainer tubes with EDTA (BD Biosciences). Bone marrow was collected from femurs by flushing with complete RPMI medium supplemented with fetal calf serum, sodium pyruvate, HEPES, 2-mercaptoethanol, non-essential amino acids, and gentamycin. Red blood cells from blood, spleen and bone marrow preparations were lysed with an ammonium chloride-based lysis buffer (*e.g.,* ACK lysis buffer), followed by washing with complete RPMI medium.

[0586] For staining of cell populations, 1 x 10^6 cells from the various tissue sources were incubated with anti-mouse CD16/CD32 (2.4G2, BD Biosciences) on ice for 10 minutes, followed by labeling with one or a combination of the following antibody cocktails for 30 min on ice.

[0587] Bone marrow: anti-mouse FITC-CD43 (1B11, BioLegend), PE-ckit (2B8, BioLegend), PeCy7-IgM (II/41, eBioscience), PerCP-Cy5.5-IgD (11-26c.2a, BioLegend), APC-eFluor780-B220 (RA3-6B2, eBioscience), A700-CD19 (1D3, BD Biosciences).

[0588] Peripheral blood and spleen: anti-mouse FiTC-κ (187.1, BD Biosciences), PE-λ (RML-42, BioLegend), PeCy7-IgM (II/41, eBioscience), PerCP-Cy5.5-IgD (11-26c.2a, BioLegend), APC-CD3 (145-2C11, BD), A700-CD19 (1D3, BD), APC-eFluor780-B220 (RA3-6B2, eBioscience). Following incubation with the labeled antibodies, cells were washed and fixed in 2% formaldehyde. Data acquisition was performed on an LSRII flow cytometer and analyzed with FlowJo. Results from a representative H/κ and H/κ-A6 mouse are shown in FIGs. 14 - 18.

[0589] The results demonstrate that B cells of H/κ-A6 mice progress through the stages of B cell development in a similar fashion to H/κ mice in the bone marrow and peripheral compartments, and show normal patterns of maturation once they enter the periphery. H/κ-A6 mice demonstrated an increased $CD43^{int}CD19^{+}$ cell population as compared to H/κ mice (FIG. 16B). This may indicate an accelerated IgM expression from the humanized heavy chain locus containing an ectopic mouse genomic fragment comprising the mouse ADAM6a and ADAM6b sequences in H/κ-A6 mice. In the periphery, B and T cell populations of H/κ-A6 mice appear normal and similar to H/κ mice.

[0590] T**estis Morphology and Sperm Characterization.** To determine if infertility in mice having humanized immunoglobulin heavy chain variable loci is due to testis and/or sperm production defects, testis morphology and sperm content of the epididymis was examined.

[0591] Briefly, testes from two groups of five mice per group (Group 1: mice homozygous for human heavy and κ light chain variable gene loci, $mADAM6^{-/-}$; Group 2: mice heterozygous for human heavy chain variable gene loci and homozygous for κ light chain variable gene loci, $mADAM6^{+/-}$) were dissected with the epididymis intact and weighed. The specimens were then fixed, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE) stain. Testis sections (2 testes per mouse, for a total of 20) were examined for defects in morphology

and evidence of sperm production, while epididymis sections were examined for presence of sperm.

[0592] In this experiment, no differences in testis weight or morphology was observed between mADAM6$^{-/-}$ mice and mADAM6$^{+/-}$ mice. Sperm was observed in all genotypes, both in the testes and the epididymis. These results establish that the absence of mouse ADAM6a and ADAM6b genes does not lead to detectable changes in testis morphology, and that sperm is produced in mice in the presence and absence of these two genes. Defects in fertility of male ADAM6$^{-/-}$ mice are therefore not likely to be due to low sperm production.

[0593] Sperm Motility and Migration. Mice that lack other ADAM gene family members are infertile due to defects in sperm motility or migration. Sperm migration is defined as the ability of sperm to pass from the uterus into the oviduct, and is normally necessary for fertilization in mice. To determine if the deletion of mouse ADAM6a and ADAM6b affects this process, sperm migration was evaluated in mADAM6$^{-/-}$mice. Sperm motility was also examined.

[0594] Briefly, sperm was obtained from testes of (1) mice heterozygous for human heavy chain variable gene loci and homozygous for human κ light chain variable gene locui (ADAM6$^{+/-}$); (2) mice homozyogous for human heavy chain variable gene loci and homozygous for human κ light chain variable gene loci (ADAM6$^{-/-}$); (3) mice homozygous for human heavy chain variable gene loci and homozygous for wild-type κ light chain (ADAM6$^{-/-}$ mκ); and, (4) wild-type C57 BL/6 mice (WT). No significant abnormalities were observed in sperm count or overall sperm motility by inspection. For all mice, cumulus dispersal was observed, indicating that each sperm sample was able to penetrate the cumulus cells and bind the zona pellucida *in vitro*. These results establish that ADAM6$^{-/-}$ mice have sperm that are capable of penetrating the cumulus and binding the zona pellucida.

[0595] Fertilization of mouse ova *in vitro* (IVF) was done using sperm from mice as described above. A slightly lower number of cleaved embryos were present for ADAM6$^{-/-}$ the day following IVF, as well as a reduced number of sperm bound to the eggs. These results establish that sperm from ADAM6$^{-/-}$ mice, once exposed to an ovum, are capable of penetrating the cumulus and binding the zona pellucida.

[0596] In another experiment, the ability of sperm from ADAM6$^{-/-}$ mice to migrate from the uterus and through the oviduct was determined in a sperm migration assay.

[0597] Briefly, a first group of five superovulated female mice were set up with five ADAM6$^{-/-}$ males. A second group of five superovulated female mice were set up with five ADAM6$^{-/-}$ males. The mating pairs were observed for copulation, and five to six hours post-copulation the uterus and attached oviduct from all females were removed and flushed for analysis. Flush solutions were checked for eggs to verify ovulation and obtain a sperm count. Sperm migration

was evaluated in two different ways. First, both oviducts were removed from the uterus, flushed with saline, and any sperm identified were counted. The presence of eggs was also noted as evidence of ovulation. Second, oviducts were left attached to the uterus and both tissues were fixed, embedded in paraffin, sectioned and stained (as described above). Sections were examined for presence of sperm, in both the uterus and in both oviducts.

[0598] For the five females mated with the five ADAM6$^{-/-}$ males, very little sperm was found in the flush solution from the oviduct. Flush solutions from oviducts of the five females mated with the five ADAM6$^{+/-}$ males exhibited a sperm level about 25- to 30-fold higher (avg, n = 10 oviducts) than present in flush solutions from the oviducts of the five females mated with the five ADAM6$^{-/-}$ males.

[0599] Histological sections of uterus and oviduct were prepared. The sections were examined for sperm presence in the uterus and the oviduct (the colliculus tubarius). Inspection of histological sections of oviduct and uterus revealed that for female mice mated with ADAM6$^{-/-}$ mice, sperm was found in the uterus but not in the oviduct. Further, sections from females mated with ADAM6$^{-/-}$ mice revealed that sperm was not found at the uterotubal junction (UTJ). In sections from females mated with ADAM6$^{+/-}$ mice, sperm was identified in the UTJ and in the oviduct.

[0600] These results establish that mice lacking ADAM6a and ADAM6b genes make sperm that exhibit an *in vivo* migration defect. In all cases, sperm was observed within the uterus, indicating that copulation and sperm release apparently occur as normal, but little to no sperm was observed within the oviducts after copulation as measured either by sperm count or histological observation. These results establish that mice lacking ADAM6a and ADAM6b genes produce sperm that exhibit an inability to migrate from the uterus to the oviduct. This defect apparently leads to infertility because sperm are unable to cross the uterine-tubule junction into the oviduct, where eggs are fertilized. Taken together, all of these results converge to the support the hypothesis that mouse ADAM6 genes help direct sperm with normal motility to migrate out of the uterus, through the uterotubal junction and the oviduct, and thus approach an egg to achieve the fertilization event. The mechanism by which ADAM6 achieves this may be directly by action of the ADAM6 proteins, or through coordinate expression with other proteins, *e.g.,* other ADAM proteins, in the sperm cell, as described below.

[0601] ADAM Gene Family Expression. A complex of ADAM proteins are known to be present as a complex on the surface of maturing sperm. Mice lacking other ADAM gene family members lose this complex as sperm mature, and exhibit a reduction of multiple ADAM proteins in mature sperm. To determine if a lack of ADAM6a and ADAM6b genes affects other ADAM proteins in a similar manner, Western blots of protein extracts from testis (immature sperm) and epididymis (maturing sperm) were analyzed to determine the expression levels of other ADAM gene family members.

[0602] In this experiment, protein extracts were analyzed from four ADAM6$^{-/-}$ and four ADAM6$^{+/-}$ mice. The results showed that expression of ADAM2 and ADAM3 were not affected in testis extracts. However, both ADAM2 and ADAM3 were dramatically reduced in epididymis extracts. This demonstrates that the absence of ADAM6a and ADAM6b in sperm of ADAM6$^{-/-}$ mice may have a direct affect on the expression and perhaps function of other ADAM proteins as sperm matures (*e.g.,* ADAM2 and ADAM3). This suggests that ADAM6a and ADAM6b are part of an ADAM protein complex on the surface of sperm, which might be critical for proper sperm migration.

Example 9. Human Heavy Chain Variable Gene Utilization in ADAM6 Rescue Mice

[0603] Selected human heavy chain variable gene usage was determined for mice homozygous for human heavy and κ light chain variable gene loci either lacking mouse ADAM6a and ADAM6b genes (mADAM6$^{-/-}$) or containing an ectopic genomic fragment encoding for mouse ADAM6a and ADAM6b genes (ADAM6$^{+/+}$; see Example 1) by a quantitative PCR assay using TAQMAN™ probes (as described above).

[0604] Briefly, CD19^{+} B cells were purified from the spleens of mADAM6$^{-/-}$ and ADAM6$^{+/+}$ mice using mouse CD19 Microbeads (Miltenyi Biotec) and total RNA was purified using the RNEASY™ Mini kit (Qiagen). Genomic RNA was removed using a RNase-free DNase on-column treatment (Qiagen). About 200 ng mRNA was reverse-transcribed into cDNA using the First Stand cDNA Synthesis kit (Invitrogen) and then amplified with the TAQMAN™ Universal PCR Master Mix (Applied Biosystems) using the ABI 7900 Sequence Detection System (Applied Biosystems). Relative expression of each gene was normalized to the mouse κ Constant (mCκ). Table 9 sets forth the sense/antisense/TAQMAN™ MGB probe combinations used in this experiment.

TABLE 9

Human V_H	Sequence (5'-3')	SEQ ID NOs:
V_H6-1	Sense: CAGGTACAGCTGCAGCAGTCA	6
	Anti-sense: GGAGATGGCACAGGTGAGTGA	7
	Probe: TCCAGGACTGGTGAAGC	8
V_H1-2	Sense: TAGTCCCAGTGATGAGAAAGAGAT	9
	Anti-sense: GAGAACACAGAAGTGGATGAGATC	10
	Probe: TGAGTCCAGTCCAGGGA	11
V_H3-23	Sense: AAAAATTGAGTGTGAATGGATAAGAGTG	12
	Anti-sense: AACCCTGGTCAGAAACTGCCA	13
	Probe: AGAGAAACAGTGGATACGT	14
	Sense: AACTACGCACAGAAGTTCCAGG	15

Human V_H	Sequence (5'-3')	SEQ ID NOs:
V_H1-69	Anti-sense: GCTCGTGGATTTGTCCGC	16
	Probe: CAGAGTCACGATTACC	17
mCκ	Sense: TGAGCAGCACCCTCACGTT	18
	Anti-sense: GTGGCCTCACAGGTATAGCTGTT	19
	Probe: ACCAAGGACGAGTATGAA	20

[0605] In this experiment, expression of all four human V_H genes was observed in the samples analyzed. Further, the expression levels were comparable between mADAM6$^{-/-}$ and ADAM6$^{+/+}$ mice. These results demonstrate that human V_H genes that were both distal to the modification site (V_H3-23 and V_H1-69) and proximal to the modification site (V_H1-2 and V_H6-1) were all able to recombine to form a functionally expressed human heavy chain. These results demonstrate that the ectopic genomic fragment comprising mouse ADAM6a and ADAM6b sequences inserted into a human heavy chain genomic sequence did not affect V(D)J recombination of human heavy chain gene segments within the locus, and these mice are able to recombine human heavy chain gene segments in normal fashion to produce functional heavy chain immunoglobulin proteins.

Example 10. Deletion of the Mouse Immunoglobulin Light Chain Loci

[0606] Various targeting constructs were made using VELOCIGENE® technology (see, *e.g.,* US Pat. No. 6,586,251 and Valenzuela et al. (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech. 21(6):652-659) to modify mouse genomic Bacterial Artificial Chromosome (BAC) libraries to inactivate the mouse κ and λ light chain loci.

[0607] Deletion of the mouse λ light chain locus. DNA from mouse BAC clone RP23-135k15 (Invitrogen) was modified by homologous recombination to inactivate the endogenous mouse λ light chain locus through targeted deletion of the Yλ-Jλ-Cλ gene clusters (FIG. 20).

[0608] Briefly, the entire proximal cluster comprising Vλ1-Jλ3-Cλ3-Jλ1-Cλ1 gene segments was deleted in a single targeting event using a targeting vector comprising a neomycin cassette flanked by *lox*P sites with a 5' mouse homology arm containing sequence 5' of the Vλ1 gene segment and a 3' mouse homology arm containing sequence 3' of the Cλ1 gene segment (FIG. 20, Targeting Vector 1).

[0609] A second targeting construct was prepared to precisely delete the distal endogenous mouse λ gene cluster containing Vλ2-Jλ2-Cλ2-Jλ4-Cλ4 except that the targeting construct contained a 5' mouse homology arm that contained sequence 5' of the Vλ2 gene segment and

a 3' mouse homology arm that contained sequence 5' to the endogenous Cλ2 gene segment (FIG. 20, Targeting Vector 2). Thus, the second targeting construct precisely deleted Vλ2-Jλ2, while leaving Cλ2-Jλ4-Cλ4 intact at the endogenous mouse λ locus. ES cells containing an inactivated endogenous λ locus (as described above) were confirmed by karyotyping and screening methods (*e.g.,* TAQMAN®) known in the art. DNA was then isolated from the modified ES cells and subjected to treatment with CRE recombinase thereby mediating the deletion of the proximal targeting cassette containing the neomycin marker gene, leaving only a single *lox*P site at the deletion point (FIG. 20, bottom).

[0610] Deletion of the mouse κ light chain locus. Several targeting constructs were made using similar methods described above to modify DNA from mouse BAC clones RP23-302g12 and RP23-254m04 (Invitrogen) by homologous recombination to inactivate the mouse κ light chain locus in a two-step process (FIG. 21).

[0611] Briefly, the Jκ gene segments (1-5) of the endogenous mouse κ light chain locus were deleted in a single targeting event using a targeting vector comprising a hygromycin-thymidine kinase (hyg-TK) cassette containing a single *lox*P site 3' to the hyg-TK cassette (FIG. 21, Jκ Targeting Vector). The homology arms used to make this targeting vector contained mouse genomic sequence 5' and 3' of the endogenous mouse Jκ gene segments. In a second targeting event, a second targeting vector was prepared to delete a portion of mouse genomic sequence upstream (5') to the most distal endogenous mouse Vκ gene segment (FIG. 21, Vκ Targeting Vector). This targeting vector contained an inverted *lox*511 site, a *lox*P site and a neomycin cassette. The homology arms used to make this targeting vector contained mouse genomic sequence upstream of the most distal mouse Vκ gene segment. The targeting vectors were used in a sequential fashion (*i.e.,* Jκ then Vκ) to target DNA in ES cells. ES bearing a double-targeted chromosome (*i.e.,* a single endogenous mouse κ locus targeted with both targeting vectors) were confirmed by karyotyping and screening methods (*e.g.,* TAQMAN™) known in the art. DNA was then isolated from the modified ES cells and subjected to treatment with Cre recombinase thereby mediating the deletion of endogenous mouse Vκ gene segments and both selection cassettes, while leaving two juxtaposed *lox* sites in opposite orientation relative to one another (FIG. 21, bottom; SEQ ID NO:59).

[0612] Thus, two modified endogenous light chain loci (κ and λ) containing intact enhancer and constant regions were created for progressively inserting unrearranged human λ germline gene segments in a precise manner using targeting vectors described below.

Example 11. Replacement of Mouse Light Chain Loci with a Human λ Light Chain Mini-Locus

[0613] Multiple targeting vectors were engineered for progressive insertion of human λ gene segments into the endogenous mouse κ and λ light chain loci using similar methods as described above. Multiple independent initial modifications were made to the endogenous light chain loci each producing a chimeric light chain locus containing hVλ and Jλ gene segments

operably linked to mouse light chain constant genes and enhancers.

[0614] A human λ mini-locus containing 12 human Yλ and one human Jλ gene segment. A series of initial targeting vectors were engineered to contain the first 12 consecutive human Vλ gene segments from cluster A and a hJλ1 gene segment or four hJλ gene segments using a human BAC clone named RP11 -729g4 (Invitrogen). FIGs. 22A and 22B show the targeting vectors that were constructed for making an initial insertion of human λ light chain gene segments at the mouse λ and κ light chain loci, respectively.

[0615] For a first set of initial targeting vectors, a 124,125 bp DNA fragment from the 729g4 BAC clone containing 12 hVλ gene segments and a hJλ1 gene segment was engineered to contain a PI-SceI site 996 bp downstream (3') of the hJλ1 gene segment for ligation of a 3' mouse homology arm. Two different sets of homology arms were used for ligation to this human fragment; one set of homology arms contained endogenous mouse λ sequences from the 135k15 BAC clone (FIG. 22A) and another set contained endogenous κ sequence 5' and 3' of the mouse Vκ and Jκ gene segments from mouse BAC clones RP23-302g12 and RP23-254m04, respectively (FIG. 22B).

[0616] For the 12/1-λ Targeting Vector (FIG. 22A), a PI-SceI site was engineered at the 5' end of a 27,847 bp DNA fragment containing the mouse Cλ2-Jλ4-Cλ4 and enhancer 2.4 of the modified mouse λ locus described in Example 10. The -28 kb mouse fragment was used as a 3' homology arm by ligation to the ~124 kb human λ fragment, which created a 3' junction containing, from 5' to 3', a hJλ1 gene segment, 996 bp of human λ sequence 3' of the hJλ□ gene segment, 1229 bp of mouse λ sequence 5' to the mouse Cλ2 gene, the mouse Cλ2 gene and the remaining portion of the ~28 kb mouse fragment. Upstream (5') from the human Vλ3-12 gene segment was an additional 1456 bp of human λ sequence before the start of the 5' mouse homology arm, which contained 23,792 bp of mouse genomic DNA corresponding to sequence 5' of the endogenous mouse λ locus. Between the 5' homology arm and the beginning of the human λ sequence was a neomycin cassette flanked by Frt sites.

[0617] Thus, the 12/1-λ Targeting Vector included, from 5' to 3', a 5' homology arm containing ~24 kb of mouse λ genomic sequence 5' of the endogenous λ locus, a 5' Frt site, a neomycin cassette, a 3' Frt site, ~123 kb of human genomic λ sequence containing the first 12 consecutive hVλ gene segments and a hJλ1 gene segment, a PI-SceI site, and a 3' homology arm containing ~28 kb of mouse genomic sequence including the endogenous Cλ2-Jλ4-Cλ4 gene segments, the mouse enhancer 2.4 sequence and additional mouse genomic sequence downstream (3') of the enhancer 2.4 (FIG. 22A).

[0618] In a similar fashion, the 12/1-κ Targeting Vector (FIG. 22B) employed the same ~124 human λ fragment with the exception that mouse homology arms containing mouse κ sequence were used such that targeting to the endogenous κ locus could be achieved by homologous recombination. Thus, the 12/1-κ Targeting Vector included, from 5' to 3', a 5' homology arm containing ~23 kb of mouse genomic sequence 5' of the endogenous κ locus, an I-CeuI site, a 5' Frt site, a neomycin cassette, a 3' Frt site, ~124 kb of human genomic λ

sequence containing the first 12 consecutive hVλ gene segments and a hJλ1 gene segment, a PI-SceI site, and a 3' homology arm containing ~28 kb of mouse genomic sequence including the endogenous the mouse Cκ gene, Eκi and Eκ3' and additional mouse genomic sequence downstream (3') of Eκ3' (FIG. 22B, 12/1-κ Targeting Vector).

[0619] Homologous recombination with either of these two initial targeting vectors created a modified mouse light chain locus (κ or λ) containing 12 hVλ gene segments and a hJλ1 gene segment operably linked to the endogenous mouse light chain constant gene and enhancers (Cκ or Cλ2 and Eκi/Eκ3' or Enh 2.4/Enh 3.1) gene which, upon recombination, leads to the formation of a chimeric λ light chain.

[0620] A human λ mini-locus with 12 human Yλ and four human Jλ gene segments. In another approach to add diversity to a chimeric λ light chain locus, a third initial targeting vector was engineered to insert the first 12 consecutive human Vλ gene segments from cluster A and hJλ1, 2, 3 and 7 gene segments into the mouse κ light chain locus (FIG. 22B, 12/4-κ Targeting Vector). A DNA segment containing hJλ1, Jλ2, Jλ3 and Jλ7 gene segments was made by *de novo* DNA synthesis (Integrated DNA Technologies) including each Jλ gene segment and human genomic sequence of ~100 bp from both the immediate 5' and 3' regions of each Jλ gene segment. A PI-SceI site was engineered into the 3' end of this ~1 kb DNA fragment and ligated to a chloramphenicol cassette. Homology arms were PCR amplified from human λ sequence at 5' and 3' positions relative to the hJλ1 gene segment of the human BAC clone 729g4. Homologous recombination with this intermediate targeting vector was performed on a modified 729g4 BAC clone that had been previously targeted upstream (5') of the human vλ3
□□
2 gene segment with a neomycin cassette flanked by Frt sites, which also contained an I-CeuI site 5' to the 5' Frt site. The double-targeted 729g4 BAC clone included from 5' to 3' an I-CeuI site, a 5' Frt site, a neomycin cassette, a 3' Frt site, a ~123 kb fragment containing the first 12 hVλ gene segments, a ~1 kb fragment containing human Jλ1, 2, 3 and 7 gene segments, a PI-SceI site, and a chloramphenicol cassette. This intermediate targeting vector was digested together with I-CeuI and PI-SceI and subsequently ligated into the modified mouse BAC clone (described above) to create the third targeting vector.

[0621] This ligation resulted in a third targeting vector for insertion of human λ sequences into the endogenous κ light chain locus, which included, from 5' to 3', a 5' mouse homology arm containing ~23 kb of genomic sequence 5' of the endogenous mouse κ locus, an I-CeuI site, a 5' Frt site, a neomycin cassette, a 3' Frt site, a ~123 kb fragment containing the first 12 hVλ gene segments, a ~1 kb fragment containing hJλ1, 2, 3 and 7 gene segments, a PI-SceI site and a 3' homology arm containing ~28 kb of mouse genomic sequence including the endogenous the mouse Cκ gene, Eκi and Eκ3' and additional mouse genomic sequence downstream (3') of Eκ3' (FIG. 22B, 12/4-κ Targeting Vector). Homologous recombination with this third targeting vector created a modified mouse κ light chain locus containing 12 hVλ gene segments and four hJλ gene segments operably linked to the endogenous mouse Cκ gene which, upon recombination, leads to the formation of a chimeric human λ/mouse κ light chain.

[0622] A human λ mini-locus with an integrated human κ light chain sequence. In a similar fashion, two additional targeting vectors similar to those engineered to make an initial insertion of human λ gene segments into the endogenous κ light chain locus (FIG. 22B, 12/1-κ and 12/4-κ Targeting Vectors) were engineered to progressively insert human λ light chain gene segments using uniquely constructed targeting vectors containing contiguous human λ and κ genomic sequences. These targeting vectors were constructed to include a ~ 23 kb human κ genomic sequence naturally located between human Vκ4-1 and Jκ1 gene segments. This human κ genomic sequence was specifically positioned in these two additional targeting vectors between human Vλ and human Jλ gene segments (FIG. 22B, 12(κ)1-κ and 12(κ)4-κ Targeting Vectors).

[0623] Both targeting vectors containing the human κ genomic sequence were made using the modified RP11-729g4 BAC clone described above (FIG. 24). This modified BAC clone was targeted with a spectinomycin selection cassette flanked by NotI and AsiSI restriction sites (FIG. 24, top left). Homologous recombination with the spectinomycin cassette resulted in a double-targeted 729g4 BAC clone which included, from 5' to 3', an I-CeuI site, a 5' Frt site, a neomycin cassette, a 3' Frt site, a ~123 kb fragment containing the first 12 hVλ gene segments, a NotI site about 200 bp downstream (3') to the nonamer sequence of the hVλ3-1 gene segment, a spectinomycin cassette and an AsiSI site. A separate human BAC clone containing human κ sequence (CTD-2366j12) was targeted two independent times to engineer restriction sites at locations between hVκ4-1 and hJκ1 gene segments to allow for subsequent cloning of a ~23 kb fragment for ligation with the hVλ gene segments contained in the double targeted modified 729g4 BAC clone (FIG. 24, top right).

[0624] Briefly, the 2366j12 BAC clone is about 132 kb in size and contains hVκ gene segments 1-6, 1-5, 2-4, 7-3, 5-2, 4-1, human κ genomic sequence downstream of the Vκ gene segments, hJκ gene segments 1-5, the hCκ and about 20 kb of additional genomic sequence of the human κ locus. This clone was first targeted with a targeting vector containing a hygromycin cassette flanked by Frt sites and a NotI site downstream (3') of the 3' Frt site. The homology arms for this targeting vector contained human genomic sequence 5' and 3' of the Vκ gene segments within the BAC clone such that upon homologous recombination with this targeting vector, the Vκ gene segments were deleted and a NotI site was engineered ~133 bp downstream of the hVκ4-1 gene segment (FIG. 24, top right). This modified 2366j12 BAC clone was targeted independently with two targeting vectors at the 3' end to delete the hJκ gene segments with a chloramphenicol cassette that also contained either a hJλ1 gene segment, a PI-SceI site and an AsiSI site or a human λ genomic fragment containing four hJλ gene segments (*supra*), a PI-SceI site and an AsiSI site (FIG. 24, top right). The homology arms for these two similar targeting vectors contained sequence 5' and 3' of the hJκ gene segments. Homologous recombination with these second targeting vectors and the modified 2366j12 BAC clone yielded a double-targeted 2366j12 clone which included, from 5' to 3', a 5' Frt site, a hygromycin cassette, a 3' Frt site, a NotI site, a 22,800 bp genomic fragment of the human κ locus containing the intergenic region between the Vκ4-1 and Jκ1 gene segments, either a hJλ1 gene segment or a human λ genomic fragment containing hJλ1, Jλ2, Jλ3 and Jλ7, a PI-SceI site and a chloramphenicol cassette (FIG. 24, top right). Two final targeting

vectors to make the two additional modifications were achieved by two ligation steps using the double-targeted 729g4 and 2366j12 clones.

[0625] Double targeted 729g4 and 2366j12 clones were digested with NotI and AsiSI yielding one fragment containing the neomycin cassette and hVλ gene segments and another fragment containing the ~23 kb genomic fragment of the human κ locus containing the intergenic region between the Vκ4-1 and Jκ1 gene segments, either a hJλ1 gene segment or a genomic fragment containing hJλ1, Jλ2, Jλ3 and Jλ7 gene segments, the PI-SceI site and the chloramphenicol cassette, respectively. Ligation of these fragments generated two unique BAC clones containing from 5' to 3' the hVλ gene segments, the human κ genomic sequence between the Vκ4-1 and Jκ1 gene segments, either a hJλ1 gene segment or a genomic fragment containing hJλ1, Jλ2, Jλ3 and Jλ7 gene segments, a PI-SceI site and a chloramphenicol cassette (FIG. 24, bottom). These new BAC clones were then digested with I-CeuI and PI-SceI to release the unique fragments containing the upstream neomycin cassette and the contiguous human λ and κ sequences and ligated into a modified mouse BAC clone 302g12 which contained from 5' to 3' mouse genomic sequence 5' of the endogenous κ locus, an I-CeuI site, a 5' Frt site, a neomycin cassette, a 3' Frt site, hVλ gene segments (3-12 to 3-1), a NotI site ~200 bp downstream of Vλ3-1, ~23 kb of human κ sequence naturally found between the human Vκ4-1 and Jκ1 gene segments, either a hJλ1 gene segment or a genomic fragment containing hJλ1, Jλ2, Jλ3 and Jλ7 gene segments, the mouse Eκi, the mouse Cκ gene and Eκ3' (FIG. 22, 12hVλ-VκJκ-hJλ1 and 12hVλ-VκJκ-4hJλ Targeting Vectors). Homologous recombination with both of these targeting vectors created two separate modified mouse κ light chain loci containing 12 hVλ gene segments, human κ genomic sequence, and either one or four hJλ gene segments operably linked to the endogenous mouse Cκ gene which, upon recombination, leads to the formation of a chimeric human λ/mouse κ light chain.

Example 12. Engineering Additional Human Yλ Genes Segments Into a Human λ Light Chain Mini-Locus

[0626] Additional hVλ gene segments were added independently to each of the initial modifications described in Example 11 using similar targeting vectors and methods (FIG. 23A, +16-λ Targeting Vector and FIG. 23B, +16-κ Targeting Vector).

[0627] Introduction of 16 additional human Yλ gene segments. Upstream (5') homology arms used in constructing targeting vectors for adding 16 additional hVλ gene segments to the modified light chain loci described in Example 11 contained mouse genomic sequence 5' of either the endogenous κ or λ light chain loci. The 3' homology arms were the same for all targeting vectors and contained human genomic sequence overlapping with the 5' end of the human λ sequence of the modifications as described in Example 11.

[0628] Briefly, two targeting vectors were engineered for introduction of 16 additional hVλ gene segments to the modified mouse light chain loci described in Example 11 (FIG. 23A and 5B, +16-λ or +16-κ Targeting Vector). A ~172 kb DNA fragment from human BAC clone RP11-

761113 (Invitrogen) containing 21 consecutive hVλ gene segments from cluster A was engineered with a 5' homology arm containing mouse genomic sequence 5' to either the endogenous κ or λ light chain loci and a 3' homology arm containing human genomic λ sequence. The 5' mouse κ or λ homology arms used in these targeting constructs were the same 5' homology arms described in Example 11 (FIG. 23A and 23B). The 3' homology arm included a 53,057 bp overlap of human genomic λ sequence corresponding to the equivalent 5' end of the ~123 kb fragment of human genomic λ sequence described in Example 11. These two targeting vectors included, from 5' to 3', a 5' mouse homology arm containing either ~23 kb of genomic sequence 5' of the endogenous mouse κ light chain locus or ~24 kb of mouse genomic sequence 5' of the endogenous λ light chain locus, a 5' Frt site, a hygromycin cassette, a 3' Frt site and 171,457 bp of human genomic λ sequence containing 21 consecutive hVλ gene segments, ~53 kb of which overlaps with the 5' end of the human λ sequence described in Example 12 and serves as the 3' homology arm for this targeting construct (FIG. 23A and 23B, +16-λ or +16-κ Targeting Vectors). Homologous recombination with these targeting vectors created independently modified mouse κ and λ light chain loci each containing 28 hVλ gene segments and a hJλ1 gene segment operably linked to endogenous mouse constant genes (Cκ or Cλ2) which, upon recombination, leads to the formation of a chimeric light chain.

[0629] In a similar fashion, the +16-κ Targeting Vector was also used to introduce the 16 additional hVλ gene segments to the other initial modifications described in Example 11 that incorporated multiple hJλ gene segments with and without an integrated human κ sequence (FIG. 22B). Homologous recombination with this targeting vector at the endogenous mouse κ locus containing the other initial modifications created mouse κ light chain loci containing 28 hVλ gene segments and hJλ1, 2, 3 and 7 gene segments with and without a human Vκ-Jκ genomic sequence operably linked to the endogenous mouse Cκ gene which, upon recombination, leads to the formation of a chimeric λ-κ light chain.

[0630] Introduction of 12 additional human Vλ gene segments. Additional hVλ gene segments were added independently to each of the modifications described above using similar targeting vectors and methods. The final locus structure resulting from homologous recombination with targeting vectors containing additional hVλ gene segments are shown in FIG. 25A and 25B.

[0631] Briefly, a targeting vector was engineered for introduction of 12 additional hVλ gene segments to the modified mouse κ and λ light chain loci described above (FIG. 23A and 23B, +12-λ or 12-κ Targeting Vectors). A 93,674 bp DNA fragment from human BAC clone RP11-22118 (Invitrogen) containing 12 consecutive hVλ gene segments from cluster B was engineered with a 5' homology arm containing mouse genomic sequence 5' to either the endogenous mouse κ or λ light chain loci and a 3' homology arm containing human genomic λ sequence. The 5' homology arms used in this targeting construct were the same 5' homology arms used for the addition of 16 hVλ gene segments described above (FIG. 23A and 23B). The 3' homology arm was made by engineering a PI-SceI site ~3431 bp 5' to the human Vλ3-29P gene segment contained in a 27,468 bp genomic fragment of human λ sequence from

BAC clone RP11-761113. This PI-SceI site served as a ligation point to join the -94 kb fragment of additional human λ sequence to the ~27 kb fragment of human λ sequence that overlaps with the 5' end of the human λ sequence in the previous modification using the +16-λ or +16-κ Targeting Vectors (FIG. 23A and 23B). These two targeting vectors included, from 5' to 3', a 5' homology arm containing either ~23 kb of mouse genomic sequence 5' of the endogenous κ light chain locus or ~24 kb of mouse genomic sequence 5' of the endogenous λ light chain locus, a 5' Frt site, a neomycin cassette, a 3' Frt site and 121,188 bp of human genomic λ sequence containing 16 hVλ gene segments and a PI-SceI site, ~27 kb of which overlaps with the 5' end of the human λ sequence from the insertion of 16 addition hVλ gene segments and serves as the 3' homology arm for this targeting construct (FIG. 23A and 23B, +12-λ or 12-κ Targeting Vectors). Homologous recombination with these targeting vectors independently created modified mouse κ and λ light chain loci containing 40 hVλ gene segments and human J┗1 operably linked to the endogenous mouse constant genes (Cκ or Cλ2) which, upon recombination, leads to the formation of a chimeric light chain (bottom of FIG. 23A and 23B).

[0632] In a similar fashion, the +12-κ Targeting Vector was also used to introduce the 12 additional hVλ gene segments to the other initial modifications that incorporated multiple hJλ gene segments with and without an integrated human κ sequence (FIG. 22B). Homologous recombination with this targeting vector at the endogenous mouse κ locus containing the other modifications created a mouse κ light chain locus containing 40 hVλ gene segments and hJλ1, 2, 3 and 7 gene segments with and without a human Vκ-Jκ genomic sequence operably linked to the endogenous mouse Cκ gene which, upon recombination, leads to the formation of a chimeric λ-κ light chain.

Example 13. Identification of targeted ES cells Bearing Human λ Light Chain Gene Segments

[0633] Targeted BAC DNA made according to the foregoing Examples was used to electroporate mouse ES cells to create modified ES cells for generating chimeric mice that express human λ light chain gene segments. ES cells containing an insertion of unrearranged human λ light chain gene segments were identified by a quantitative TAQMAN® assay. Specific primers sets and probes were design for insertion of human λ sequences and associated selection cassettes (gain of allele, GOA), loss of endogenous mouse sequences and any selection cassettes (loss of allele, LOA) and retention of flanking mouse sequences (allele retention, AR). For each additional insertion of human λ sequences, additional primer sets and probes were used to confirm the presence of the additional human λ sequences as well as the previous primer sets and probes used to confirm retention of the previously targeted human sequences. Table 10 sets forth the primers and associated probes used in the quantitative PCR assays. Table 11 sets forth the combinations used for confirming the insertion of each section of human λ light chain gene segments in ES cell clones.

[0634] ES cells bearing the human λ light chain gene segments are optionally transfected with a construct that expresses FLP in order to remove the Frt'ed neomycin cassette introduced by

the insertion of the targeting construct containing human Vλ5-52 - Vλ1-40 gene segments (FIG. 23A and 23B). The neomycin cassette may optionally be removed by breeding to mice that express FLP recombinase (*e.g.,* US 6,774,279). Optionally, the neomycin cassette is retained in the mice.

TABLE 10

Primer	SEQ ID NO:	Probe	SEQ ID NO:
hL2F	60	hL2P	82
hL2R	61		
hL3F	62	hL3P	83
hL3R	63		
NeoF	64	NeoP	84
NeoR	65		
61hJ1F	66	61hJ1P	85
61hJ1R	67		
67hT1F	68	67hT1P	86
67hT1R	69		
67hT3F	70	67hT3P	87
67hT3R	71		
HygF	72	HygP	88
HygR	73		
MKD2F	74	MKD2P	89
MKD2R	75		
MKP8F	76	MKP8P	90
MKP8R	77		
MKP15F	78	MKP15P	91
MKP15R	79		
MK20F	80	-	-
MKP4R	81		
68h2F	92	68h2P	96
68h2R	93		
68h5F	94	68h5P	97
68h5R	95		
mL1F	133	mL1 P	141
mL1R	134		
mL2F	135	mL2P	142
mL2R	136		

Primer	SEQ ID NO:	Probe	SEQ ID NO:
mL11F	137	mL11P	143
mL11R	138		
mL12F	139	mL12P	144
mL12R	140		

TABLE 11

Modification	Assay	Forward/Reverse Primer Set	Probe	Sequence Location
Insertion of 12 hVλ & hJλ1	GOA	hL2F/hL2R	hL2P	hVλ3-12 - hVλ3-1
		hL3F/hL3R	hL3P	
		61hJ1F/61hJ1R	61hJ1P	hJλ sequence
		NeoF/NeoR	NeoP	Neomycin cassette
	LOA	MK20F/MKP4R	-	*lox*511/*lox*P sequence of inactivated κ locus
		HygF/HygR	HygP	Hygromycin cassette from inactivated λ locus
		mL1 F/mL1R	mL1P	Mouse Vλ1-Cλ1 Cluster
		mL2F/mL2R	mL2P	
		mL11F/mL11R	mL11P	Mouse Vλ2-Cλ2 Cluster
		mL12F/mL12R	mL12P	
	AR/LOA	MKD2F/MKD2R	MKD2P	Mouse sequence in 5' Vκ locus
		MKP15F/MKP15R	MKP15P	Mouse sequence in 3' Vκ locus
Insertion of 16 hVλ	GOA	67hT1 F/67hT1R	67hT1 P	hVλ3-27 - hVλ3-12
		67hT3F/67hT3R	67hT3P	
		HygF/HygR	HygP	Hygromycin cassette
	LOA	NeoF/NeoR	NeoP	Neomycin cassette
		mL1 F/mL1R	mL1 P	Mouse Vλ1-Cλ1 Cluster
		mL2F/mL2R	mL2P	
		mL11F/mL11R	mL11P	Mouse Vλ2-Cλ2 Cluster
		mL12F/mL12R	mL12P	
	AR	hL2F/hL2R	hL2P	hVλ3-12 - hVλ3-1
		hL3F/hL3R	hL3P	
	AR/LOA	MKD2F/MKD2R	MKD2P	Mouse sequence in 5' Vκ locus
		MKP15F/MKP15R	MKP15P	Mouse sequence in 3' Vκ locus

Modification	Assay	Forward/Reverse Primer Set	Probe	Sequence Location
Insertion of 12 hVλ	GOA	68h2F/68h2R	68h2P	hVλ5-52 - hVλ1-40
		68h5F/68h5R	68h5P	
		NeoF/NeoR	NeoP	Neomycin cassette
	LOA	HygF/HygR	HygP	Hygromycin cassette
		mL1 F/mL1R	mL1 P	Mouse Vλ1-Cλ1 Cluster
		mL2F/mL2R	mL2P	
		mL11F/mL1R	mL11P	Mouse Vλ2-Cλ2
		mL12F/mL12R	mL12P	Cluster
	AR	hL2F/hL2R	hL2P	hVλ3-12 - hVλ3-1
		hL3F/hL3R	hL3P	
		67hT1 F/67hT1R	67hT1P	hVλ3-27 - hVλ3-12
		67hT3F/67hT3R	67hT3P	
	AR/LOA	MKD2F/MKD2R	MKD2P	Mouse sequence in 5' Vκ locus
		MKP15F/MKP15R	MKP15P	Mouse sequence in 3' Vκ locus

Example 14. Generation of Mice Expressing Human λ Light Chain From an Endogenous Light Chain Locus

[0635] Targeted ES cells described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, *e.g.,* US Pat. No. 7,294,754 and Poueymirou et al. (2007) F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99. VELOCIMICE® (F0 mice fully derived from the donor ES cell) independently bearing human λ gene segments were identified by genotyping using a modification of allele assay (Valenzuela *et al., supra*) that detected the presence of the unique human λ gene segments (*supra*).

[0636] κ:λ light chain usage of mice bearing human λ light chain gene segments. Mice homozygous for each of three successive insertions of hVλ gene segments with a single hJλ gene segment (FIG. 23B) and mice homozygous for a first insertion of hVλ gene segments with either a single hJλ gene segment or four human Jλ gene segments including a human Vκ-Jκ genomic sequence (FIG. 22B) were analyzed for κ and λ light chain expression in splenocytes using flow cytometry.

[0637] Briefly, spleens were harvested from groups of mice (ranging from three to seven

animals per group) and grinded using glass slides. Following lysis of red blood cells (RBCs) with ACK lysis buffer (Lonza Walkersville), splenocytes were stained with fluorescent dye conjugated antibodies specific for mouse CD19 (Clone 1D3; BD Biosciences), mouse CD3 (17A2; Biolegend), mouse Igκ (187.1; BD Biosciences) and mouse Igλ (RML-42; Biolegend). Data was acquired using a BD™ LSR II flow cytometer (BD Biosciences) and analyzed using FLOWJO™ software (Tree Star, Inc.). Table 12 sets forth the average percent values for B cells ($CD19^+$), κ light chain ($CD19^+Ig\kappa^+Ig\lambda^-$), and λ light chain ($CD19^+Ig\kappa^-Ig\lambda^+$) expression observed in splenocytes from groups of animals bearing each genetic modification.

[0638] In a similar experiment, B cell contents of the splenic compartment from mice homozygous for a first insertion of 12 hVλ and four hJλ gene segments including a human Vκ-Jκ genomic sequence operably linked to the mouse Cκ gene (bottom of FIG. 22B) and mice homozygous for 40 hVλ and one hJλ gene segment (bottom of FIG. 23B or top of FIG. 25B) were analyzed for Igκ and Igλ expression using flow cytometry (as described above). FIG. 26A shows the Igλ and Igκ expression in $CD19^+$ B cells for a representative mouse from each group. The number of $CD19^+$ B cells per spleen was also recorded for each mouse (FIG. 26B).

[0639] In another experiment, B cell contents of the spleen and bone marrow compartments from mice homozygous for 40 hVλ, and four hJλ, gene segments including a human Vκ-Jκ genomic sequence operably linked to the mouse Cκ gene (bottom of FIG. 26B) were analyzed for progression through B cell development using flow cytometry of various cell surface markers.

[0640] Briefly, two groups (N=3 each, 9-12 weeks old, male and female) of wild type and mice homozygous for 40 hVλ and four hJλ gene segments including a human Vκ-Jκ genomic sequence operably linked to the mouse Cκ gene were sacrificed and spleens and bone marrow were harvested. Bone marrow was collected from femurs by flushing with complete RPMI medium (RPMI medium supplemented with fetal calf serum, sodium pyruvate, Hepes, 2-mercaptoethanol, non-essential amino acids, and gentamycin). RBCs from spleen and bone marrow preparations were lysed with ACK lysis buffer (Lonza Walkersville), followed by washing with complete RPMI medium. $1x10^6$ cells were incubated with anti-mouse CD16/CD32 (2.4G2, BD Biosciences) on ice for 10 minutes, followed by labeling with a selected antibody panel for 30 min on ice.

[0641] Bone marrow panel: anti-mouse FITC-CD43 (1B11, BioLegend), PE-ckit (2B8, BioLegend), PeCy7-IgM (II/41, eBioscience), PerCP-Cy5.5-IgD (11-26c.2a, BioLegend), APC-B220 (RA3-6B2, eBioscience), APC-H7-CD19 (ID3, BD) and Pacific Blue-CD3 (17A2, BioLegend).

[0642] Bone marrow and spleen panel: anti-mouse FITC-Igκ (187.1, BD), PE-Igλ, (RML-42, BioLegend), PeCy7-IgM (II/41, ebioscience), PerCP-Cy5.5-IgD (11-26c.2a, BioLegend), Pacific Blue-CD3 (17A2, BioLegend), APC- B220 (RA3-6B2, eBioscience), APC-H7-CD19 (ID3, BD).

[0643] Following staining, cells were washed and fixed in 2% formaldehyde. Data acquisition was performed on a FACSCANTOII™ flow cytometer (BD Biosciences) and analyzed with FLOWJO™ software (Tree Star, Inc.). FIGs. 27A - 27D show the results for the splenic compartment of one representative mouse from each group. FIGs. 28A - 28E show the results for the bone marrow compartment of one representative mouse from each group. Table 13 sets forth the average percent values for B cells ($CD19^+$), κ light chain ($CD19^+Ig\kappa^+Ig\lambda^-$), and λ light chain ($CD19^+Ig\kappa^-Ig\lambda^+$) expression observed in splenocytes from groups of animals bearing various genetic modifications. Table 14 sets forth the average percent values for B cells ($CD19^+$), mature B cells ($B220^{hi}IgM^+$), immature B cells ($B220^{int}IgM^+$), immature B cells expressing κ light chain ($B220^{int}IgM^+Ig\kappa^+$) and immature B cells expressing λ light chain ($B220^{int}IgM^+Ig\lambda^+$) observed in bone marrow of wild type and mice homozygous for 40 hVλ and four hJλ, gene segments including a human Vκ-Jκ genomic sequence operably linked to the mouse Cκ gene. This experiment was repeated with additional groups of the mice described above and demonstrated similar results (data not shown).

TABLE 12

Genotype	B cells	$Ig\kappa^+$	$Ig\lambda^+$
Wild Type	46.2	91.0	3.6
12 hVλ+hJλ1	28.3	10.4	62.5
12 hVλ-VκJκ-hJλ1	12.0	11.0	67.5
12 hVλ-VκJκ-4hJλ	41.8	17.2	68.4
28 hVλ+hJλ1	22.0	13.3	51.1
40 hVλ+hJλ1	28.2	24.3	53.0

TABLE 13

Genotype	B cells	$Ig\kappa^+$	$Ig\lambda^+$
Wild Type	49.8	91.2	3.5
40 hVλ-VκJκ-4hJλ	33.3	41.6	43.1

TABLE 14

Genotype	B cells	Mature B cells	Immature B cells	Immature $Ig\kappa^+$ B cells	Immature $Ig\lambda^+$ B cells
Wild Type	62.2	9.2	12.0	79.0	8.84
40hVλ-VκJκ-4hJλ	60.43	2.59	7.69	38.29	43.29

[0644] Human Vλ gene usage in mice bearing human λ light chain gene segments. Mice heterozygous for a first insertion of human λ sequences (hVλ3-12 - hVλ3-1 and hJλ1, FIG. 23B) and homozygous for a third insertion of human λ sequences (hVλ5-52 - hVλ3-1 and hJλ1, FIG. 23B) were analyzed for human λ light chain gene usage by reverse-transcriptase polymerase chain reaction (RT-PCR) using RNA isolated from splenocytes.

[0645] Briefly, spleens were harvested and perfused with 10 mL RPMI-1640 (Sigma) with 5% HI-FBS in sterile disposable bags. Each bag containing a single spleen was then placed into a STOMACHER™ (Seward) and homogenized at a medium setting for 30 seconds. Homogenized spleens were filtered using a 0.7µm cell strainer and then pelleted with a centrifuge (1000 rpm for 10 minutes) and RBCs were lysed in BD PHARM LYSE™ (BD Biosciences) for three minutes. Splenocytes were diluted with RPMI-1640 and centrifuged again, followed by resuspension in 1 mL of PBS (Irvine Scientific). RNA was isolated from pelleted splenocytes using standard techniques known in the art.

[0646] RT-PCR was performed on splenocyte RNA using primers specific for human hVλ gene segments and the mouse Cκ gene (Table 15). PCR products were gel-purified and cloned into pCR2.1-TOPO TA vector (Invitrogen) and sequenced with primers M13 Forward (GTAAAACGAC GGCCAG; SEQ ID NO:113) and M13 Reverse (CAGGAAACAG CTATGAC; SEQ ID NO:114) located within the vector at locations flanking the cloning site. Eighty-four total clones derived from the first and third insertions of human λ sequences were sequenced to determine hVλ gene usage (Table 16). The nucleotide sequence of the hVλ-hJλ1-mCκ junction for selected RT-PCR clones is shown in FIG. 29.

[0647] In a similar fashion, mice homozygous for a third insertion of human λ light chain gene sequences (*i.e.* 40 hVλ gene segments and four hJλ gene segments including a human Vκ-Jκ genomic sequence, bottom of FIG. 25B) operably linked to the endogenous mouse Cκ gene were analyzed for human λ light chain gene usage by RT-PCR using RNA isolated from splenocytes (as described above). The human λ light chain gene segment usage for 26 selected RT-PCR clones are shown in Table 17. The nucleotide sequence of the hVλ-hJλ-mCκ junction for selected RT-PCR clones is shown in FIG. 30.

[0648] In a similar fashion, mice homozygous for a first insertion of human λ light chain gene segments (12 hVλ gene segments and hJλ1, FIG. 22A & FIG. 23A) operably linked to the endogenous mouse Cλ2 gene were analyzed for human λ light chain gene usage by RT-PCR using RNA isolated from splenocytes (as described above). The primers specific for hVλ gene segments (Table 15) were paired with one of two primers specific for the mouse Cλ2 gene; Cλ2-1 (SEQ ID NO:162) or Cλ2-2 (SEQ ID NO:163).

[0649] Multiple hVλ gene segments rearranged to hλ1 were observed from the RT-PCR clones from mice bearing human λ light chain gene segments at the endogenous mouse λ light chain locus. The nucleotide sequence of the hVλ-hJλ-mCλ2 junction for selected RT-PCR clones is shown in FIG. 31.

TABLE 15

5' hVλ Primer	Sequence (5'-3')	SEQ ID NO:
VLL-1	CCTCTCCTCC TCACCCTCCT	98
VLL-1n	ATGRCCDGST YYYCTCTCCT	99
VLL-2	CTCCTCACTC AGGGCACA	100

5' hVλ Primer	Sequence (5'-3')	SEQ ID NO:
VLL-2n	ATGGCCTGGG CTCTGCTSCT	101
VLL-3	ATGGCCTGGA YCSCTCTCC	102
VLL-4	TCACCATGGC YTGGRYCYCM YTC	103
VLL-4.3	TCACCATGGC CTGGGTCTCC TT	104
VLL-5	TCACCATGGC CTGGAMTCYT CT	105
VLL-6	TCACCATGGC CTGGGCTCCA CTACTT	106
VLL-7	TCACCATGGC CTGGACTCCT	107
VLL-8	TCACCATGGC CTGGATGATG CTT	108
VLL-9	TAAATATGGC CTGGGCTCCT CT	109
VLL-10	TCACCATGCC CTGGGCTCTG CT	110
VLL-11	TCACCATGGC CCTGACTCCT CT	111
3' Mouse Cκ Primer	Sequence (5'-3')	SEQ ID NO:
mIgKC3'-1	CCCAAGCTTA CTGGATGGTG GGAAGATGGA	112

TABLE 16

hVλ	Observed No. of Clones
3-1	2
4-3	3
2-8	7
3-9	4
3-10	3
2-14	1
3-19	1
2-23	7
3-25	1
1-40	9
7-43	2
1-44	2
5-45	8
7-46	3
9-49	6
1-51	3

TABLE 17

Clone	hVλ	hJλ
1-3	1-44	7

Clone	hVλ	hJλ
1-5	1-51	3
2-3	9-49	7
2-5	1-40	1
2-6	1-40	7
3b-5	3-1	7
4a-1	4-3	7
4a-5	4-3	7
4b-1	1-47	3
5-1	3-10	3
5-2	1-40	7
5-3	1-40	7
5-4	7-46	2
5-6	1-40	7
5-7	7-43	3
6-1	1-40	1
6-2	1-40	2
6-7	1-40	3
7a-1	3-10	7
7a-2	9-49	2
7a-7	3-10	7
7b-2	7-43	3
7b-7	7-46	7
7b-8	7-43	3
11a-1	5-45	2
11a-2	5-45	7

[0650] FIG. 29 shows the sequence of the hVλ-hJλ1-mCκ junction for RT-PCR clones from mice bearing a first and third insertion of hVλ gene segments with a single hJλ gene segment. The sequences shown in FIG. 29 illustrate unique rearrangements involving different hVλ gene segments with hJλ1 recombined to the mouse Cκ gene. Heterozygous mice bearing a single modified endogenous κ locus containing 12 hVλ gene segments and hJλ1 and homozygous mice bearing two modified endogenous κ loci containing 40 hVλ gene segments and hJλ1 were both able to produce human λ gene segments operably linked to the mouse Cκ gene and produce B cells that expressed human λ light chains. These rearrangements demonstrate that the chimeric loci were able to independently rearrange human λ gene segments in multiple, independent B cells in these mice. Further, these modifications to the endogenous κ light chain

locus did not render any of the hVλ gene segments inoperable or prevent the chimeric locus from recombining multiple hVλ and a hJλ (Jλ1) gene segment during B cell development as evidenced by 16 different hVλ gene segments that were observed to rearrange with hJλ1 (Table 16). Further, these mice made functional antibodies containing rearranged human Vλ-Jλ gene segments operably linked to mouse Cκ genes as part of the endogenous immunoglobulin light chain repertoire.

[0651] FIG. 30 shows the sequence of the hVλ-hJλ-mCκ junction for selected RT-PCR clones from mice homozygous for 40 hVλ and four hJλ gene segments including a human Vκ-Jκ genomic sequence. The sequences shown in FIG. 30 illustrate additional unique rearrangements involving multiple different hVλ gene segments, spanning the entire chimeric locus, with multiple different hJλ gene segments rearranged and operably linked to the mouse Cκ gene. Homozygous mice bearing modified endogenous κ loci containing 40 hVλ and four hJλ gene segments were also able to produce human λ gene segments operably linked to the mouse Cκ gene and produce B cells that expressed human λ light chains. These rearrangements further demonstrate that the all stages of chimeric loci were able to independently rearrange human λ gene segments in multiple, independent B cells in these mice. Further, these additional modifications to the endogenous κ light chain locus demonstrates that each insertion of human λ gene segments did not render any of the hVλ and/or Jλ gene segments inoperable or prevent the chimeric locus from recombining the hVλ and Jλ gene segments during B cell development as evidenced by 12 different hVλ gene segments that were observed to rearrange with all four hJλ gene segments (Table 17) from the 26 selected RT-PCR clone. Further, these mice as well made functional antibodies containing human Vλ-Jλ gene segments operably linked to mouse Cκ regions as part of the endogenous immunoglobulin light chain repertoire.

[0652] FIG. 31 shows the sequence of the hVλ-hJλ-mCλ2 junction for three individual RT-PCR clones from mice homozygous for 12 hVλ gene segments and hJλ1. The sequences shown in FIG. 31 illustrate additional unique rearrangements involving different hVλ gene segments, spanning the length of the first insertion, with hJλ1 rearranged and operably linked to the mouse Cλ2 gene (2D1 = Vλ2-8Jλ1; 2D9 = Vλ3-10Jλ1; 3E15 = Vλ3-1Jλ1). One clone demonstrated a nonproductive rearrangement due to N additions at the hVλ-hJλ junction (2D1, FIG. 31). This is not uncommon in V(D)J recombination, as the joining of gene segments during recombination has been shown to be imprecise. Although this clone represents an unproductive recombinant present in the light chain repertoire of these mice, this demonstrates that the genetic mechanism that contributes to junctional diversity among antibody genes is operating normally in these mice and leading to an antibody repertoire containing light chains with greater diversity.

[0653] Homozygous mice bearing modified endogenous λ loci containing 12 hVλ gene segments and hJλ1 were also able to produce human λ gene segments operably linked to an endogenous mouse Cλ gene and produce B cells that expressed reverse chimeric λ light chains containing hVλ regions linked to mouse Cλ regions. These rearrangements further demonstrate that human λ light chain gene segments placed at the other light chain locus (*i.e.,*

the λ locus) were able to independently rearrange human λ gene segments in multiple, independent B cells in these mice. Further, the modifications to the endogenous λ light chain locus demonstrate that the insertion of human λ gene segments did not render any of the hVλ and/or hJλ1 gene segments inoperable or prevent the chimeric locus from recombining the hVλ and hJλ1 gene segments during B cell development. Further, these mice also made functional antibodies containing human Vλ-Jλ gene segments operably linked to a mouse Cλ region as part of the endogenous immunoglobulin light chain repertoire.

[0654] As shown in this Example, mice bearing human λ light chain gene segments at the endogenous κ and λ light chain loci are capable of rearranging human λ light chain gene segments and expressing them in the context of a mouse Cκ and/or Cλ region as part of the normal antibody repertoire of the mouse because a functional light chain is required at various checkpoints in B cell development in both the spleen and bone marrow. Further, early subsets of B cells (*e.g.,* pre-, pro- and transitional B cells) demonstrate a normal phenotype in these mice as compared to wild type littermates (FIGs. 27D, 28A and 28B). A small deficit in bone marrow and peripheral B cell populations was observed, which may be attributed to a deletion of a subset of auto-reactive immature B cells and/or a suboptimal association of human λ light chain with mouse heavy chain. However, the Igκ/Igλ usage observed in these mice demonstrates a situation that is more like human light chain expression than that observed in mice.

Example 15. Breeding of Mice Expressing Human λ Light Chains From an Endogenous Light Chain Locus

[0655] To optimize the usage of the human λ gene segments at an endogenous mouse light chain locus, mice bearing the unrearranged human λ gene segments are bred to another mouse containing a deletion in the opposing endogenous light chain locus (either κ or λ). For example, human λ gene segments positioned at the endogenous κ locus would be the only functional light chain gene segments present in a mouse that also carried a deletion in the endogenous λ light chain locus. In this manner, the progeny obtained would express only human λ light chains as described in the foregoing examples. Breeding is performed by standard techniques recognized in the art and, alternatively, by commercial companies, *e.g.,* The Jackson Laboratory. Mouse strains bearing human λ light chain gene segments at the endogenous κ locus and a deletion of the endogenous λ light chain locus are screened for presence of the unique reverse-chimeric (human-mouse) λ light chains and absence of endogenous mouse λ light chains.

[0656] Mice bearing an unrearranged human λ light chain locus are also bred with mice that contain a replacement of the endogenous mouse heavy chain variable gene locus with the human heavy chain variable gene locus (see US 6,596,541, Regeneron Pharmaceuticals, the VELOCIMMUNE® genetically engineered mouse). The VELOCIMMUNE® mouse includes, in part, having a genome comprising human heavy chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces antibodies comprising

a human heavy chain variable region and a mouse heavy chain constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy chains of the antibodies can be isolated and operably linked to DNA encoding the human heavy chain constant regions. The DNA can then be expressed in a cell capable of expressing the fully human heavy chain of the antibody. Upon a suitable breeding schedule, mice bearing a replacement of the endogenous mouse heavy chain locus with the human heavy chain locus and an unrearranged human λ light chain locus at the endogenous κ light chain locus is obtained. Antibodies containing somatically mutated human heavy chain variable regions and human λ light chain variable regions can be isolated upon immunization with an antigen of interest.

Example 16. Generation of Antibodies From Mice Expressing Human Heavy Chains and Human λ Light Chains

[0657] After breeding mice that contain the unrearranged human λ light chain locus to various desired strains containing modifications and deletions of other endogenous Ig loci (as described above), selected mice are immunized with an antigen of interest.

[0658] Generally, a VELOCIMMUNE® mouse containing one of the single rearranged human germline light chain regions is challenged with an antigen, and lymphatic cells (such as B-cells) are recovered from serum of the animals. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies containing human heavy chain and human λ light chain that are specific to the antigen used for immunization. DNA encoding the variable regions of the heavy chains and the λ light chains may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Due to the presence of the additional hVλ gene segments as compared to the endogenous mouse λ locus, the diversity of the light chain repertoire is dramatically increased and confers higher diversity on the antigen-specific repertoire upon immunization. The resulting cloned antibody sequences may be subsequently produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes (*e.g.,* B cells).

[0659] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As described above, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, *etc.* The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody containing a somatically mutated human heavy chain and a human λ light chain derived from an unrearranged human λ light chain locus of the invention. Suitable human constant regions include, for example wild type or modified IgG1, IgG2, IgG3, or IgG4.

Example 17. Breeding of ADAM6 Mice and Human λ Variable Mice

[0660] Any of the mice described herein that comprises a modification of an endogenous ADAM6 gene or ortholog or homolog thereof, and further comprises a gene that confers ADAM6 function on the mouse, is bred with a mouse comprising a modification that comprises a human λ variable segment (*e.g.,* a V and a J segment) operably linked to a human or mouse λ or κ constant gene. The mouse comprising the human λ variable segment can have the variable segment present at a modified endogenous λ or κ locus, or on a transgene. The mice are bred and the progeny are further interbred, if needed, and progeny are screened for fertile mice that exhibit the ADAM6 function and that also express the human λ sequence in the context of a human or mouse λ or κ constant region, as the case may be.

[0661] A mouse comprising a humanized heavy chain variable locus (human V, D, and J segments replacing all or substantially all mouse V, D, and J segments) that further comprises an ectopic ADAM6 sequence (or a sequence of an ortholog or homolog of ADAM6 that confers ADAM6 function on the mouse) is bred with a mouse that comprises a replacement of all or substantially all light chain V and J segments with human λ light chain V and J segment at the mouse λ locus and/or the mouse κ locus. Progeny are further bred as needed, and mice that express an antibody comprising a human V_H fused with a heavy chain constant sequence, and a cognate human λ V_L fused with a λ or a κ light chain constant sequence are identified.

[0662] The mice are exposed to an antigen of interest and allowed to generate an immune response. Antibodies specific to the antigen of interest are identified, and human V_H sequences and human λ variable sequences (including human λ variable sequences linked to mouse κ constant regions) are identified and employed to make a human antibody by engineering the variable domain sequences in combination with human constant region genes.

[0663] In one instance, a mouse is created by breeding that comprises a replacement of all or substantially all mouse heavy chain V, D, and J segments with human V, D, and J segments at the endogenous mouse heavy chain locus, and that comprises a light chain allele that comprises a replacement of all or substantially all λ light chain variable sequences with one or more human λ variable sequences at an endogenous mouse λ locus operably linked to a λ constant sequence, and that comprises a light chain allele that comprises a replacement of all or substantially all κ light chain variable sequences at an endogenous κ locus with one or more human λ variable sequences. The animal is exposed to an antigen of interest and allowed to mount an immune response. Antibodies that bind the antigen of interest are identified that comprise human heavy chain variable domains cognate with human λ variable domains on a mouse λ or mouse κ constant region are identified. Nucleic acid sequences encoding the variable domains are employed to make a fully human antibody by engineering the variable sequences in combination with human constant region sequences.

[0664] Mice as described in this example comprise one or more of the Vκ-Jκ intergenic regions described in the text and the figures herein.

SEQUENCE LISTING

[0665]

<110> Macdonald, Lynn
Stevens, Sean
Gurer, Cagan
Hosiawa, Karolina A.
Andrew J. Murphy

<120> Humanized Light Chain Mice

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Glu Val Val Ile Pro Arg Lys Glu Ile Tyr His Thr Lys Gly Leu Gln
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Ala Gln Arg Leu Leu Ser Tyr Ser Leu Arg Phe Arg Gly Gln Arg His
65                  70                  75                  80
Ile Ile His Leu Arg Arg Lys Thr Leu Ile Trp Pro Arg His Leu Leu
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            100                 105                 110
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            180                 185                 190
Ser Ser Arg Leu Glu Tyr Ala Asp Gly Ala Pro Arg Leu Ser Ser Lys
        195                 200                 205
Asn Tyr Ala Thr His Pro Ala Ala Ile Lys Gly His Phe Gln Ala Thr
    210                 215                 220
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            260                 265                 270

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        275                 280                 285
Phe Tyr Glu Arg Val Phe Tyr Ala His Phe Arg Pro Asp Ala Gly Ala
    290                 295                 300
Ile Ile Asn Lys Asn Ser Pro Gly Asp Asp Ala Val Asn Pro Ala Glu
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            340                 345                 350
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    450                 455                 460
Ala Asn Cys Thr Tyr Ser Pro Ser Gly Thr Leu Cys Arg Pro Ile Gln
465                 470                 475                 480
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Tyr Cys Tyr Lys Gly Asn Cys Thr Asp Arg Asn Ile Gln Cys Met Glu
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Pro Pro Lys Cys Lys Glu Glu Gly His Ser Gly Ser Ile Asp Ser Gly
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705                 710                 715                 720
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Glu Val Val Ile Pro Arg Lys Glu Ile Tyr His Thr Lys Gly Leu Gln
    50                  55                  60
Ala Gln Arg Leu Leu Ser Tyr Ser Leu His Phe Arg Gly Gln Arg His
65                  70                  75                  80
Ile Ile His Leu Arg Arg Lys Thr Leu Ile Trp Pro Arg His Leu Leu
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aggtagcgac tcgagataac ttcgtataat gtatgctata cgaagttatc catggtaagc 180
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<223> s=c or g

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REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US6586251B [0338] [0348] [0533] [0606]
- US6596541B [0338] [0414] [0656]
- US7105348B [0338] [0414]
- US7605237B [0476]
- US6998514B [0483]
- US7435871B [0483]
- US76598442B [0582]
- US7576259B [0582]
- US7294754B [0582] [0635]
- US6774279B [0634]
- WO61578097A [0665]

Non-patent literature cited in the description

- **WATERSTON, R.H. et al.**Initial sequencing and comparative analysis of the mouse genomeNature, 2002, vol. 420, 520-562 [0338]
- **BRIL, W.S. et al.**Tolerance to factor VIII in a transgenic mouse expressing human factor VIII cDNA carrying an Arg(593) to Cys substitutionThromb Haemost, 2006, vol. 95, 341-347 [0338]
- **HOMANICS, G.E. et al.**Production and characterization of murine models of classic and intermediate maple syrup urine diseaseBMC Med Genet, 2006, vol. 7, 33- [0338]
- **JAMSAI, D. et al.**A humanized BAC transgenic/knockout mouse model for HbE/beta-thalassemiaGenomics, 2006, vol. 88, 3309-15 [0338]
- **PAN, Q. et al.**Different role for mouse and human CD3delta/epsilon heterodimer in preT cell receptor (preTCR) function: human CD3delta/epsilon heterodimer restores the defective preTCR function in CD3gamma- and CD3gammadelta-deficient miceMol Immunol, 2006, vol. 43, 1741-1750 [0338]
- **ALT, F.W.BLACKWELL, T.K.YANCOPOULOS, G.D.**Immunoglobulin genes in transgenic miceTrends Genet, 1985, vol. 1, 231-236 [0339]
- **GREEN, L.L. et al.**Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACsNat Genet, 1994, vol. 7, 13-21 [0339]
- **LONBERG, N.**Human antibodies from transgenic animalsNat Biotechnol, 2005, vol. 23, 1117-1125 [0339]
- **LONBERG, N. et al.**Antigen-specific human antibodies from mice comprising four distinct genetic modificationsNature, 1994, vol. 368, 856-859 [0339]
- **JAKOBOVITS, A. et al.**From XenoMouse technology to panitumumab, the first fully

human antibody product from transgenic miceNat Biotechnol, 2007, vol. 25, 1134-1143 [0339]

- **TOMIZUKA, K. et al.**Double trans-chromosomic mice: maintenance of two individual human chromosome fragments containing Ig heavy and kappa loci and expression of fully human antibodiesProc Natl Acad Sci U S A, 2000, vol. 97, 722-727 [0339]
- **GIBSON, T.B. et al.**Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancerClin Colorectal Cancer, 2006, vol. 6, 29-31 [0339]
- **KIM, Y.H. et al.**Clinical efficacy of zanolimumab (HuMax-CD4): two Phase II studies in refractory cutaneous **T**-cell lymphomaBlood, 2007, vol. 109, 114655-62 [0339]
- **MAKER, A.V. et al.**Tumor regression and autoimmunity in patients treated with cytotoxic **T** lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II studyAnn Surg Oncol, 2005, vol. 12, 1005-1016 [0339]
- **MCCLUNG, M.R.LEWIECKI, E.M. et al.**Denosumab in postmenopausal women with low bone mineral densityN Engl J Med, 2006, vol. 354, 821-831 [0339]
- **GARRETT, F.E. et al.**Chromatin architecture near a potential 3' end of the igh locus involves modular regulation of histone modifications during B-Cell development and in vivo occupancy at CTCF sitesMol Cell Biol, 2005, vol. 25, 1511-1525 [0339]
- **MANIS, J.P. et al.**Elucidation of a downstream boundary of the 3' IgH regulatory regionMol Immunol, 2003, vol. 39, 753-760 [0339]
- **PAWLITZKY, I. et al.**Identification of a candidate regulatory element within the 5' flanking region of the mouse Igh locus defined by pro-B cell-specific hypersensitivity associated with binding of PU.1, Pax5, and E2AJ Immunol, 2006, vol. 176, 6839-6851 [0339]
- **HOMBACH, J. et al.**Molecular components of the B-cell antigen receptor complex of the IgM classNature, 1990, vol. 343, 760-762 [0339]
- **RAO, S.P. et al.**Differential expression of the inhibitory IgG Fc receptor FcgammaRIIB on germinal center cells: implications for selection of high-affinity B cellsJ Immunol, 2002, vol. 169, 1859-1868 [0339]
- **BRAMBELL, F.W. et al.**A **T**heoretical Model of Gamma-Globulin CatabolismNature, 1964, vol. 203, 1352-1354 [0339]
- **JUNGHANS, R.P.ANDERSON, C.L.T**he protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptorProc Natl Acad Sci U S A, 1996, vol. 93, 5512-5516 [0339]
- **HJELM, F. et al.**Antibody-mediated regulation of the immune responseScand J Immunol, 2006, vol. 64, 177-184 [0339]
- **NIMMERJAHN, F.RAVETCH, J.V.**Fc-receptors as regulators of immunityAdv Immunol, 2007, vol. 96, 179-204 [0339]
- **VALENZUELA, D.M. et al.**High-throughput engineering of the mouse genome coupled with high-resolution expression analysisNat Biotechnol, 2003, vol. 21, 652-659 [0348]
- **ZHANG, Y.BUCHHOLZ, F.MUYRERS, J.P.STEWART, A.F.**A new logic for DNA engineering using recombination in Escherichia coliNat Genet, 1998, vol. 20, 123-128 [0528]
- **SCHWARTZ, D.C.CANTOR, C.**Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresisCell, 1984, vol. 37, 67-75 [0528]

- **NEUBERGER, M.S.**Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cellsEmbo J, 1983, vol. 2, 1373-1378 [0534]
- **KATAOKA, T.KAWAKAMI, T.TAKAHASHI, N.HONJO, T.**Rearrangement of immunoglobulin gamma 1-chain gene and mechanism for heavy-chain class switchProc Natl Acad Sci U S A, 1980, vol. 77, 919-923 [0534]
- **MORTENSEN, R.M. et al.**Production of homozygous mutant ES cells with a single targeting constructMol Cell Biol, 1992, vol. 12, 2391-2395 [0536]
- **ZHENG, B. et al.**Engineering mouse chromosomes with Cre-loxP: range, efficiency, and somatic applicationsMol Cell Biol, 2000, vol. 20, 648-655 [0536]
- **MCLEOD, M. et al.**Identification of the crossover site during FLP-mediated recombination in the Saccharomyces cerevisiae plasmid 2 microns circleMol Cell Biol, 1986, vol. 6, 3357-3367 [0537]
- **BUCHHOLZ, F. et al.**Improved properties of FLP recombinase evolved by cycling mutagenesisNat Biotechnol, 1998, vol. 16, 657-662 [0537]
- **WEICHHOLD, G.M. et al.T**he human immunoglobulin kappa locus consists of two copies that are organized in opposite polarityGenomics, 1993, vol. 16, 503-511 [0539]
- **SCHAIBLE, G. et al.T**he immunoglobulin kappa locus: polymorphism and haplotypes of Caucasoid and non-Caucasoid individualsHum Genet, 1993, vol. 91, 261-267 [0539]
- **GREEN, L.L.JAKOBOVITS, A.**Regulation of B cell development by variable gene complexity in mice reconstituted with human immunoglobulin yeast artificial chromosomesJ Exp Med, 1998, vol. 188, 483-495 [0545]
- **BRUGGEMANN, M. et al.**A repertoire of monoclonal antibodies with human heavy chains from transgenic micePNAS, 1989, vol. 86, 6709-6713 [0547]
- **TUAILLON, N. et al.**Human immunoglobulin heavy-chain minilocus recombination in transgenic mice: gene-segment use in mu and gamma transcriptsProc Natl Acad Sci U S A, 1993, vol. 90, 3720-3724 [0547]
- **WANG, X.STOLLAR, B.D.**Human immunoglobulin variable region gene analysis by single cell RT-PCRJ Immunol Methods, 2000, vol. 244, 217-225 [0553]
- **BROCHET, X.LEFRANC, M.P.GIUDICELLI, V.**IMGT/V-QUEST: the highly customized and integrated system for IG and TR standardized V-J and V-D-J sequence analysisNucleic Acids Res, 2008, vol. 36, W503-508 [0554]
- **CHEN, J. et al.**RAG-2-deficient blastocyst complementation: an assay of gene function in lymphocyte developmentProc Natl Acad Sci U S A, 1993, vol. 90, 4528-4532 [0555]
- **FEENEY, A.J.**Factors that influence formation of B cell repertoireImmunol Res, 2000, vol. 21, 195-202 [0557]
- **MATSUDA, F. et al.T**he complete nucleotide sequence of the human immunoglobulin heavy chain variable region locusJ Exp Med, 1998, vol. 188, 2151-2162 [0557]
- **HARLOW, E.LANE, D.**Antibodies: A Laboratory ManualCold Spring Harbor Press19880000 [0568]
- **LIE, Y.S.PETROPOULOS, C.J.**Advances in quantitative PCR technology: 5'nuclease assaysCurr Opin Biotechnol, 1998, vol. 9, 143-48 [0581]
- **RODRIGUEZ, C.I. et al.**High-efficiency deleter mice show that FLPe is an alternative to Cre-loxPNature Genetics, 2000, vol. 25, 139-140 [0583]
- **VALENZUELA et al.**High-throughput engineering of the mouse genome coupled with

high-resolution expression analysisNature Biotech., 2003, vol. 21, 6652-659 [0606]

- **POUEYMIROU et al.**F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analysesNature Biotech., 2007, vol. 25, 191-99 [0635]

PATENTKRAV

1. Mus, der omfatter:

(a) ét eller flere ikke-omarrangerede humane Vλ-gensegmenter og ét eller flere ikke-omarrangerede humane Jλ-gensegmenter ved et endogent immunoglobulin kappa-letkæde-locus fra musen, hvor immunoglobulin-kappa-letkæde-locusset omfatter et muse-Cκ-område;

(b) ét eller flere humane V_H-gensegmenter, ét eller flere humane D_H-gensegmenter og ét eller flere humane J_H-gensegmenter ved et endogent immunoglobulin-tungkæde-locus fra musen; og

(c) en modifikation af et immunoglobulin-tungkæde-locus, hvor modifikationen eliminerer endogen ADAM6-funktion, der er forbundet med en reduktion af fertilitet hos hanmus, hvor musen endvidere omfatter en nukleinsyresekvens, der koder for et muse-ADAM6a-protein eller et ortholog eller homolog eller funktionelt fragment deraf, der hvert kan fungere til at forbedre eller genoprette reduktionen af fertilitet hos en hanmus, og en nukleinsyresekvens, der koder for et muse-ADAM6b-protein, eller et ortholog eller homolog eller funktionelt fragment deraf, der hvert kan fungere til at forbedre eller genoprette reduktionen af fertilitet hos en hanmus, hvor nukleinsyresekvenserne, der koder for ADAM6-proteiner, orthologer, homologer eller funktionelle fragmenter deraf, er til stede i de humane tungkædegensegmenter.

2. Mus ifølge det foregående krav, hvor musen omfatter 12 til 40 humane Vλ-gensegmenter og mindst ét humant Jλ-gensegment.

3. Mus ifølge krav 2, hvor:

(a) musen omfatter 12 humane Vλ-gensegmenter og mindst ét humant Jλ-gensegment;

(b) musen omfatter 28 humane Vλ-gensegmenter og mindst ét humant Jλ-gensegment; eller

(c) musen omfatter 40 humane Vλ-gensegmenter og mindst ét humant Jλ-gensegment.

4. Mus ifølge et hvilket som helst af de foregående krav hvor:

(a) det mindst ene humane Jλ-gensegment er udvalgt fra Jλ1, Jλ2, Jλ3, Jλ7 og en kombination deraf; og/eller

(b) musen omfatter mindst fire humane Jλ-gensegmenter.

5. Mus ifølge et hvilket som helst af de foregående krav, hvor musen mangler et endogent variabelt kappa-letkædeområde ved det endogene immunoglobulin-kappa-letkæde-locus.

6. Mus ifølge et hvilket som helst af de foregående krav, der endvidere omfatter et humant Vκ-Jκ–intergenområde fra et humant κ-letkæde-locus, hvor det humane Vκ-Jκ-intergen-område er sammenhængende med ét eller flere ikke-omarrangerede Vλ-gensegmenter og med ét eller flere ikke-omarrangerede Jλ-gensegmenter, hvor fortrinsvis det humane intergen-Vκ-Jκ-område er placeret mellem et Vλ-gensegment og et Jλ-gensegment, og hvor det humane intergen-Vκ-Jκ-område omfatter SEQ ID NO: 158.

7. Mus ifølge et hvilket som helst af de foregående krav, der er en hanmus.

8. Isoleret celle eller væv fra musen ifølge et hvilket som helst af de foregående krav.

9. Anvendelse af musen ifølge et hvilket som helst af krav 1 til 7 til at frembringe:

(i) et inverteret kimærisk antistof;

(ii) et fuldt, humant antistof;

(iii) et fuldt, humant Fab-fragment eller

(iv) et fuldt, humant F(ab)2-fragment.

10. Fremgangsmåde til frembringelse af et antistof, hvilken fremgangsmåde omfatter:

(a) eksponering af musen ifølge et hvilket som helst af krav 1 til 7 for et antigen;

(b) at lade musen udvikle et immunrespons på antigenet; og

(c) isolering fra musen af (b) af et antistof, der specifikt genkender antigenet, hvor antistoffet omfatter en letkæde afledt af et hVλ, et hJλ og muse-Cκ-området, eller isolering fra musen af (b) en celle, der omfatter et immunoglobulindomæne, der

specifikt genkender antigenet, eller identificering i musen fra (b) af en nukleinsyresekvens, der koder for et variabelt tung- og/eller letkædedomæne, der binder antigenet.

11. Fremgangsmåde til frembringelse af et humant antistof, hvilken fremgangsmåde omfatter eksponering af musen ifølge et hvilket som helst af krav 1 til 7 for et antigen, at lade musen opbygge et immunrespons, der omfatter frembringelse af et antistof, der specifikt binder antigenet, identificering i en B-celle fra musen af en omarrangeret nukleinsyresekvens, der koder for et variabelt humant tungkædedomæne, og en omarrangeret nukleinsyresekvens, der koder for en kognat human variabel letkæde-domænesekvens af et antistof, hvor antistoffet specifikt binder antigenet, og anvendelse af nukleinsyresekvenserne, der koder for de variable humane tungkæde- og variable humane letkædedomæner bundet henholdsvis til en nukleinsyresekvens, der koder for et konstant humant tungkæde-domæne, og en nukleinsyresekvens, der koder for et konstant humant letkædedomæne, for at frembringe et ønsket antistof.

12. Fremgangsmåde til opnåelse af en nukleinsyresekvens, der koder for et variabelt tung- og/eller letkædedomæne omfattende:

(a) eksponering af musen ifølge et hvilket som helst af krav 1 til 7 for et antigen;

(b) identificering i en B-celle fra musen af:

(i) et omarrangeret letkæde-immunoglobulingen, hvor det omarrangerede letkæde-immunoglobulingen omfatter mindst et humant variabelt λ-letkædeområde-gensegment bundet til et muse-Cκ-område; eller

(ii) et omarrangeret letkæde-immunoglobulingen fra (b)(i) og et omarrangeret tungkæde-immunoglobulingen, hvor det omarrangerede tungkæde-immunoglobulingen koder for en tungkæde, der parres med letkæden kodet for af det omarrangerede letkæde-immunoglobulingen fra (b)(i); og

(c) kloning af en nukleinsyresekvens, der koder for et variabelt tung- og/eller letkædedomæne fra musens B-celle, hvor det variable tung- og/eller letkædedomæne er fra et antistof, der omfatter et humant Vλ og et muse-Cκ.

DRAWINGS

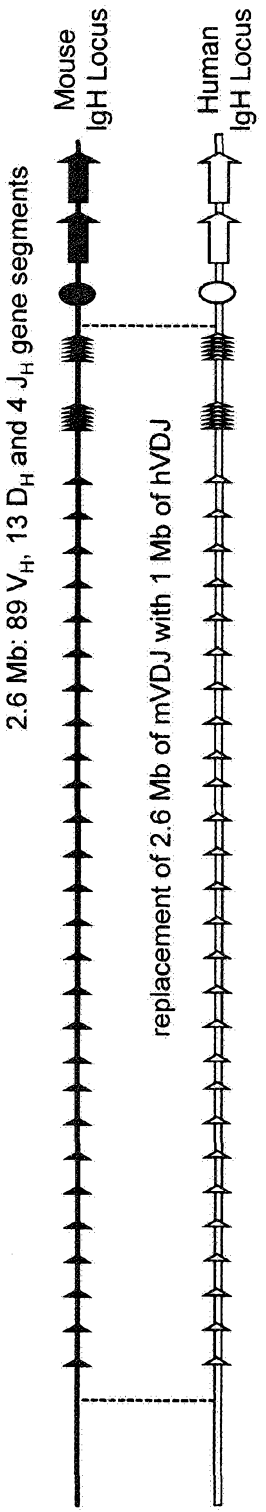

FIG. 1A

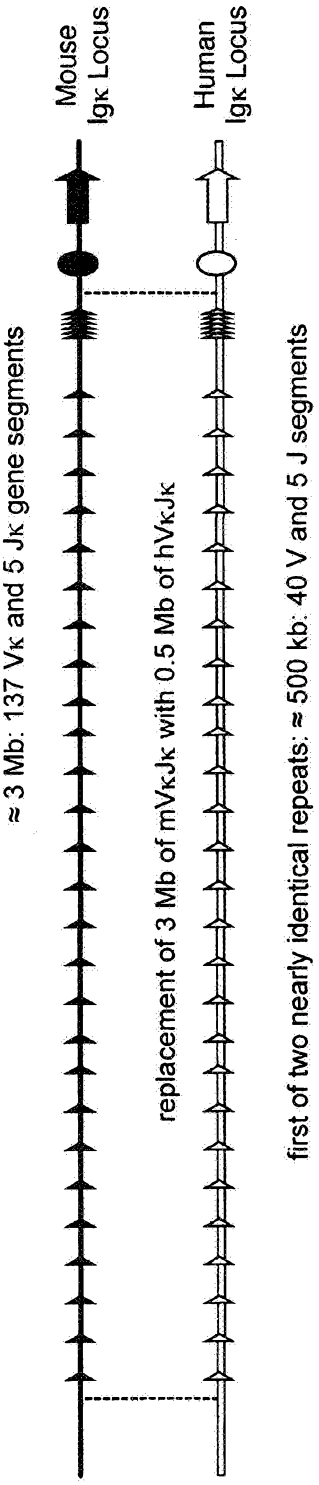
Mouse
Igκ Locus
Human
Igκ Locus
≈ 3 Mb: 137 Vκ and 5 Jκ gene segments
replacement of 3 Mb of mVκJκ with 0.5 Mb of hVκJκ
first of two nearly identical repeats: ≈ 500 kb: 40 V and 5 J segments

FIG. 1B

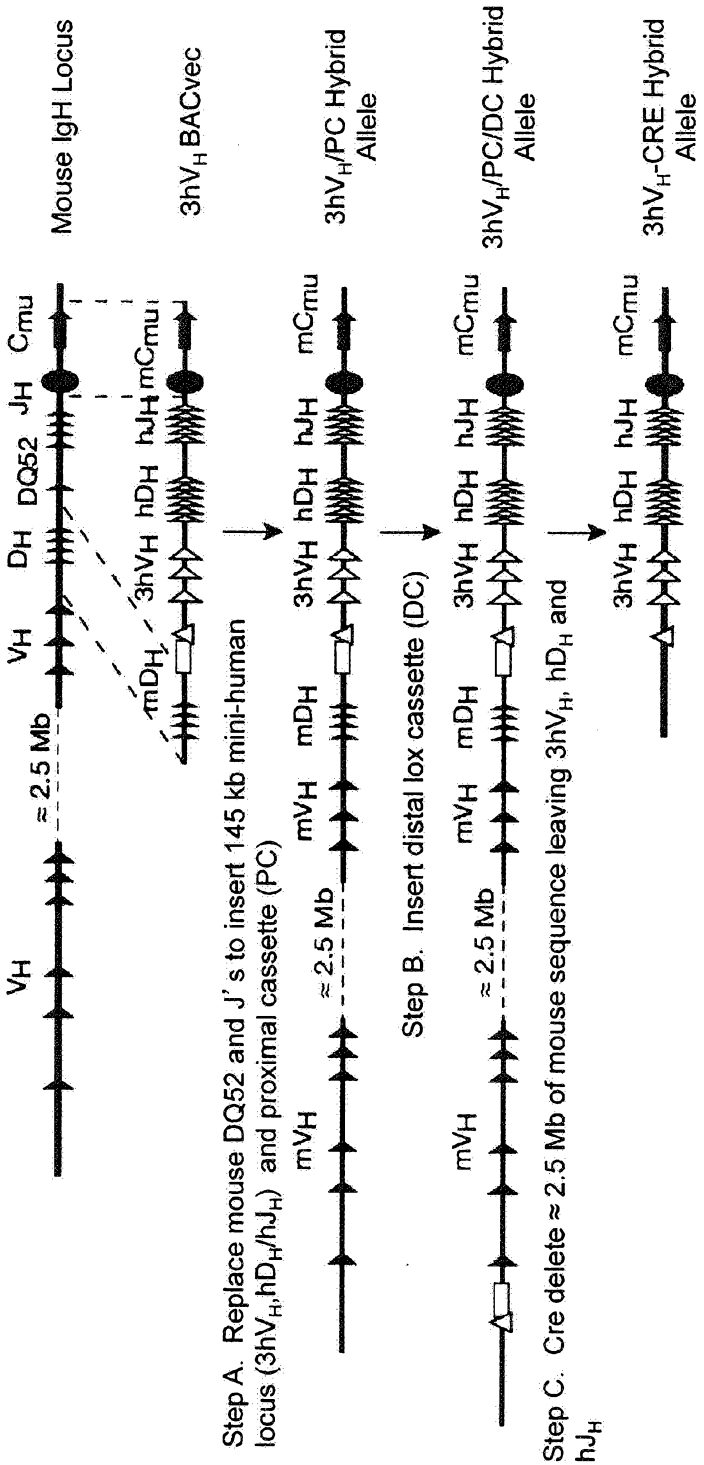
Mouse IgH Locus
3hVH BACvec
3hVH/PC Hybrid Allele
3hVH/PC/DC Hybrid Allele
3hVH-CRE Hybrid Allele
VH
≈ 2.5 Mb
DH
DQ52
JH
Cmu
mDH
3hVH
hDH
hJH
mCmu
mVH
Step A. Replace mouse DQ52 and J's to insert 145 kb mini-human locus (3hVH,hDH/hJH) and proximal cassette (PC)
Step B. Insert distal lox cassette (DC)
Step C. Cre delete ≈ 2.5 Mb of mouse sequence leaving 3hVH, hDH and hJH

FIG. 2A

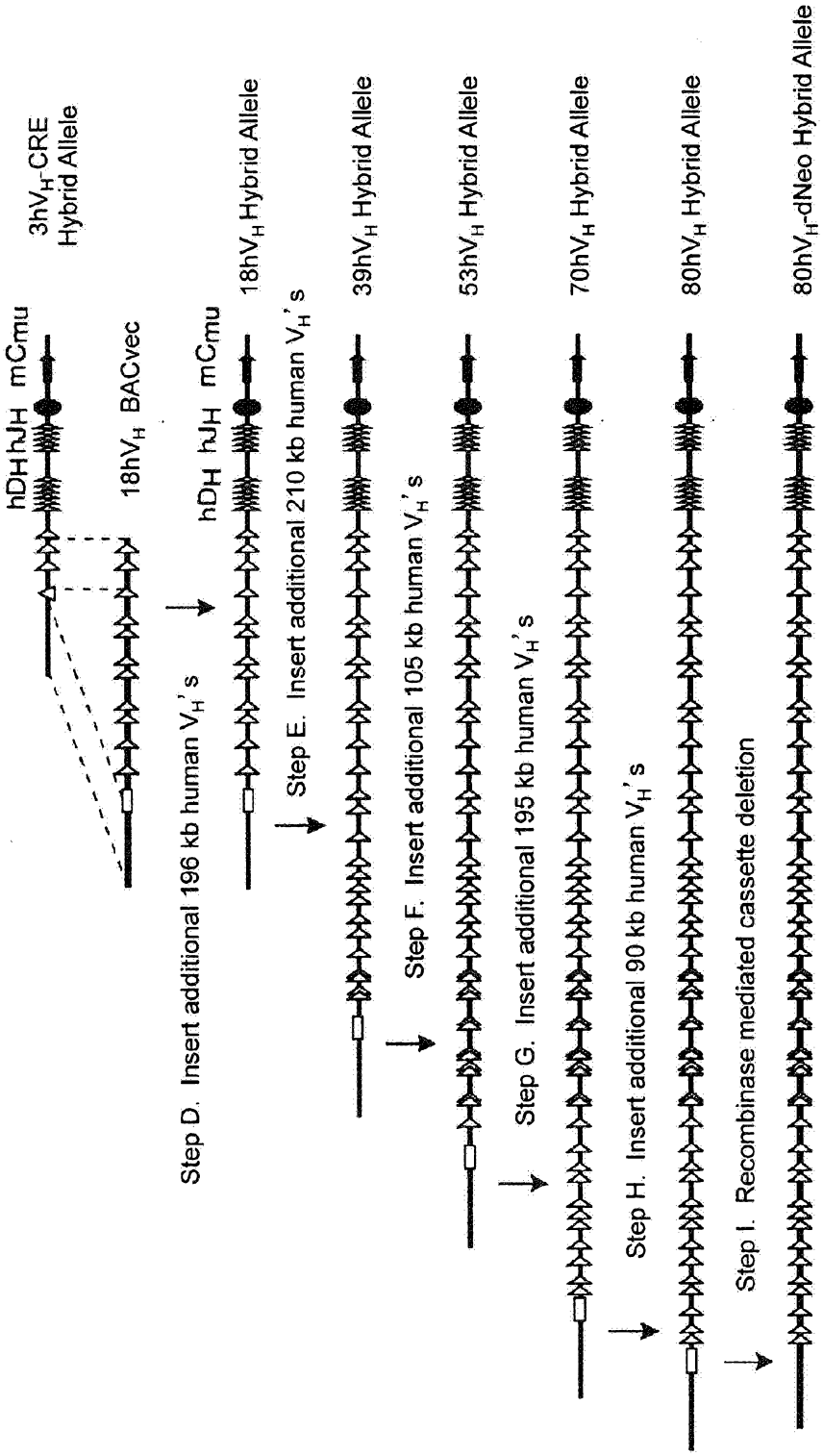
hDHhJH mCmu
3hVH-CRE Hybrid Allele
18hVH BACvec
Step D. Insert additional 196 kb human VH's
hDH hJH mCmu
18hVH Hybrid Allele
Step E. Insert additional 210 kb human VH's
39hVH Hybrid Allele
Step F. Insert additional 105 kb human VH's
53hVH Hybrid Allele
Step G. Insert additional 195 kb human VH's
70hVH Hybrid Allele
Step H. Insert additional 90 kb human VH's
80hVH Hybrid Allele
Step I. Recombinase mediated cassette deletion
80hVH-dNeo Hybrid Allele

FIG. 2B

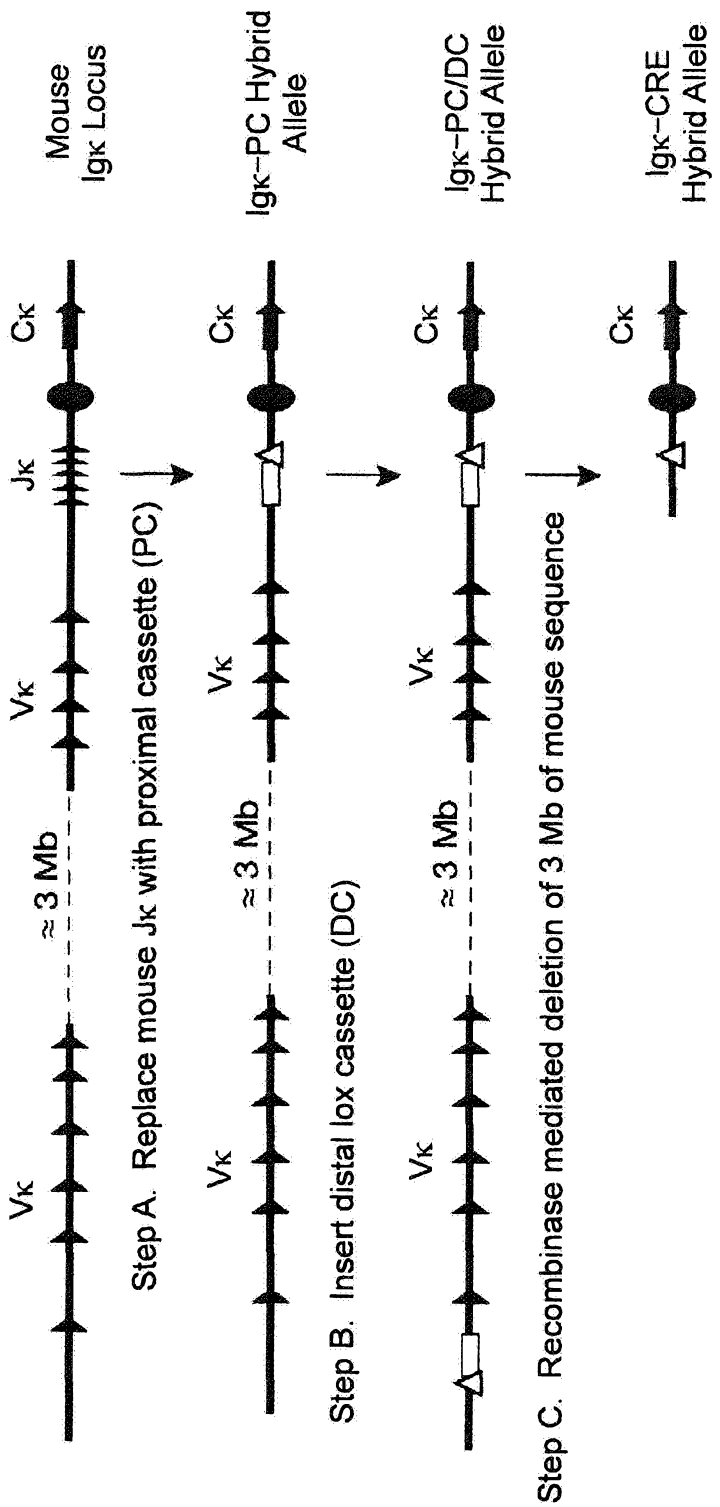
Mouse
Igκ Locus
Vκ
≈ 3 Mb
Vκ
Jκ
Cκ
Step A. Replace mouse Jκ with proximal cassette (PC)
Igκ–PC Hybrid
Allele
Vκ
≈ 3 Mb
Vκ
Cκ
Step B. Insert distal lox cassette (DC)
Igκ–PC/DC
Hybrid Allele
Vκ
≈ 3 Mb
Vκ
Cκ
Step C. Recombinase mediated deletion of 3 Mb of mouse sequence
Igκ–CRE
Hybrid Allele
Cκ

FIG. 2C

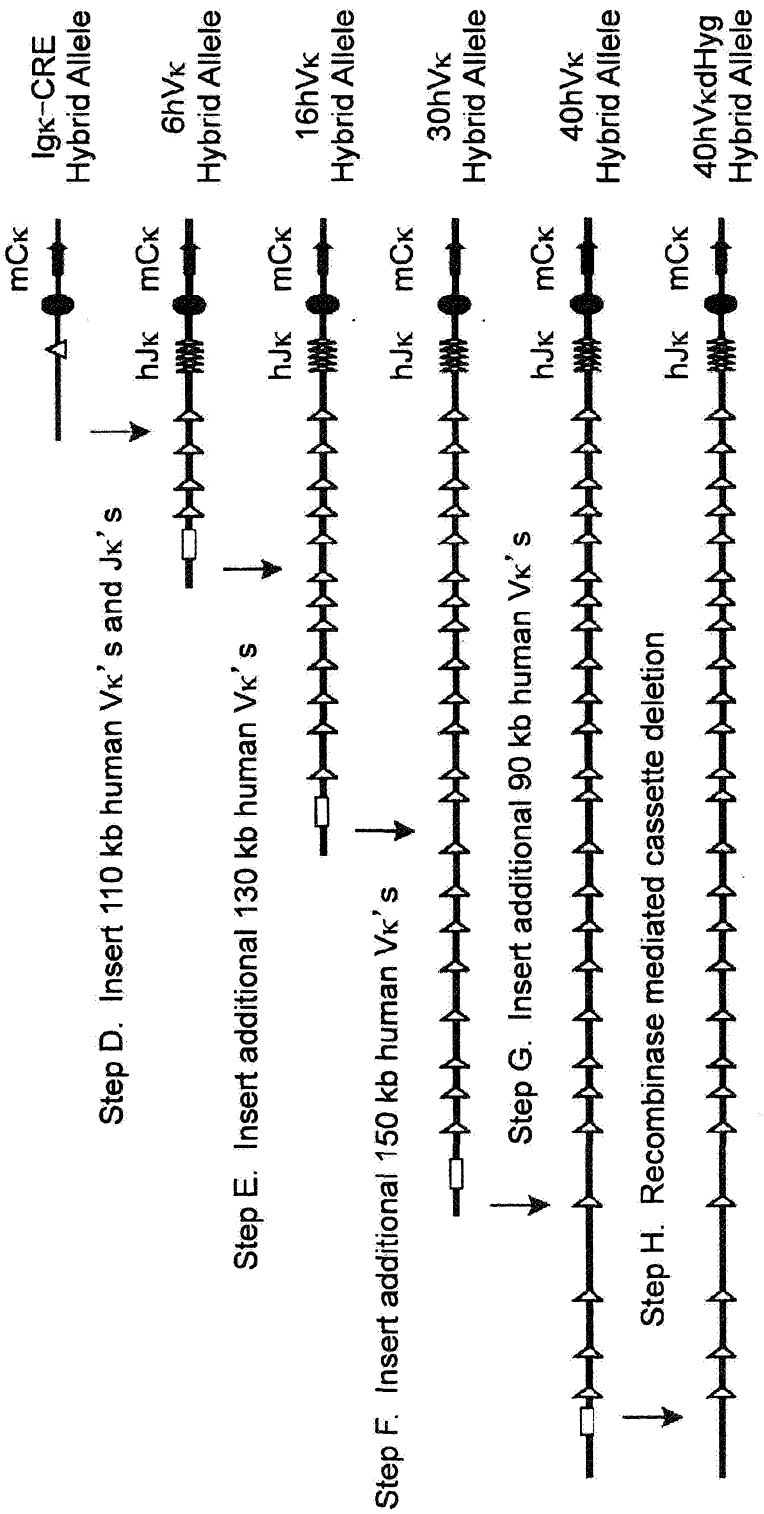
Igκ–CRE Hybrid Allele
6hVκ Hybrid Allele
16hVκ Hybrid Allele
30hVκ Hybrid Allele
40hVκ Hybrid Allele
40hVκdHyg Hybrid Allele
mCκ
hJκ
Step D. Insert 110 kb human Vκ's and Jκ's
Step E. Insert additional 130 kb human Vκ's
Step F. Insert additional 150 kb human Vκ's
Step G. Insert additional 90 kb human Vκ's
Step H. Recombinase mediated cassette deletion

FIG. 2D

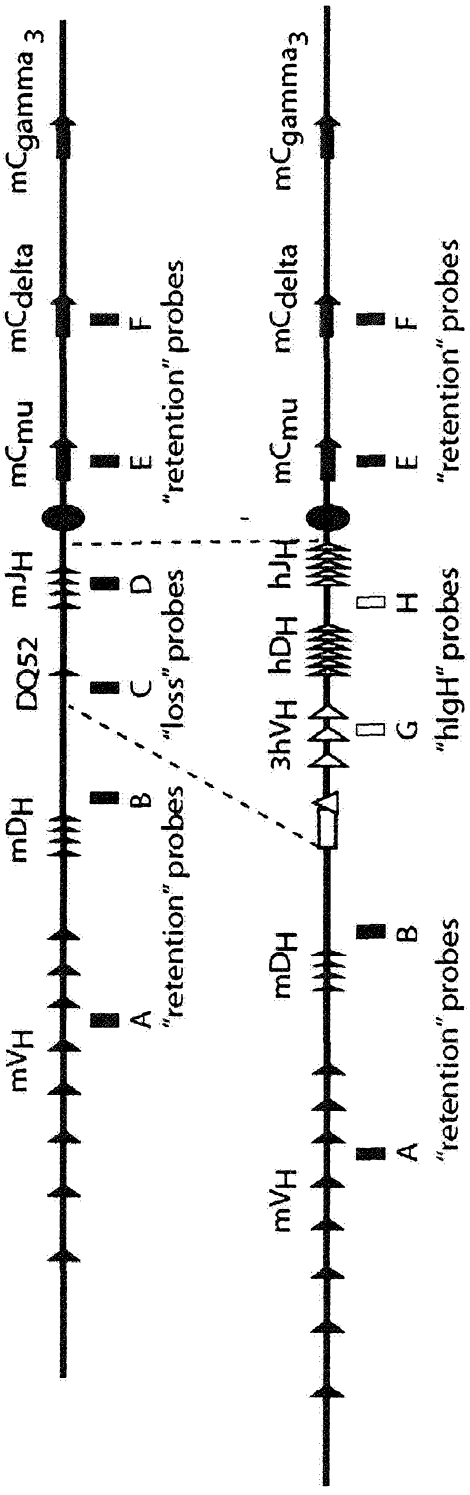
mVH
mDH
DQ52
mJH
mCmu
mCdelta
mCgamma3
A
B
C
D
E
F
"retention" probes
"loss" probes
"retention" probes
3hVH
hDH
hJH
mCmu
mCdelta
mCgamma3
G
H
E
F
"highH" probes
"retention" probes
mDH
mVH
A
B
"retention" probes

FIG. 3A

		A	B	C	D	E	F	G	H
Parental ES	Theoretical copy number	2	2	2	2	2	2	0	0
	Observed copy number	1.9	1.8	2.1	1.8	1.9	1.8	<0.01	<0.04
Modified ES	Theoretical copy number	2	2	1	1	2	2	1	1
	Observed copy number	1.9	2.4	1.0	1.0	2.0	1.9	+	+

FIG. 3B

	copy number	D	H
WT Mice	Theoretical	2	0
	Observed 1	1.71	< 0.01
	Observed 2	2.07	< 0.01
	Observed 3	2.16	< 0.01
	Observed 4	1.88	< 0.01
Het Mice	Theoretical	1	1
	Observed 1	1.22	1.04
	Observed 2	0.94	1.02
	Observed 3	0.85	0.95
	Observed 4	1.02	1.00
Homo Mice	Theoretical	0	2
	Observed 1	< 0.01	2.37
	Observed 2	< 0.01	2.22
	Observed 3	< 0.01	2.43
	Observed 4	< 0.01	1.93

FIG. 3C

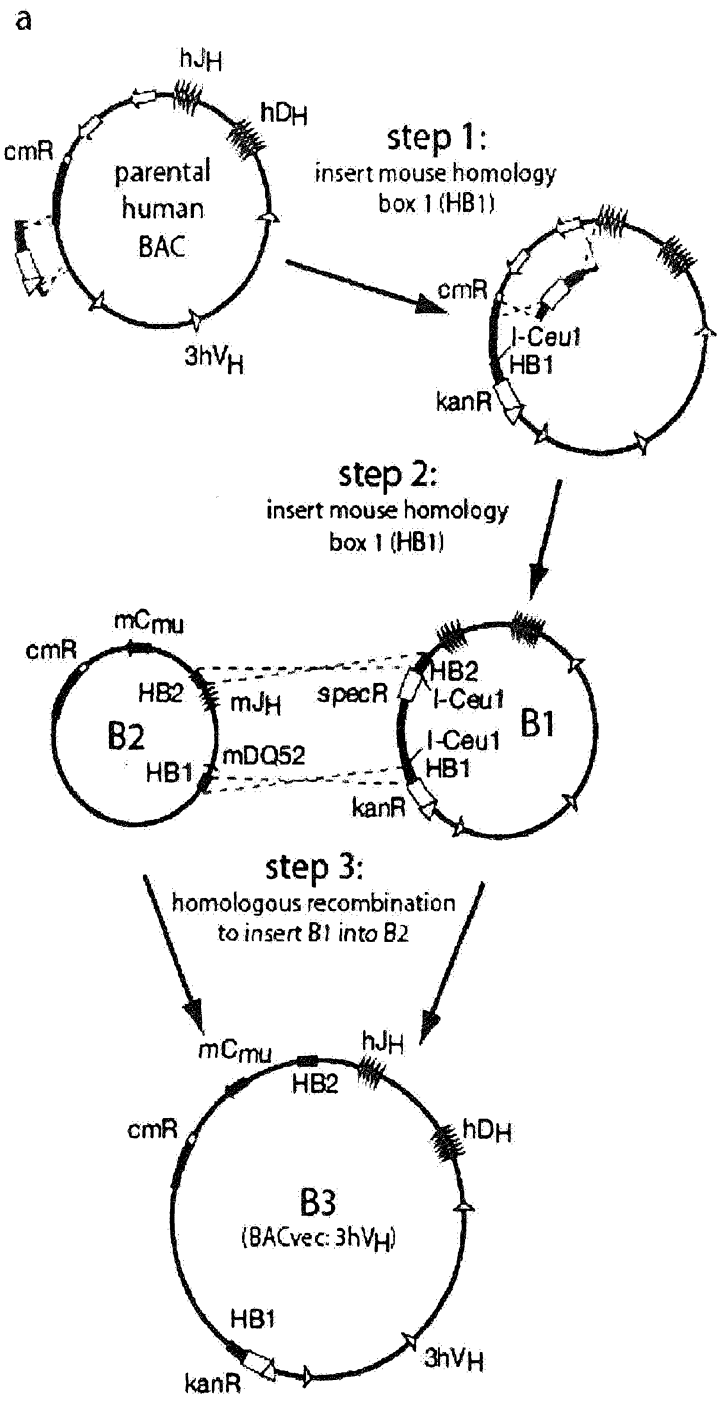
a
hJH
hDH
cmR
parental human BAC
3hVH
step 1:
insert mouse homology box 1 (HB1)
cmR
I-CeuI
HB1
kanR
step 2:
insert mouse homology box 1 (HB1)
mCmu
cmR
HB2
mJH
specR
HB2
I-CeuI
B2
B1
I-CeuI
mDQ52
HB1
HB1
kanR
step 3:
homologous recombination to insert B1 into B2
mCmu
hJH
HB2
cmR
hDH
B3
(BACvec: 3hVH)
HB1
3hVH
kanR

FIG. 4A

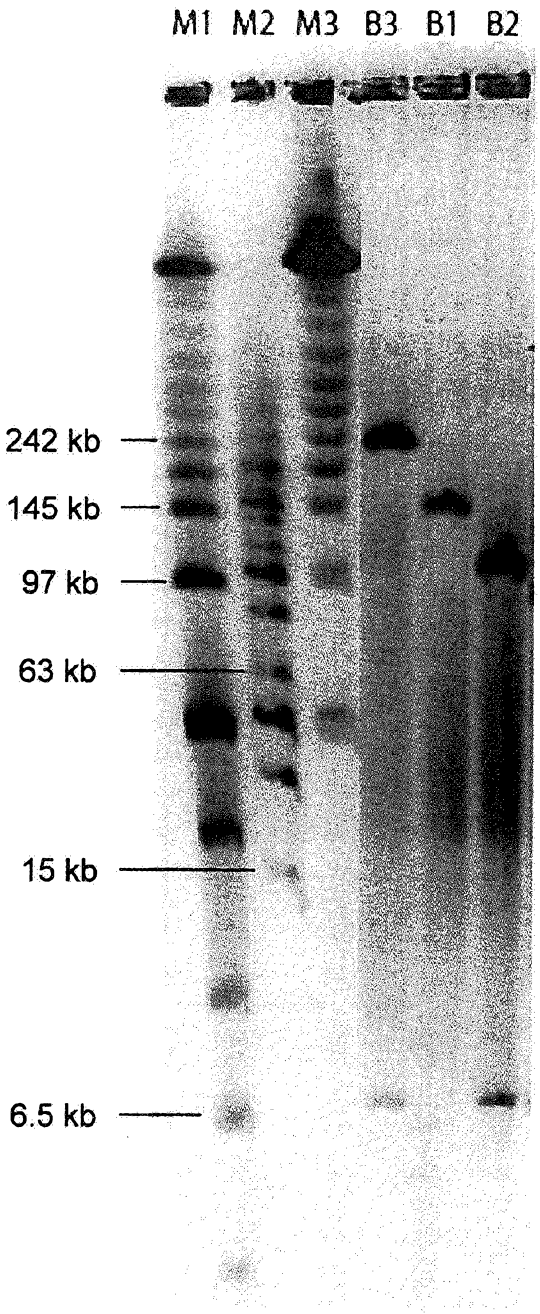

FIG. 4B

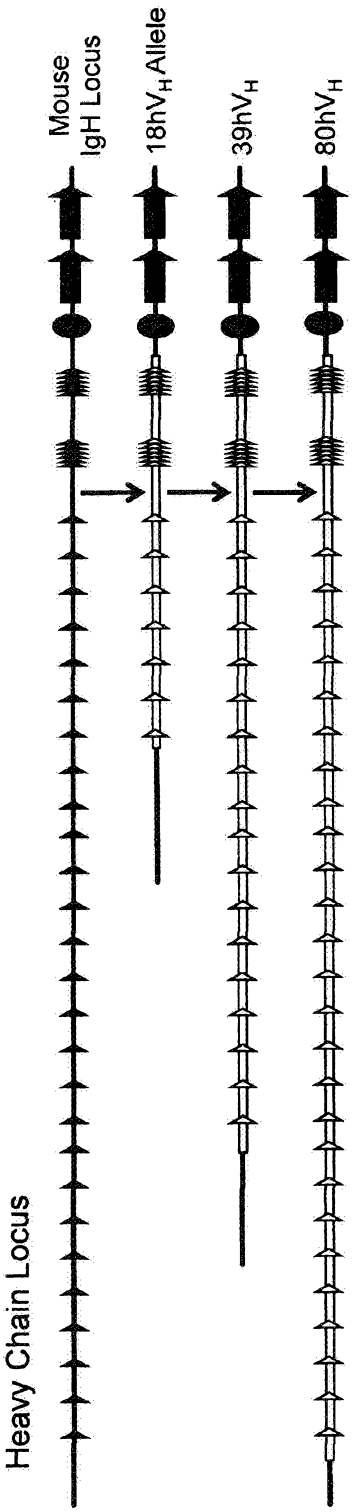

FIG. 5A

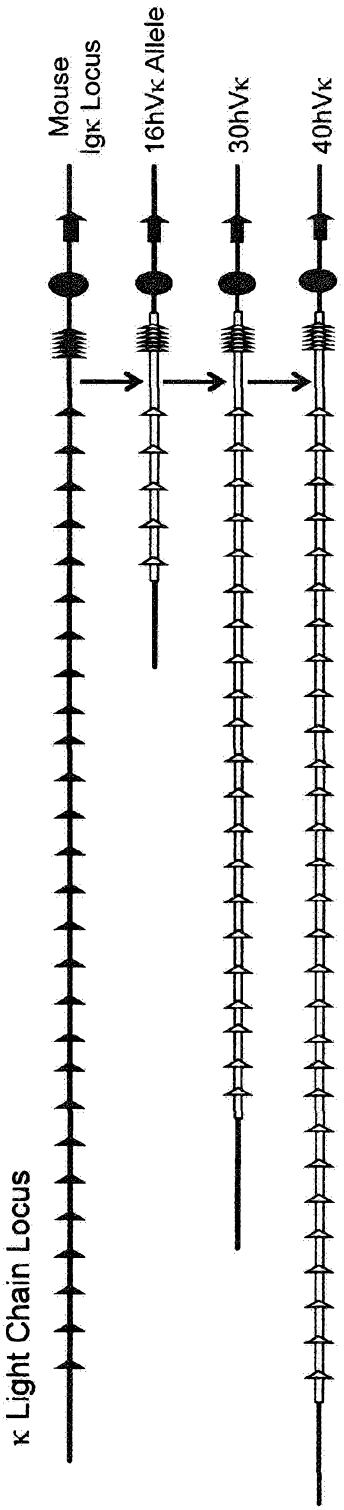
κ Light Chain Locus
Mouse Igκ Locus
16hVκ Allele
30hVκ
40hVκ

FIG. 5B

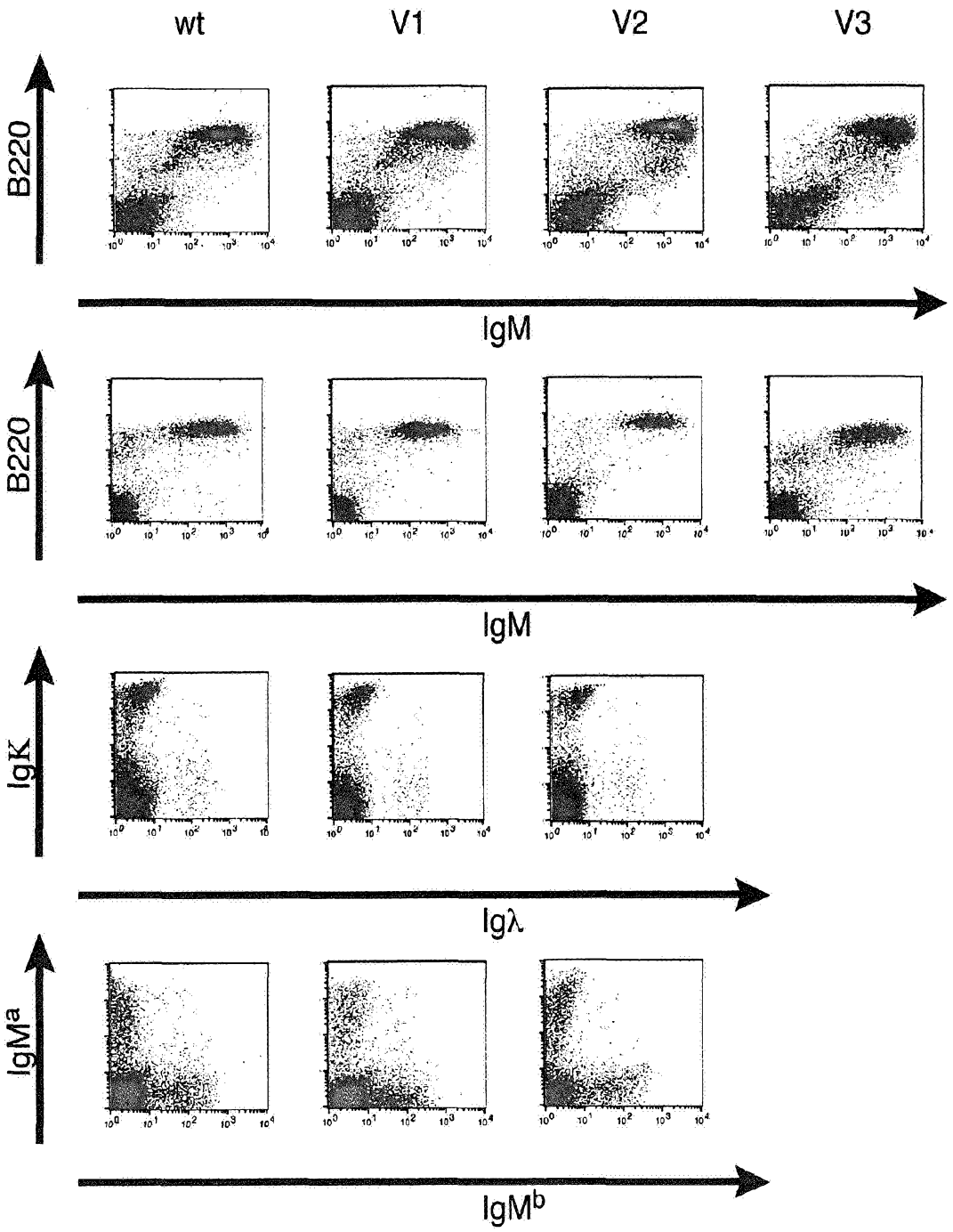

FIG. 6

3'V_H	N	D_H	N	5' J_H
		(D_H 1-26) GGTATAGTGGGAGCTACTAC		
(3-72) GCTAG		TAGTGGGgcCTAC	AGGC	CTTTTGATATC(3)
(3-9) GCAAAAG	CCCAGGGG	AGTGGGAGCTACTAC	ACCT	ATGCTTTTGATATC(3)
(3-7) GCGAGAGA	G	GGTATAGTGGGAaCTACT	GAGG	ACTTTGAtTAC(4)
(4-59) GCGAGAG	GGAC	AGTGGGAGC	CCT	CTTTGACTAC(4)
(3-23) GCGAAA	CC	TAGTGGGAGCTACT	C	CTGGTTCGACCCC(5)
		(D_H 1-7) GGTATAACTGGAACTAC		
(4-34) GCGAGAGG	AGGAG	GGTATAACTGGAACT	CGA	ATGCTTTTGATATC(3)
(1-2) GCGAGAG	GA	TATAACTGGA		ACTACTTTGACTAC(4)
(3-23) GCGAAAGA		GTATAACTGGAACcAC	TGG	TACTTTGACTAC(4)
(3-7) GCGAGAGA	G	ATAACTGGAAC	CCC	CTTTGACTAC(4)
(4-59) GCGAG	GGGA	TATAACTGGAACT	TTTCTTTT	TTTGACTAC(4)
(4-39) GCGAGA	GG	TAACTGGAACT	CTCTGGG	CTTTGACTAC(4)
	(D_H 3-10)	GTATTACTATGGTTCGGGGAGTTATTATAAC		
(3-30) GCGA	AAAGGGC	TACTATGGTTCGGGGAG	CTC	TTGACTAC(4)
(1-2) GCGAGAGA		TATTACTATGGTTCGGGGAGTTATTATAAC	GAAGGT	CTACGGTATGGACGTC(6)
	(D_H 6-6)	GAGTATAGCAGCTCGTCC		
(1-2) GCGAGAGA		GTATAGCAG		CTTTGACTAC(4)
(3-48) GCGAGA	GA	GAGTATAGCAGCTCGT	TG	TGACTAC(4)
(3-13) GCAAGAGA	GG	ATAGgAGCTCGcCC	CTCGGG	TACTTTGACTAC(4)
	(D_H 7-27)	CTAACTGGGGA		
(3-7) GCGAGAGA	TCT	TGGGGA	AGG	CTAC(4)
(3-15) ACCAC	CCA	TAACTGGGGA	GGG	TTTGACTAC(4)
(3-48) GCGAGA	GATA	GGGGA		CCg(5)

FIG. 7A

3'Vκ		N	5' Jκ
(1-6)	CAACAGAGTTAtAGTACCCCTCC	GGA	GACG(1)
(1-9)	CAACAGCTTAATAGTTACCCTC		GGACG(1)
(1-9)	CAACAGCTTAATAGTTACC		ATTCACT(3)
(1-9)	CAACAttTTAATAGTTACCC		GCTCACT(4)
(3-15)	CAGCAGTATAATAACTGGCCTC		TCACT(4)
(1-17)	CTACAGCATAATAGTTACCC		GTGGACG(1)
(1-17)	CTACAGCATAATAGTTACCCTC		GGACG(1)
(3-20)	CAGCAGTATGGTAGCTCACCTC		GGACG(1)
(2-30)	ATGCAAGGTACACACTGGCC		GTGGACG(1)
(2-30)	ATGCAAGGTtCACACTGGCC		GTACACT(2)
(2-30)	ATGCAAGGTACACACTGGCC		GCTCACT(4)
(1-33)	CAACAGTATGATAATCTCCCTCC		CACT(3)
(1-33)	CAACAGTATGATAATCTCCC		ATTCACT(3)
(1-33)	CAACAGTATGATAATCTCCC	CG	TCACT(4)
(1-33)	CAACAGTATGATAATCTCCC		GATCACC(5)
(1-37)	CAACGGAtTTACAATGCC	GA	CACC(5)
(1-39)	CAACAGAGTTACAGTACCCC	CA	TGTACACT(2)
(1-39)	CAACAGAGTTACAGTACCCCTC		TCACT(4)
(1-39)	CAACAGAGTTACAGTACtCCTCC		CACT(4)

FIG. 7B

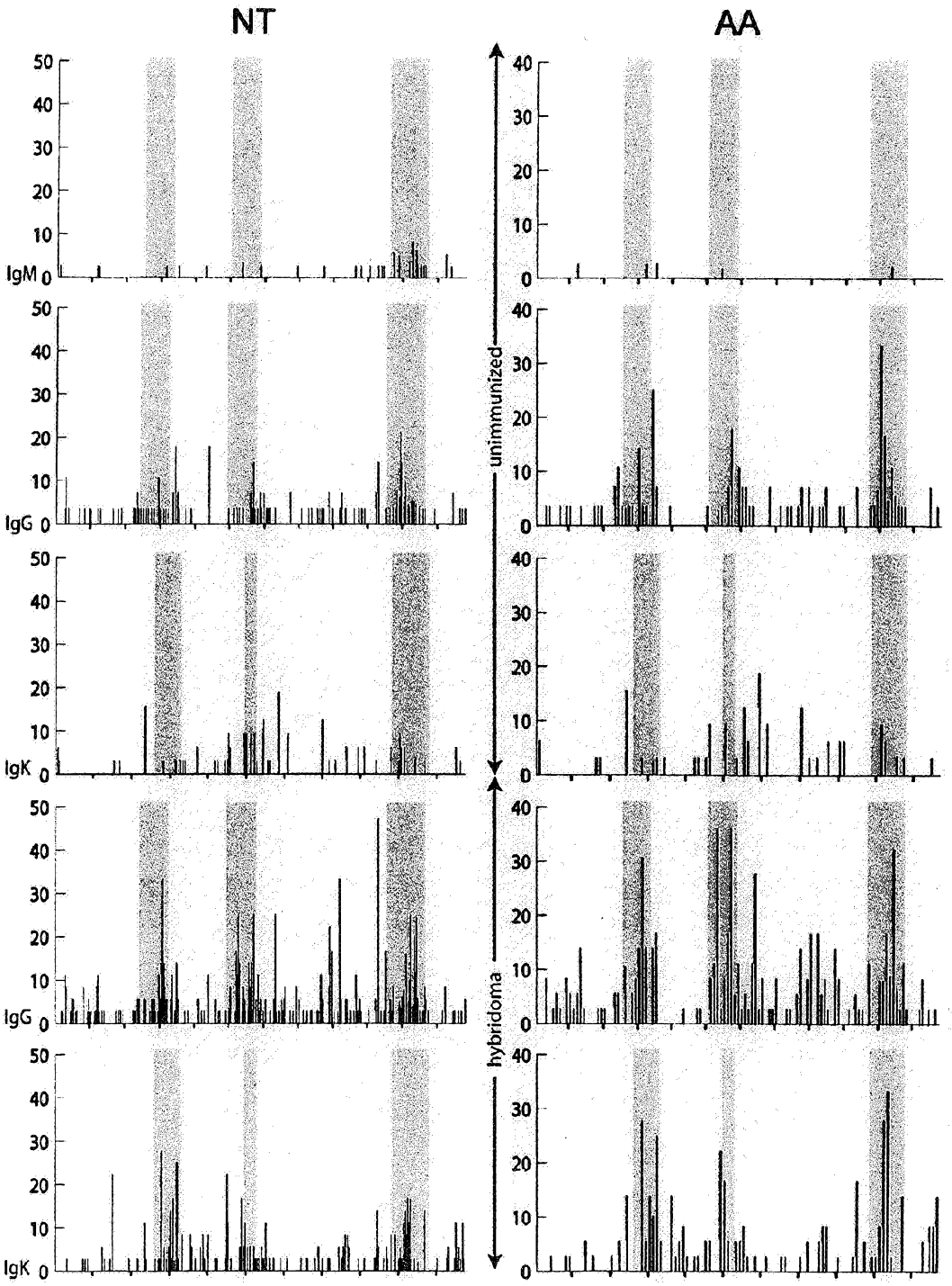
NT
AA
IgM
IgG
IgK
IgG
IgK
unimmunized
hybridoma
0
10
20
30
40
50

FIG. 8

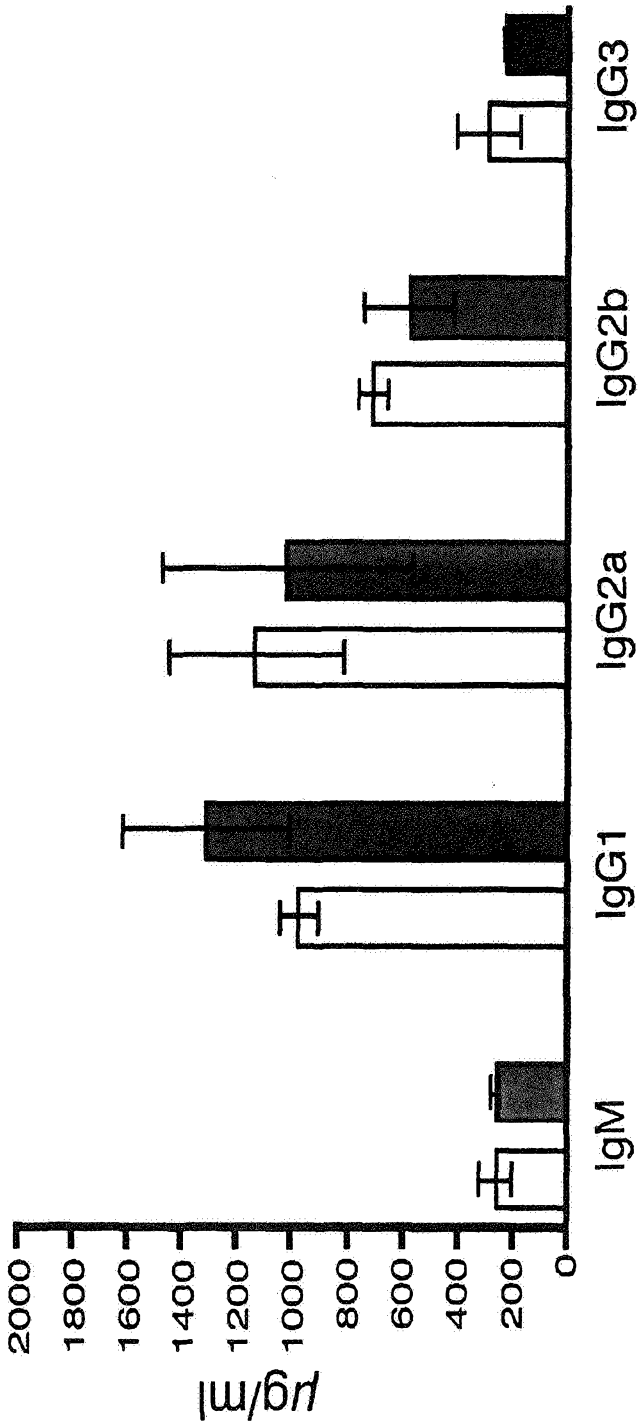
2000
1800
1600
1400
1200
1000
800
600
400
200
0
µg/ml
IgM
IgG1
IgG2a
IgG2b
IgG3

FIG. 9A

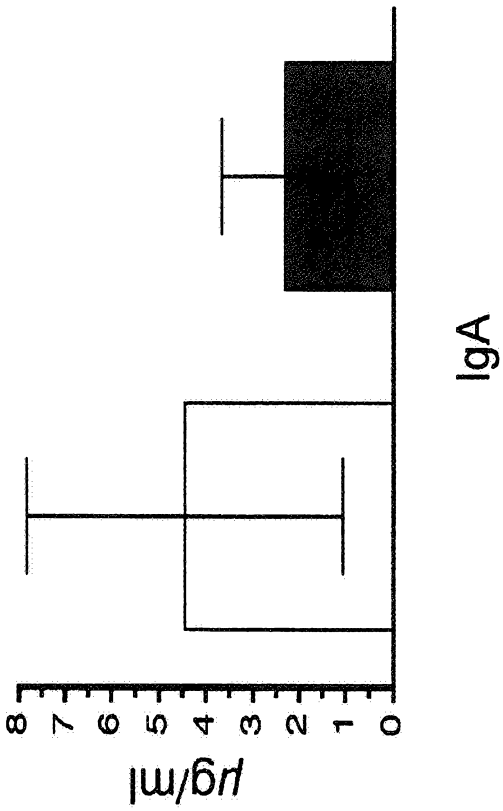

FIG. 9B

FIG. 9C

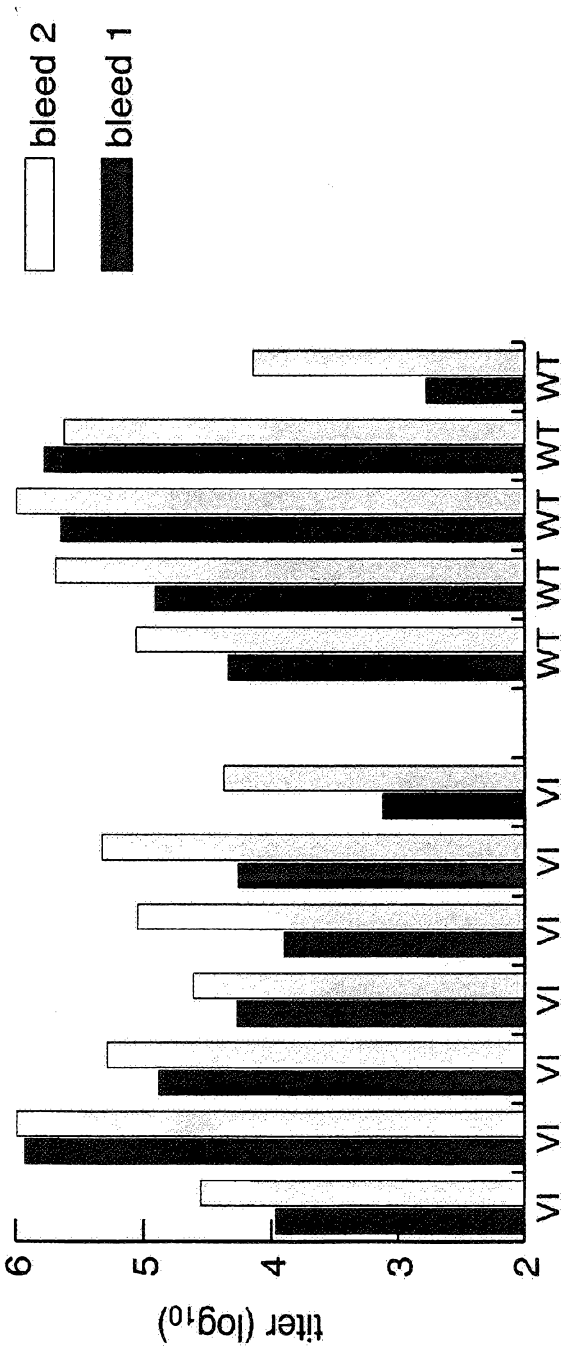
bleed 2
bleed 1
titer (log10)
6
5
4
3
2
VI
VI
VI
VI
VI
VI
VI
VI
WT
WT
WT
WT
WT
WT

FIG. 10A

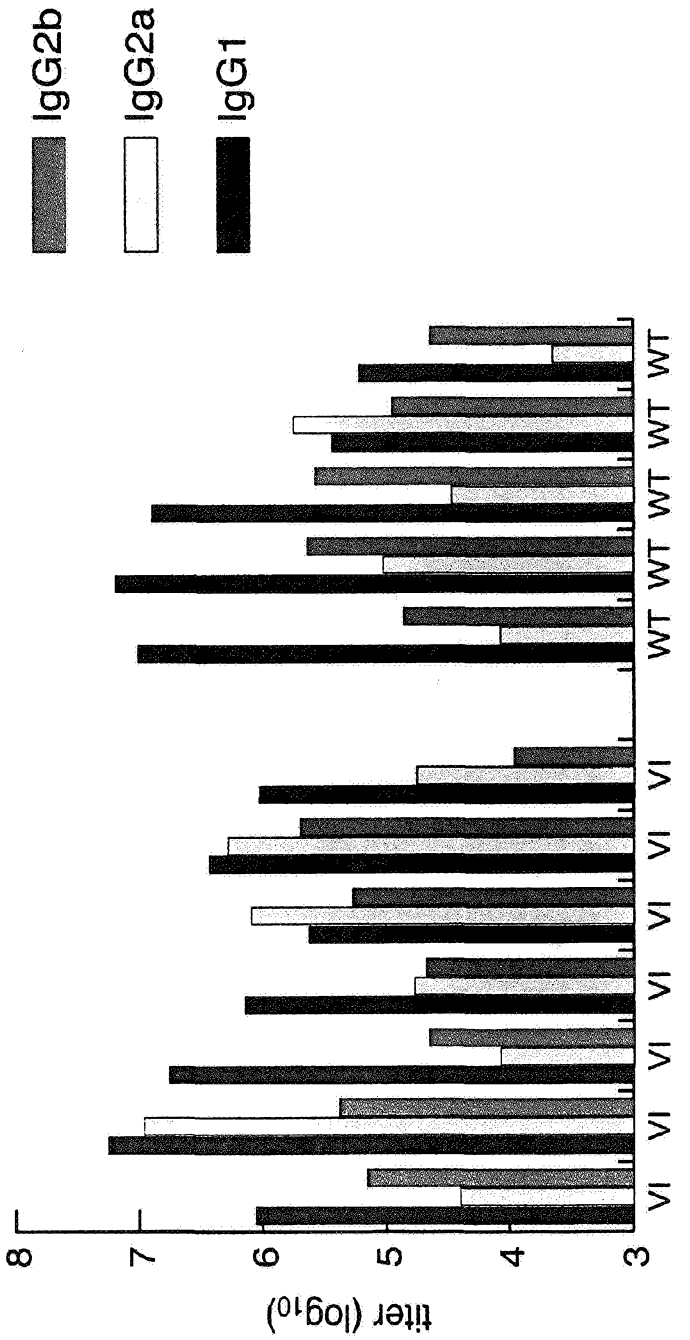
IgG2b
IgG2a
IgG1
titer (log10)
8
7
6
5
4
3
VI
VI
VI
VI
VI
VI
VI
VI
WT
WT
WT
WT
WT
WT

FIG. 10B

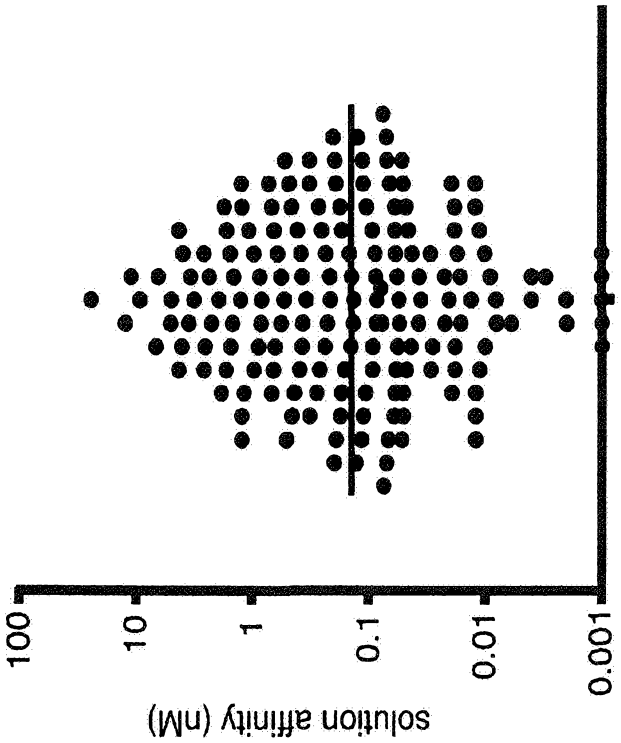

FIG. 11A

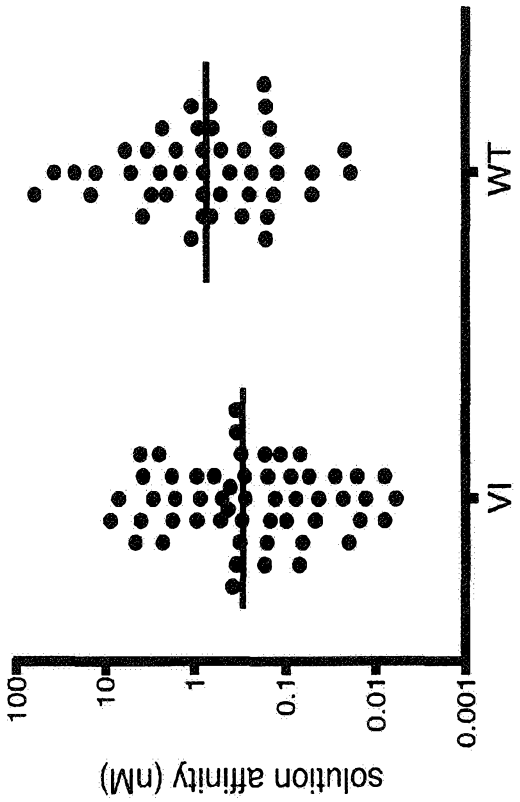

FIG. 11B

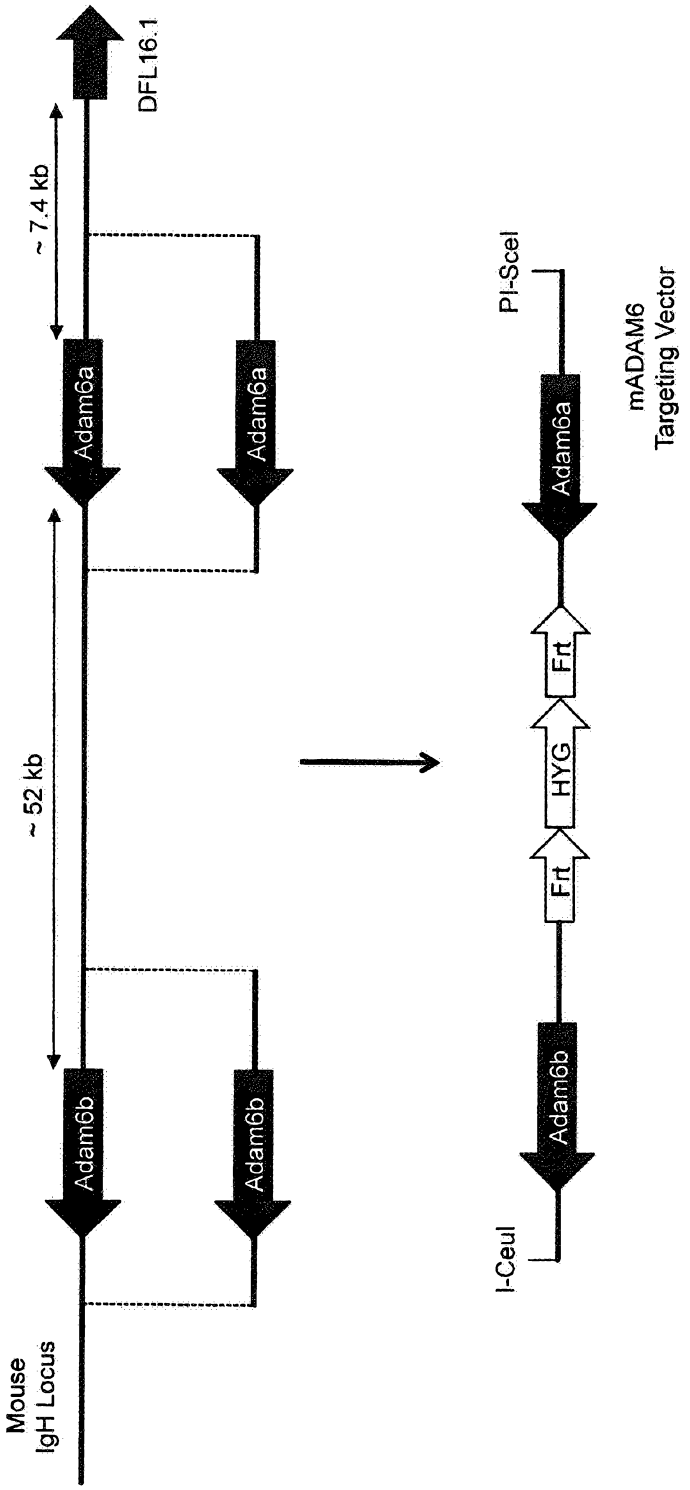
DFL16.1
~ 7.4 kb
Adam6a
Adam6a
~ 52 kb
Adam6b
Adam6b
Mouse
IgH Locus
PI-SceI
Adam6a
Frt
HYG
Frt
Adam6b
I-CeuI
mADAM6
Targeting Vector

FIG. 12

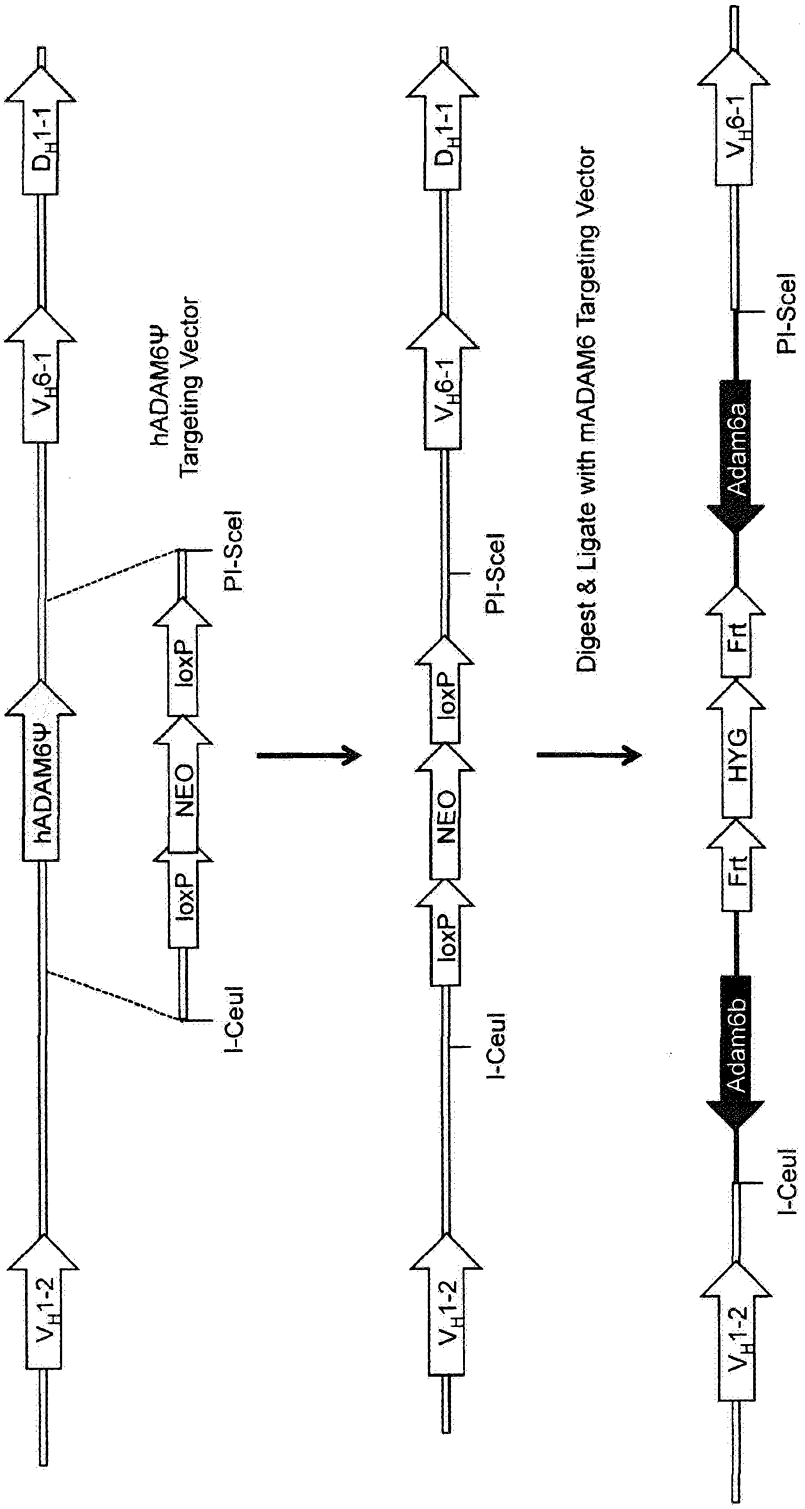
V_H1-2
hADAM6Ψ
V_H6-1
D_H1-1
I-CeuI
loxP
NEO
loxP
PI-SceI
hADAM6Ψ Targeting Vector
Digest & Ligate with mADAM6 Targeting Vector
Adam6b
Frt
HYG
Frt
Adam6a

FIG. 13

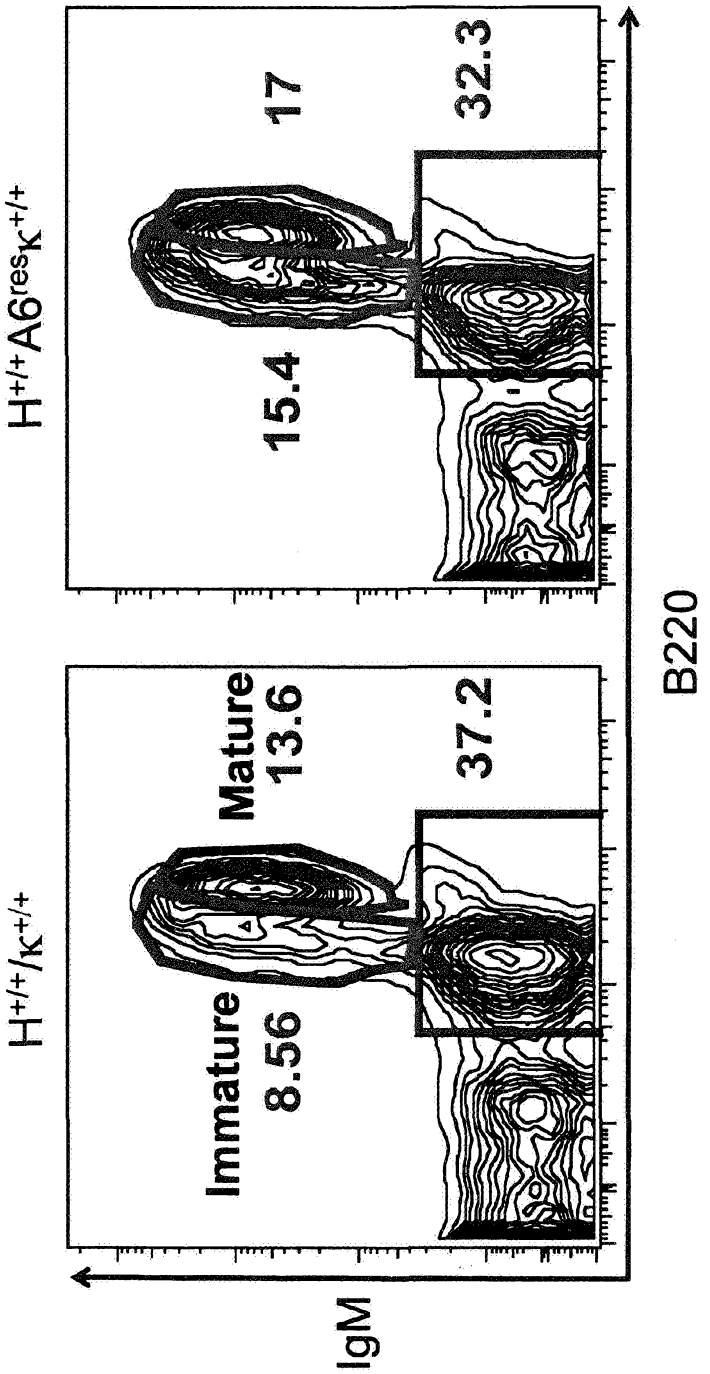

FIG. 14A

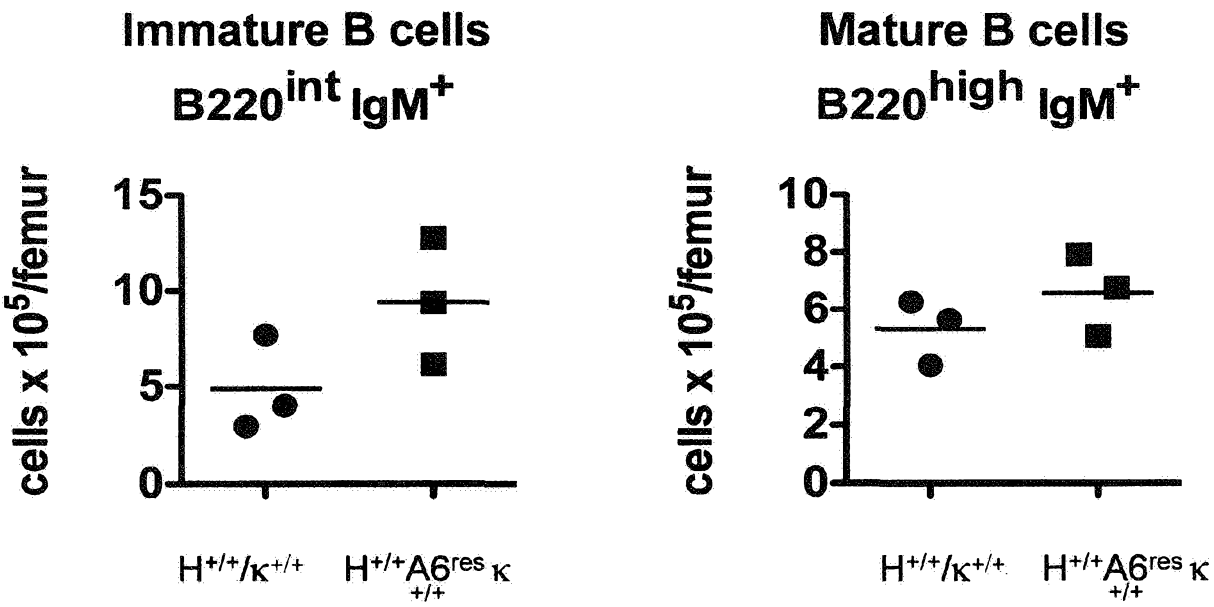

FIG. 14B

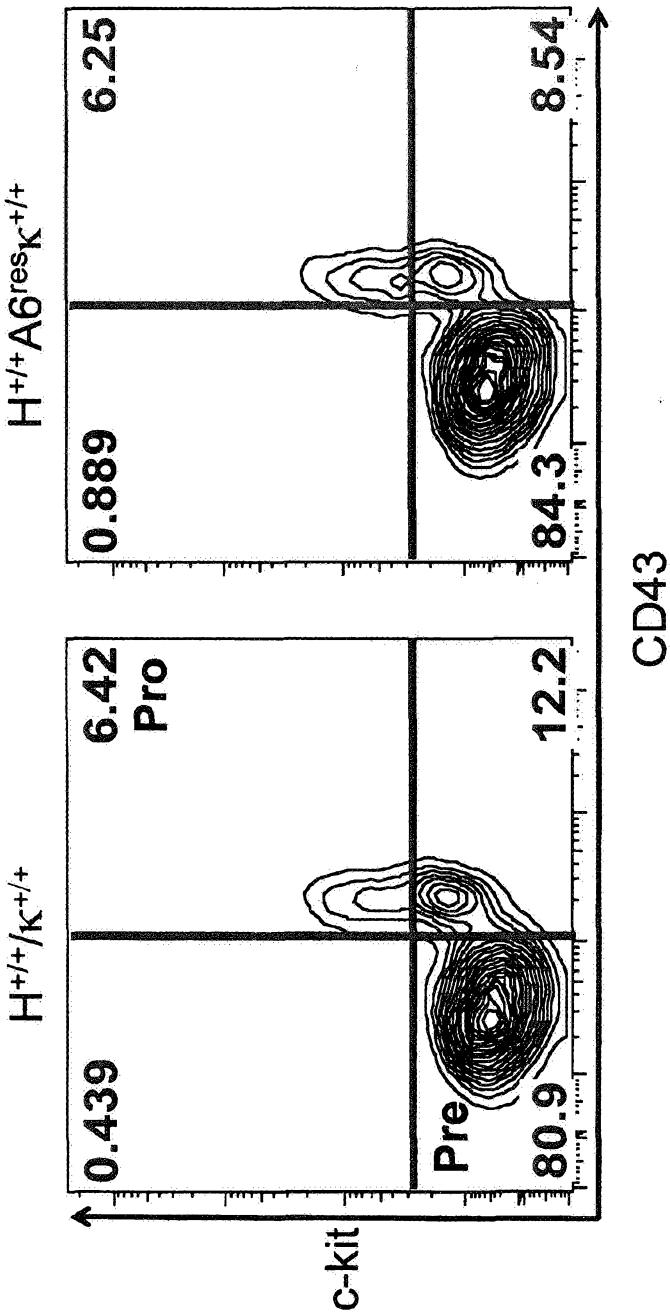

FIG. 15A

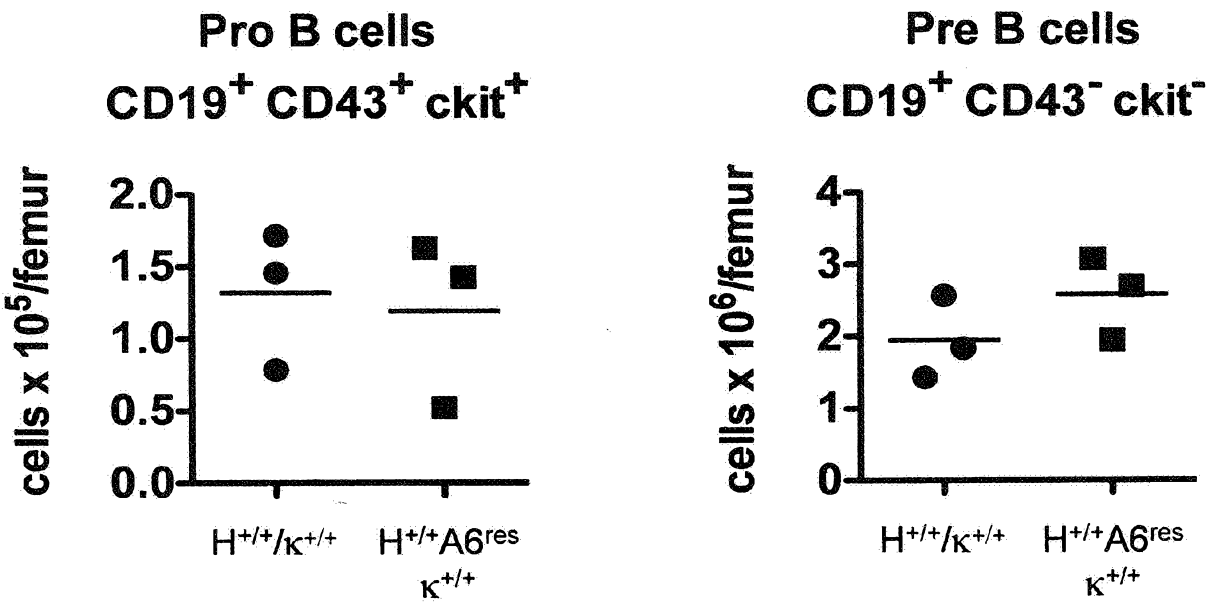

FIG. 15B

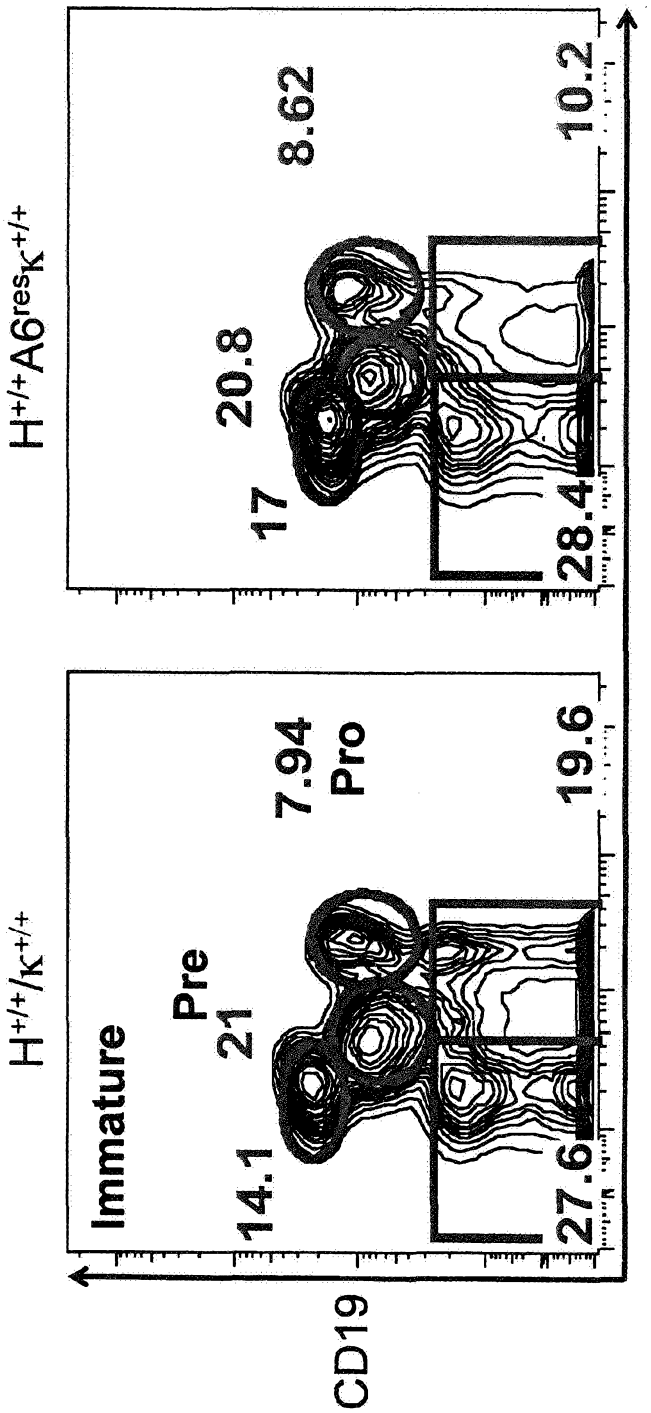
H+/+/κ+/+
H+/+A6resκ+/+
Immature
Pre
21
14.1
7.94
Pro
19.6
27.6
20.8
17
8.62
10.2
28.4
CD19
CD43

FIG. 16A

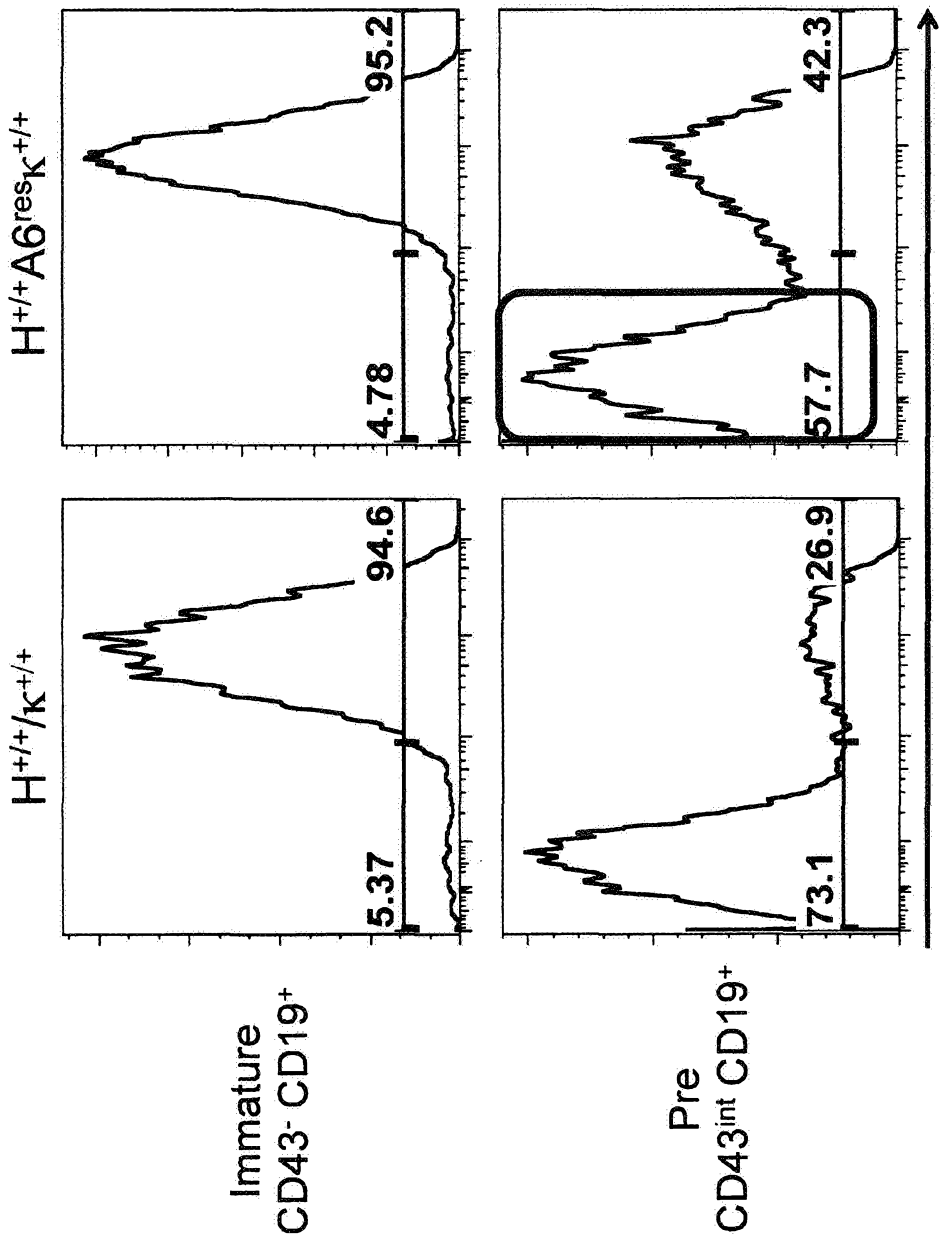
H+/+/κ+/+
H+/+A6resκ+/+
Immature
CD43- CD19+
Pre
CD43int CD19+
5.37
94.6
4.78
95.2
73.1
26.9
57.7
42.3
IgM

FIG. 16B

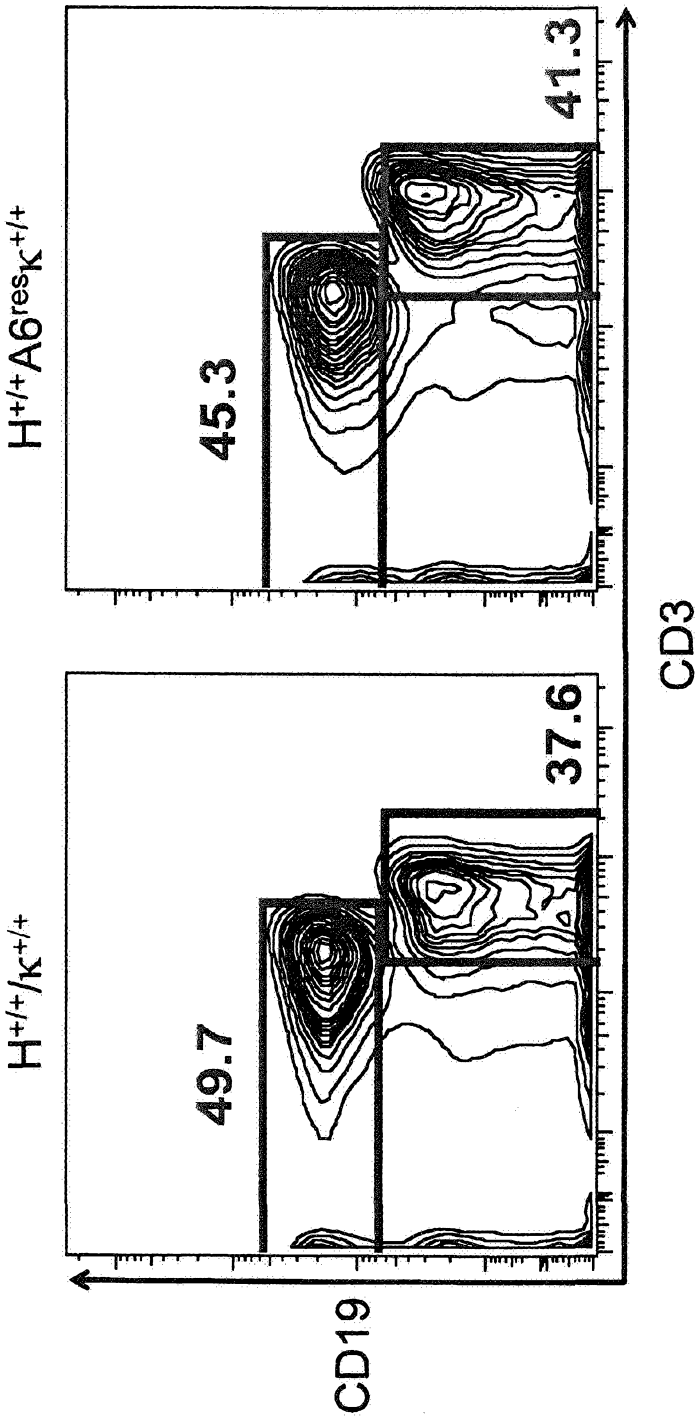

FIG. 17A

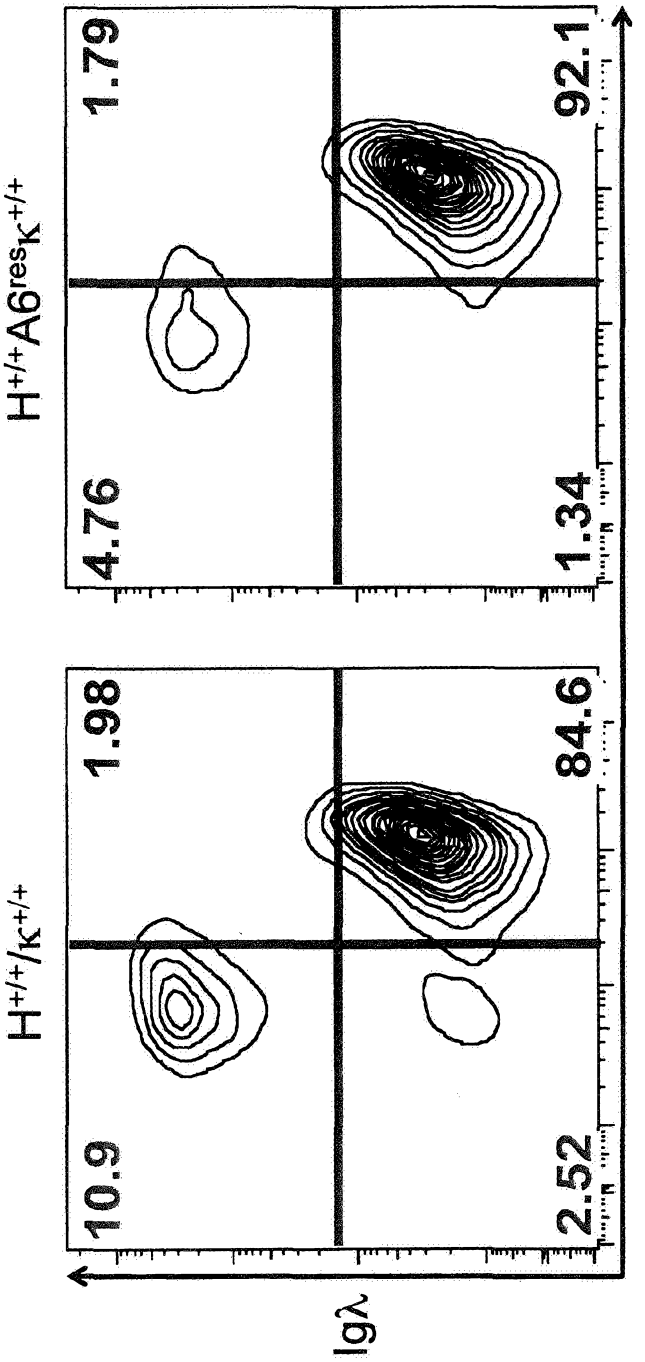

FIG. 17B

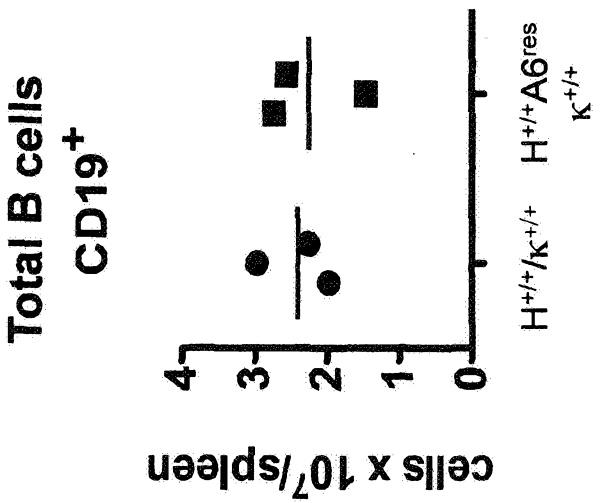

FIG. 17C

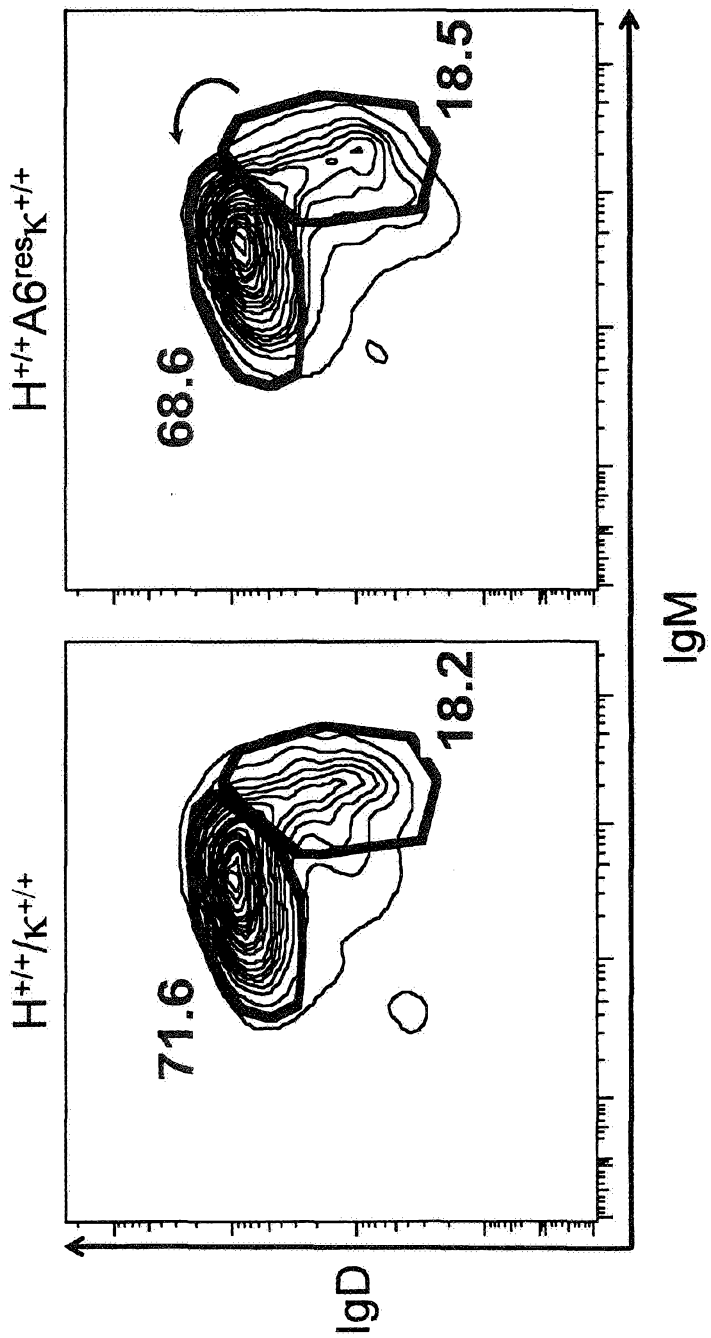

FIG. 18A

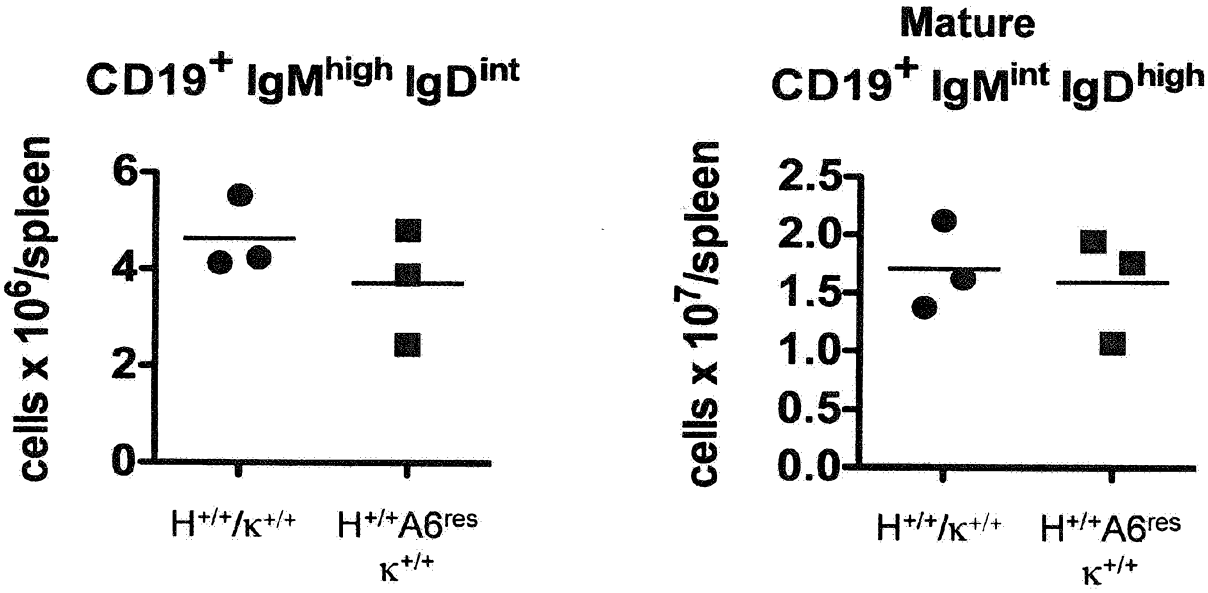

FIG. 18B

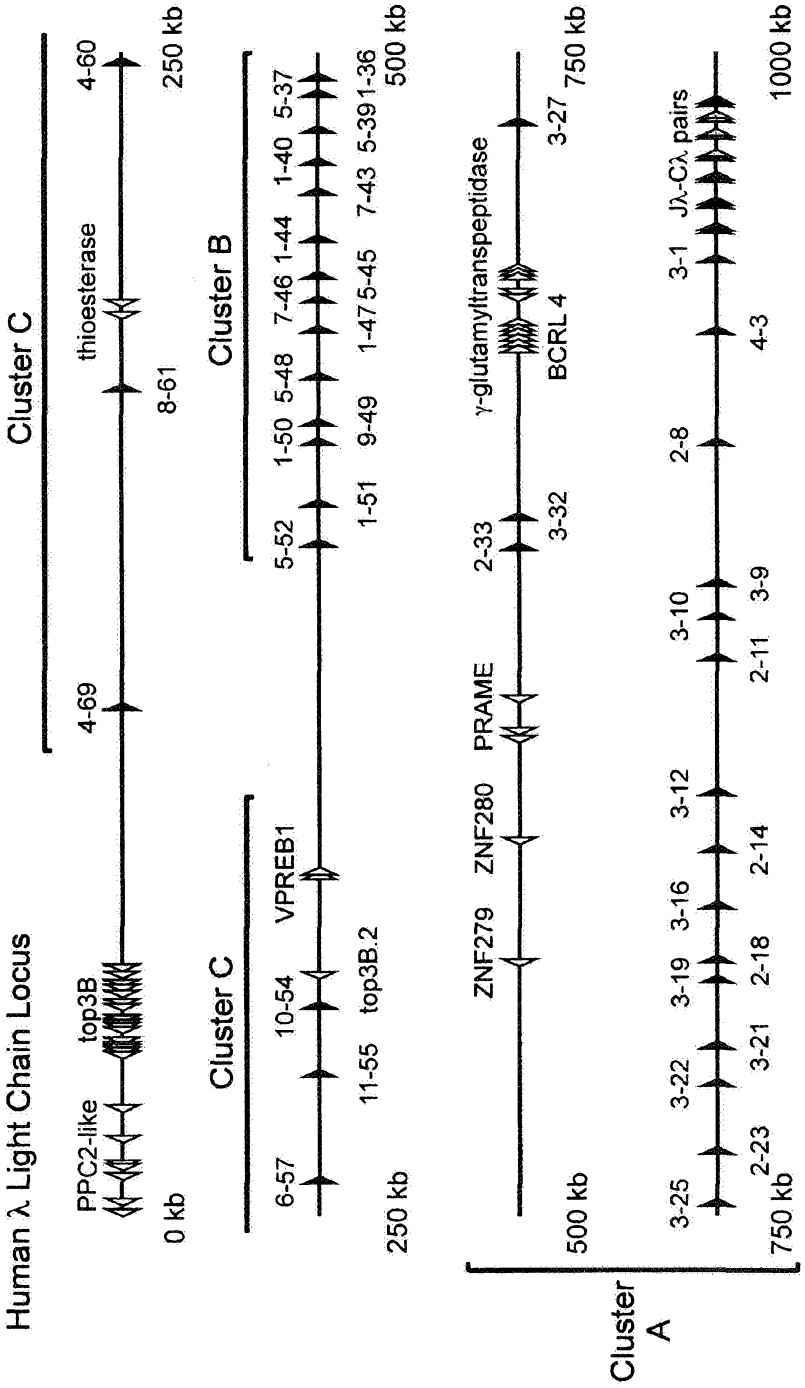
Human λ Light Chain Locus
Cluster C
PPC2-like
top3B
4-69
thioesterase
4-60
0 kb
8-61
250 kb
Cluster B
Cluster C
6-57
10-54
VPREB1
5-52
1-50
5-48
7-46
1-44
1-40
5-37
11-55
top3B.2
1-51
9-49
1-47
5-45
7-43
5-39
1-36
250 kb
500 kb
ZNF279
ZNF280
PRAME
2-33
γ-glutamyltranspeptidase
3-32
BCRL 4
3-27
500 kb
750 kb
Cluster A
3-25
3-22
3-19
3-16
3-12
3-10
2-8
3-1
Jλ-Cλ pairs
2-23
3-21
2-18
2-14
2-11
3-9
4-3
750 kb
1000 kb

FIG. 19

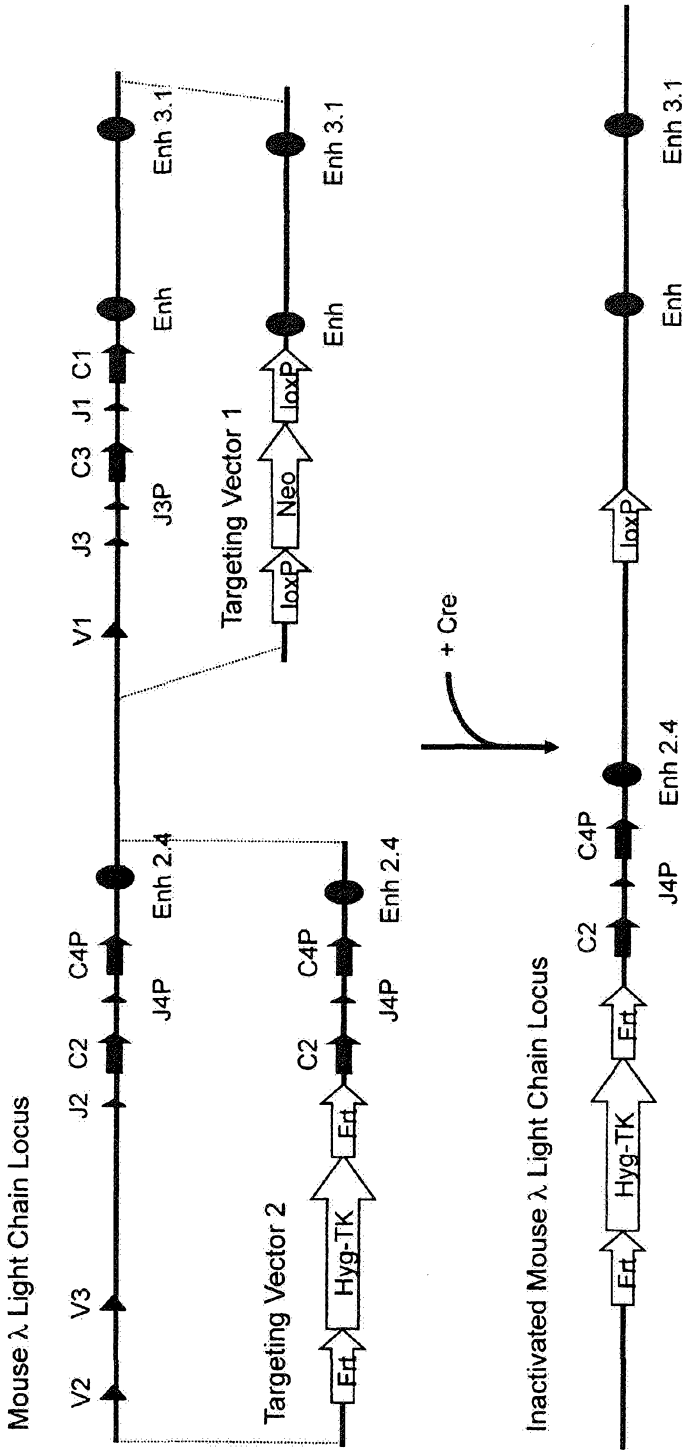
Mouse λ Light Chain Locus
V2
V3
J2
C2
J4P
C4P
Enh 2.4
Enh
J3P
J3
V1
C3
J1
C1
Enh 3.1
Targeting Vector 2
Frt
Hyg-TK
Frt
Targeting Vector 1
loxP
Neo
loxP
+ Cre
Inactivated Mouse λ Light Chain Locus
Frt
Hyg-TK
Frt
loxP

FIG. 20

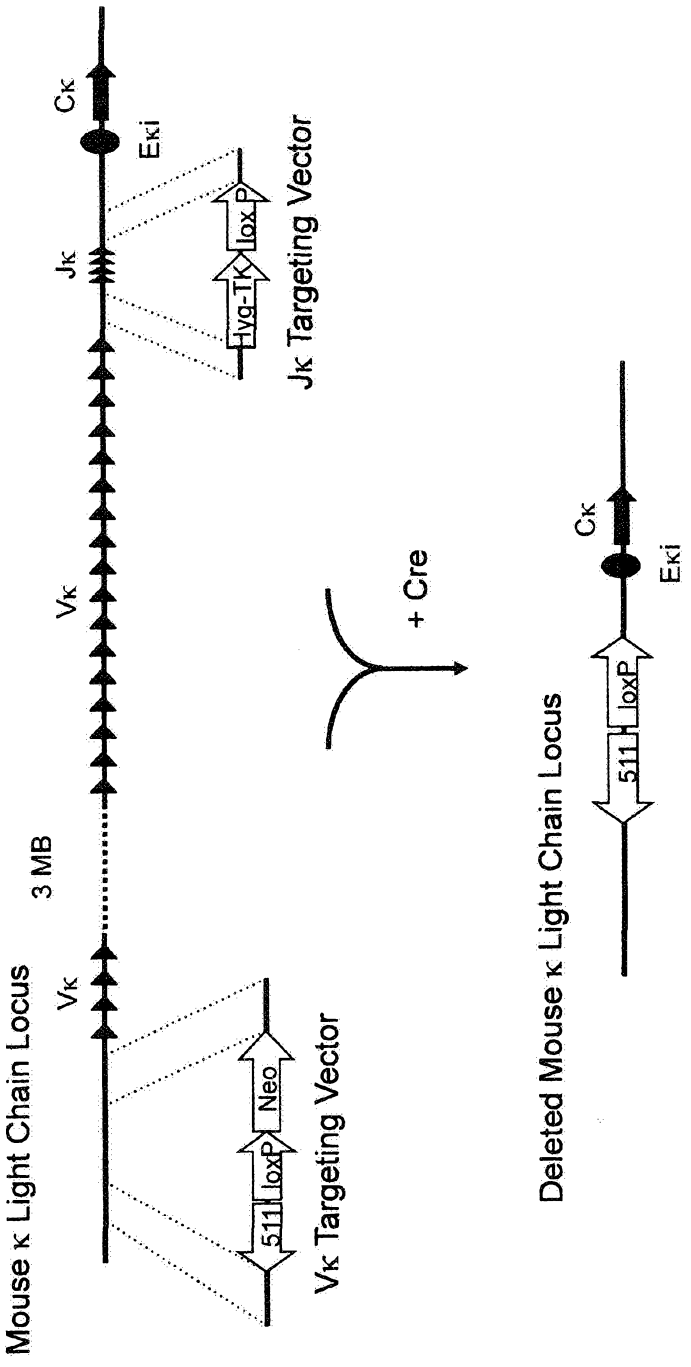
Mouse κ Light Chain Locus
3 MB
Vκ
Vκ
Jκ
Eκi
Cκ
511
loxP
Neo
Vκ Targeting Vector
Ivg-TK
loxP
Jκ Targeting Vector
+ Cre
Deleted Mouse κ Light Chain Locus
511
loxP
Cκ
Eκi

FIG. 21

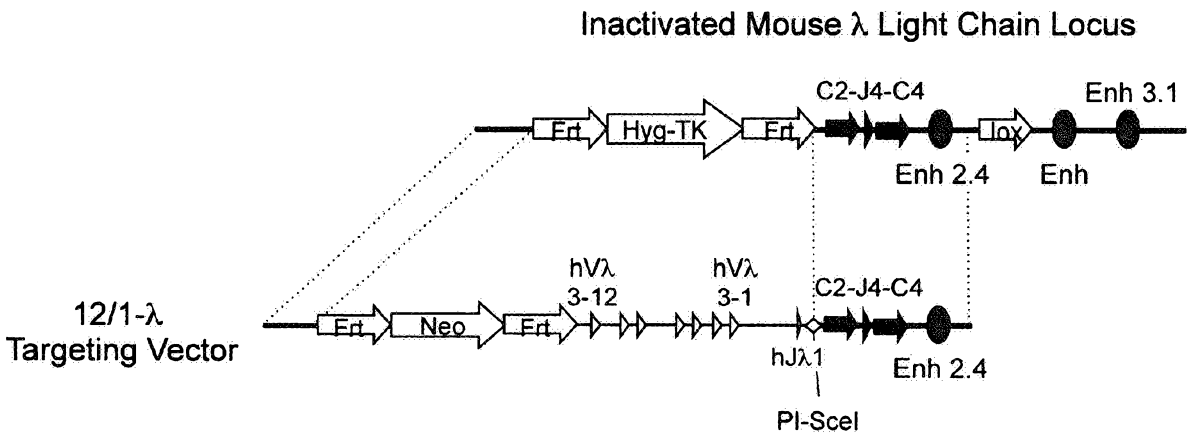

FIG. 22A

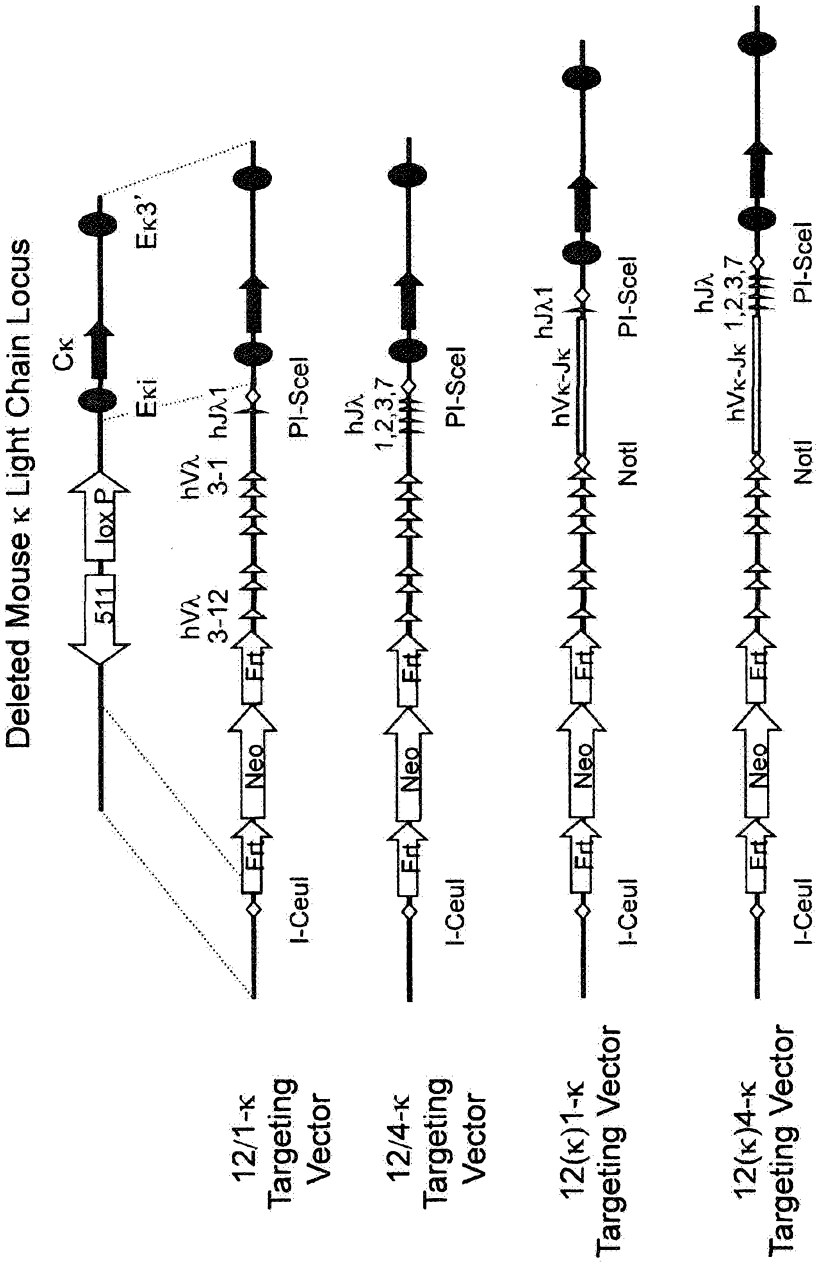
Deleted Mouse κ Light Chain Locus
Cκ
511
lox P
Eκi
Eκ3'
hVλ 3-12
hVλ 3-1
hJλ1
Frt
Neo
Frt
I-CeuI
PI-SceI
12/1-κ Targeting Vector
hJλ 1,2,3,7
12/4-κ Targeting Vector
hVκ-Jκ
12(κ)1-κ Targeting Vector
NotI
hJλ 1,2,3,7
12(κ)4-κ Targeting Vector

FIG. 22B

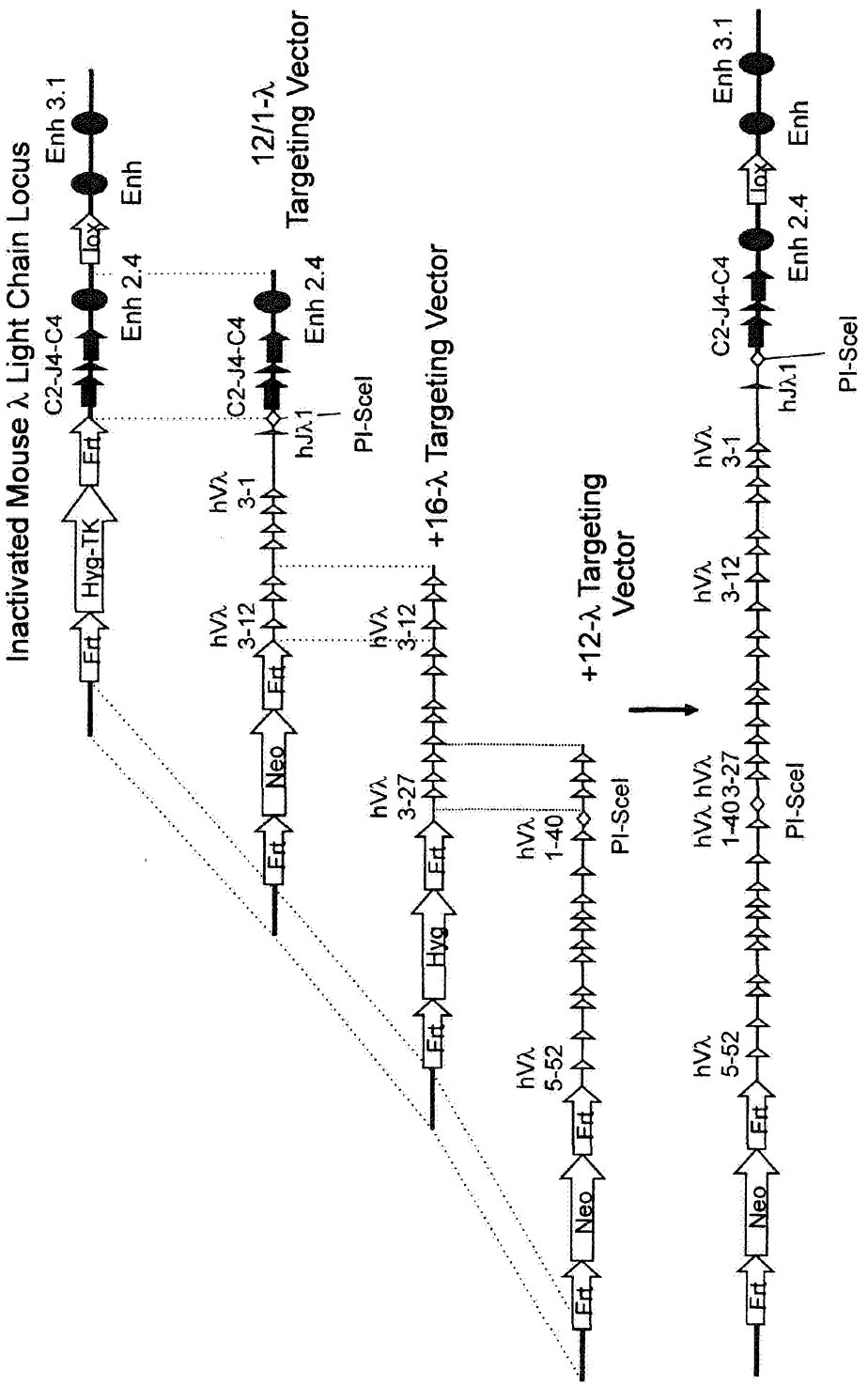
Inactivated Mouse λ Light Chain Locus
C2-J4-C4
Enh 3.1
Enh 2.4
Enh
Ert
Hyg-TK
lox
12/1-λ Targeting Vector
hVλ 3-1
hJλ1
PI-SceI
hVλ 3-12
Neo
+16-λ Targeting Vector
hVλ 3-27
Hyg
hVλ 1-40
hVλ 5-52
+12-λ Targeting Vector
hVλ 1-40 3-27

FIG. 23A

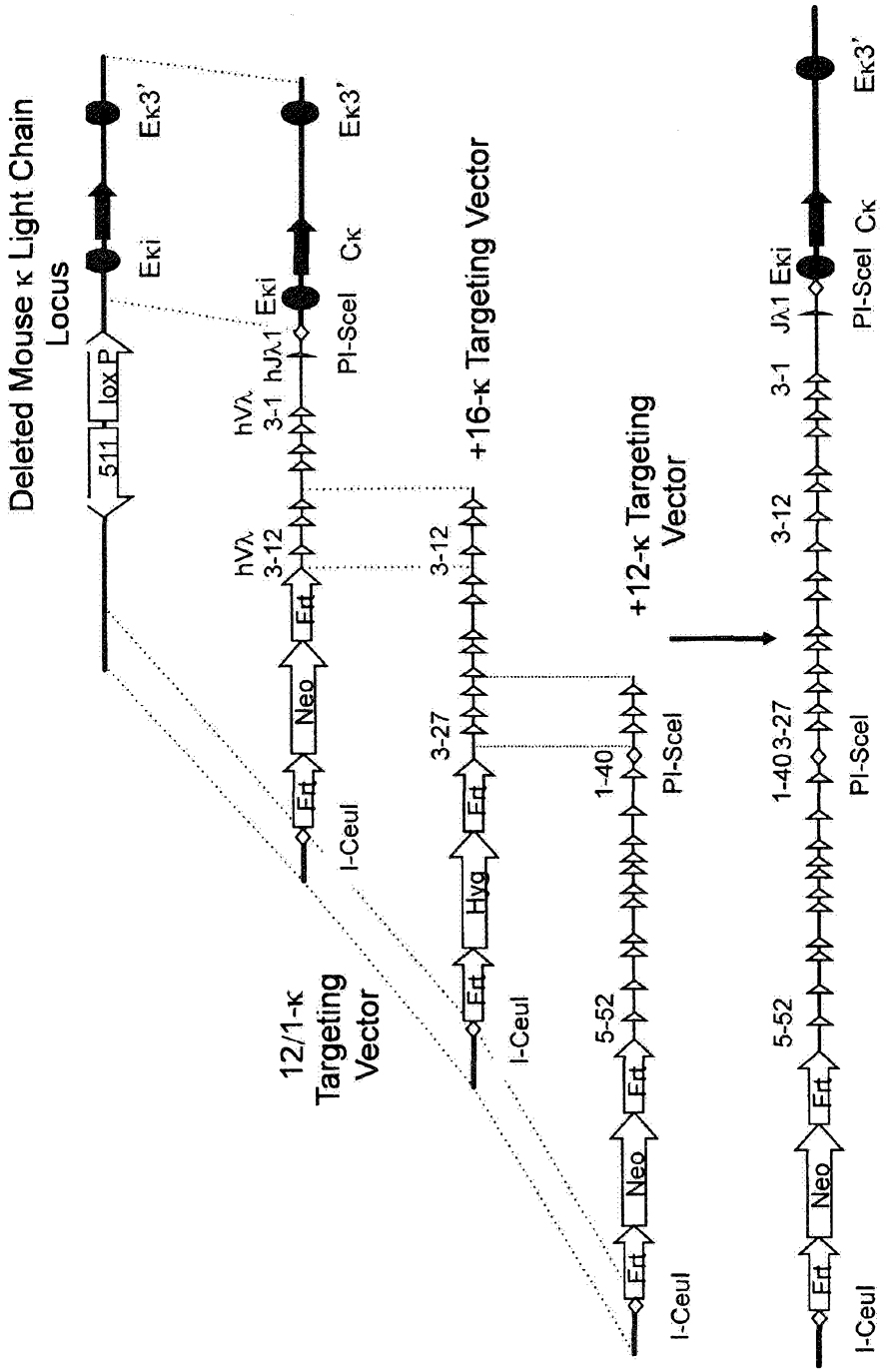
Deleted Mouse κ Light Chain Locus
511
lox P
Eκi
Eκ3'
hVλ 3-1
hJλ1
Eκi
PI-SceI
Cκ
Eκ3'
12/1-κ Targeting Vector
hVλ 3-12
Ert
Neo
Ert
I-CeuI
+16-κ Targeting Vector
3-12
3-27
Ert
Hyg
Ert
I-CeuI
+12-κ Targeting Vector
1-40
PI-SceI
5-52
Ert
Neo
Ert
I-CeuI
3-12
1-40 3-27
PI-SceI
5-52
Ert
Neo
Ert
I-CeuI
3-1 Jλ1 Eκi
PI-SceI Cκ
Eκ3'

FIG. 23B

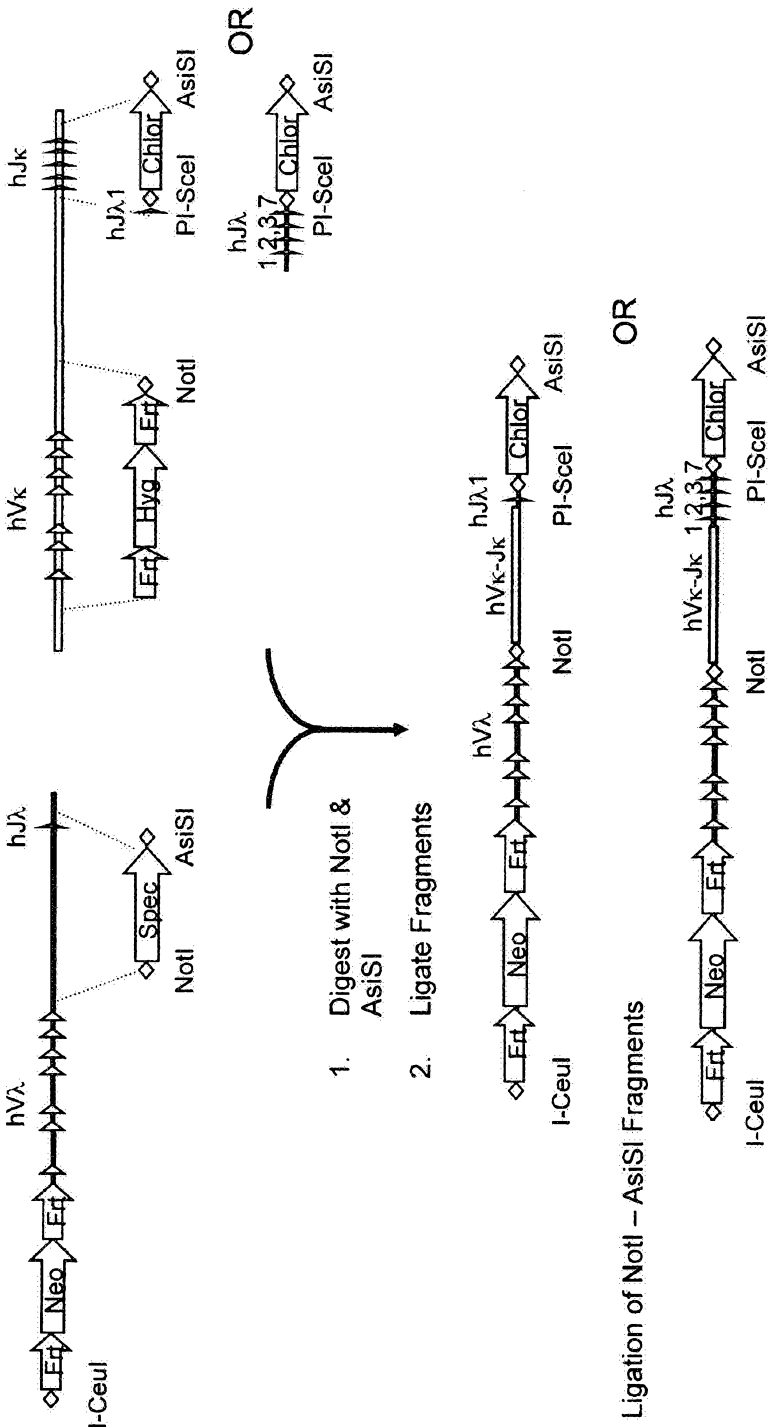
hVλ
Ert
Neo
Ert
I-CeuI
hJλ
Spec
NotI
AsiSI
hVκ
Ert
Hyg
Ert
NotI
hJκ
hJλ1
Chlor
PI-SceI
AsiSI
OR
hJλ 1,2,3,7
Chlor
PI-SceI
AsiSI
1. Digest with NotI & AsiSI
2. Ligate Fragments
hVλ
Ert
Neo
Ert
I-CeuI
NotI
hVκ-Jκ
hJλ1
Chlor
PI-SceI
AsiSI
OR
Ligation of NotI – AsiSI Fragments
hVλ
Ert
Neo
Ert
I-CeuI
NotI
hVκ-Jκ
hJλ 1,2,3,7
Chlor
PI-SceI
AsiSI

FIG. 24

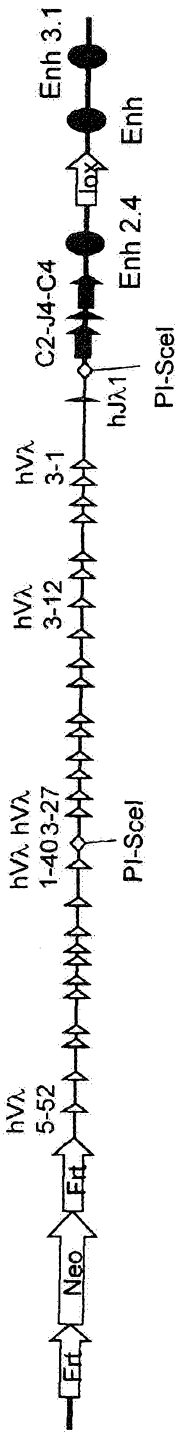
Enh 3.1
Enh
Lox
Enh 2.4
C2-J4-C4
PI-SceI
hJλ1
hVλ 3-1
hVλ 3-12
hVλ hVλ 1-40 3-27
PI-SceI
hVλ 5-52
Ert
Neo
Ert

FIG. 25A

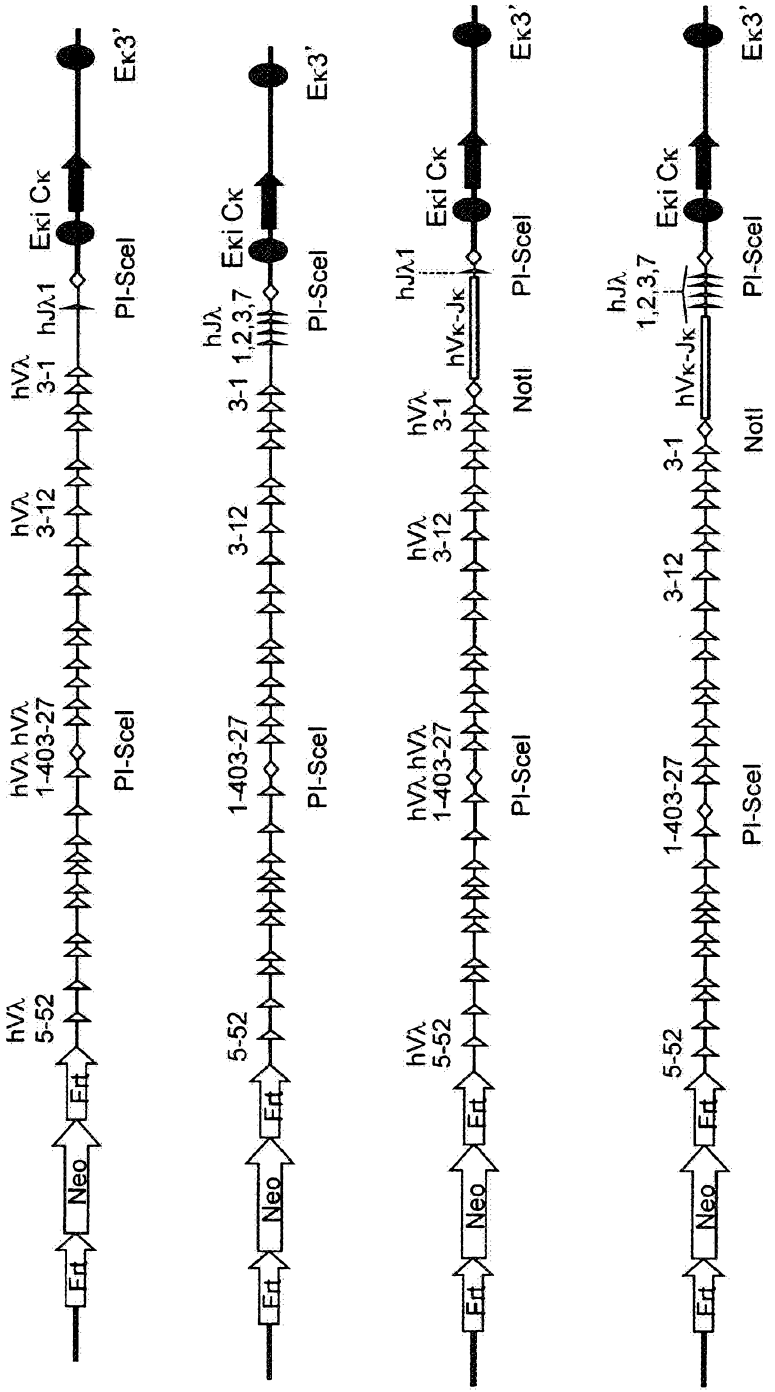
hVλ 5-52
Frt
Neo
Frt
hVλ 1-403-27
hVλ 3-12
hVλ 3-1
hJλ1
Eκi Cκ
PI-SceI
Eκ3'
hJλ 1,2,3,7
hVκ-Jκ
NotI

FIG. 25B

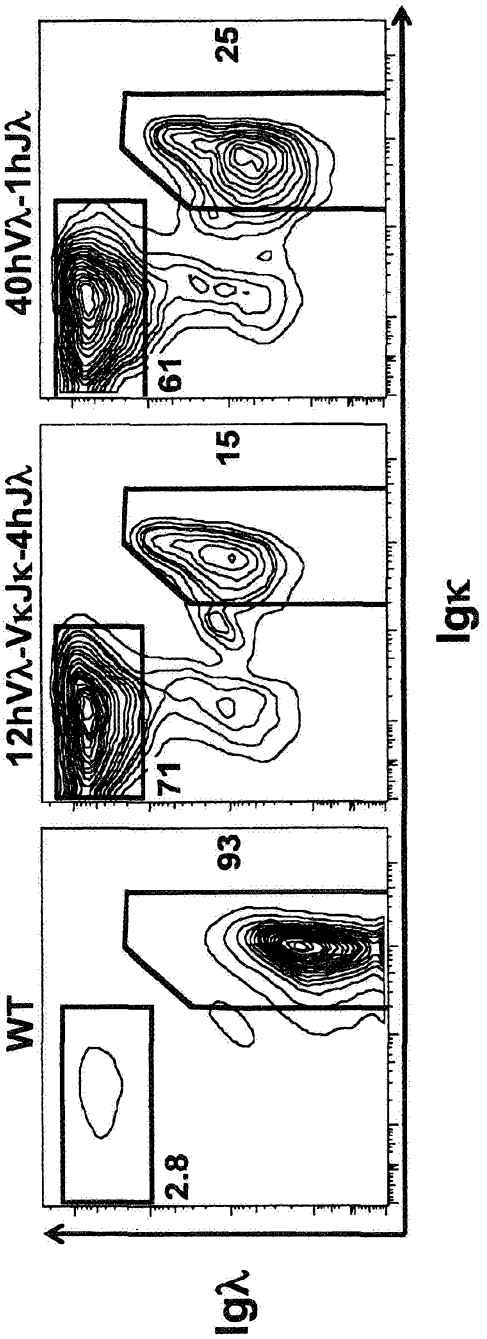

FIG. 26A

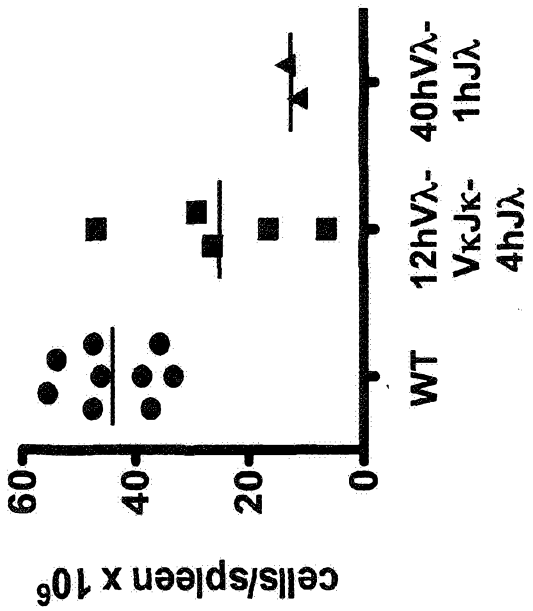

FIG. 26B

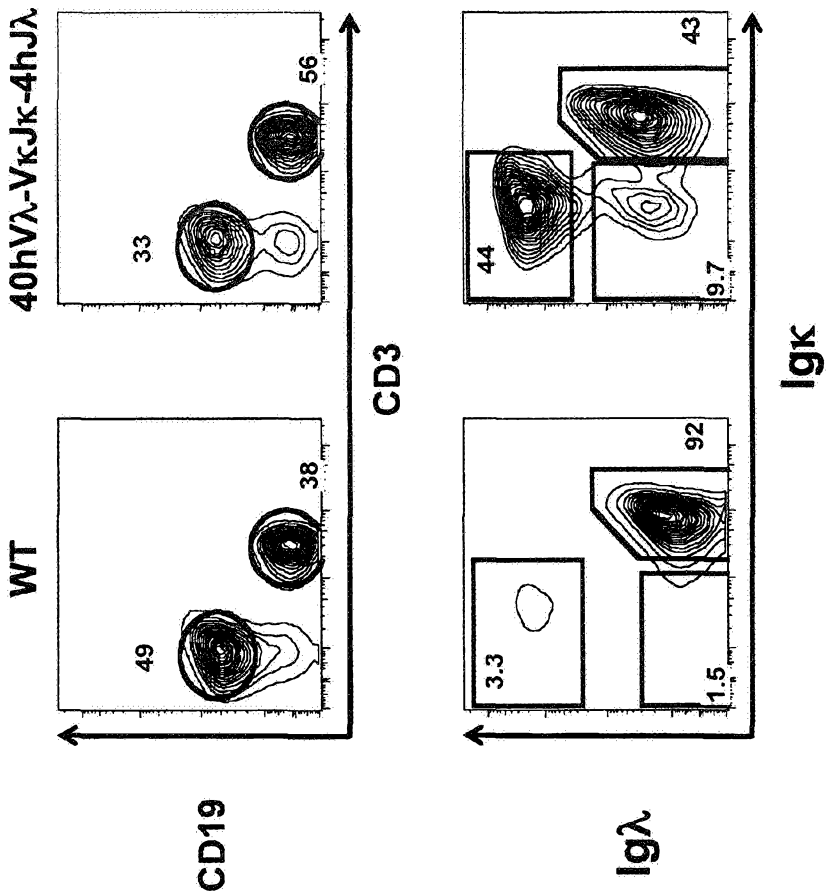

FIG. 27A

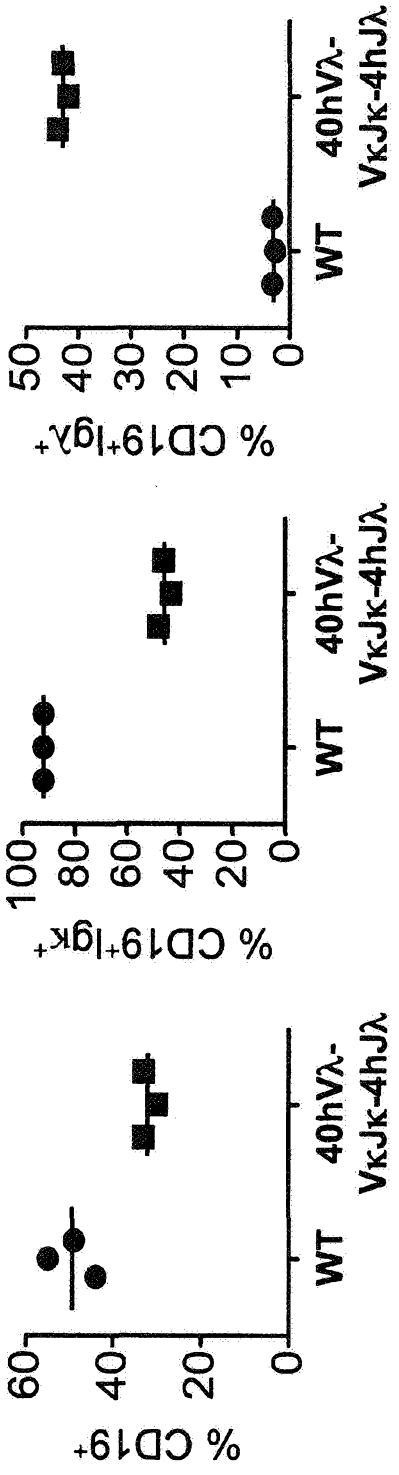
% CD19+
60
40
20
0
WT
40hVλ-VκJκ-4hJλ
% CD19+IgK+
100
80
60
40
20
0
WT
40hVλ-VκJκ-4hJλ
% CD19+Igλ+
50
40
30
20
10
0
WT
40hVλ-VκJκ-4hJλ

FIG. 27B

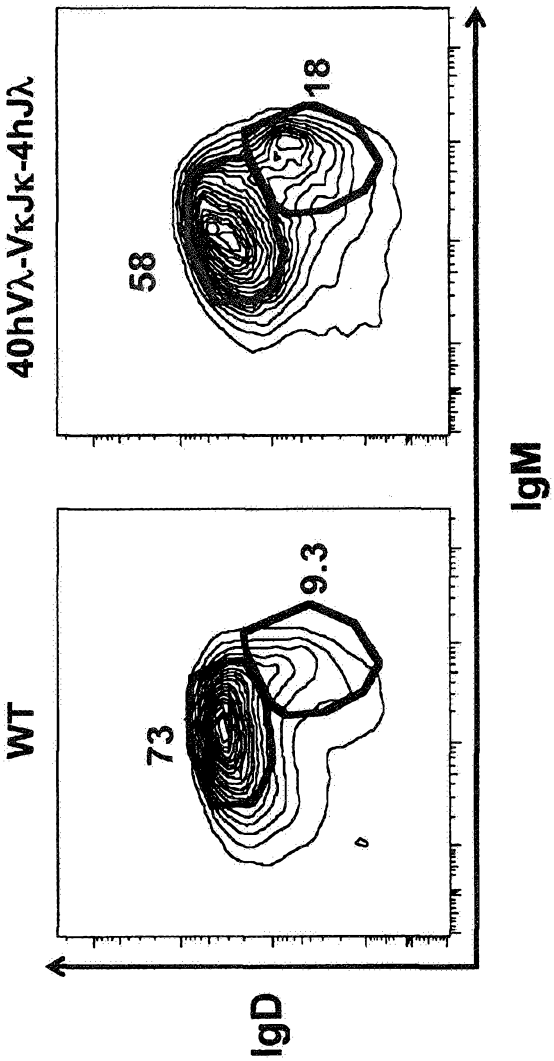

FIG. 27C

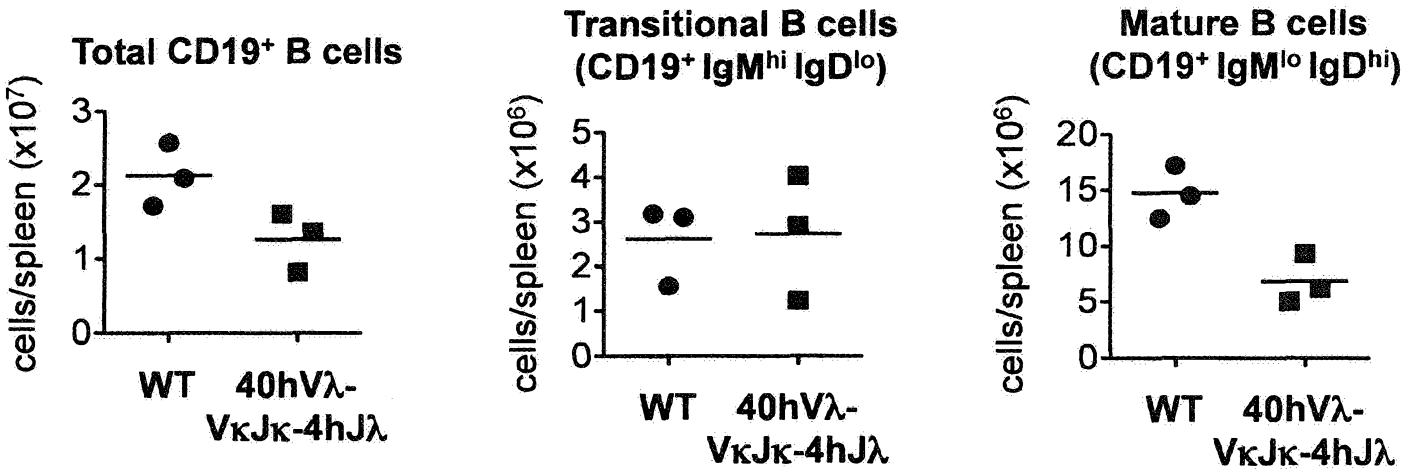

FIG. 27D

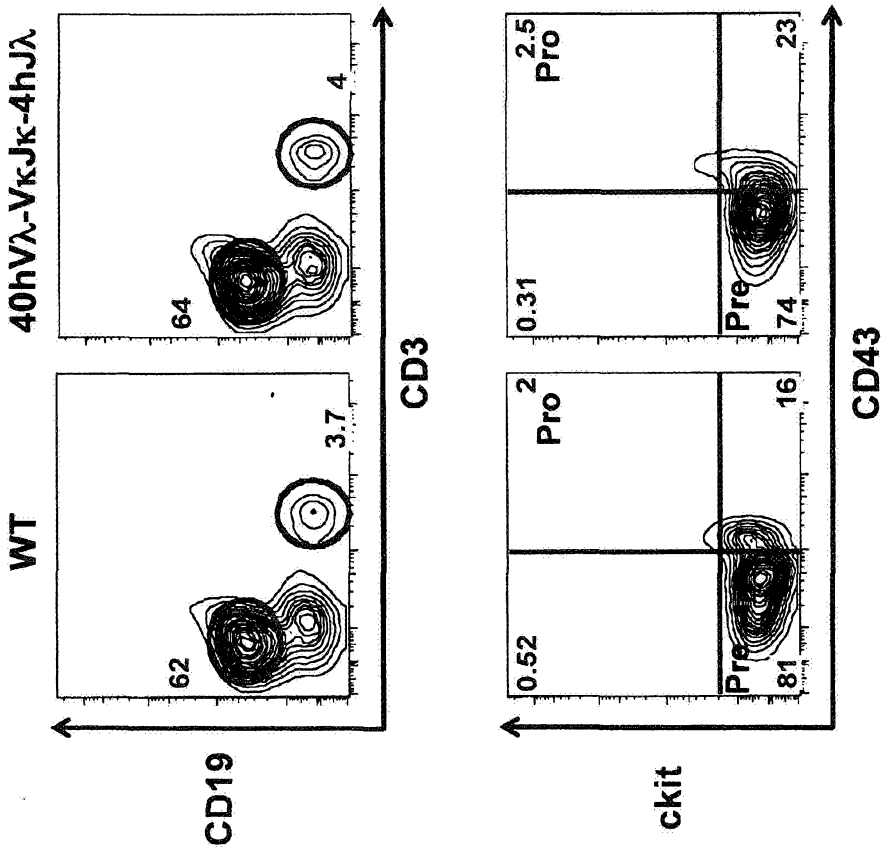
WT
40hVλ-VκJκ-4hJλ
CD19
CD3
62
3.7
64
4
ckit
CD43
0.52
Pro 2
16
Pre
81
0.31
Pro 2.5
23
Pre
74

FIG. 28A

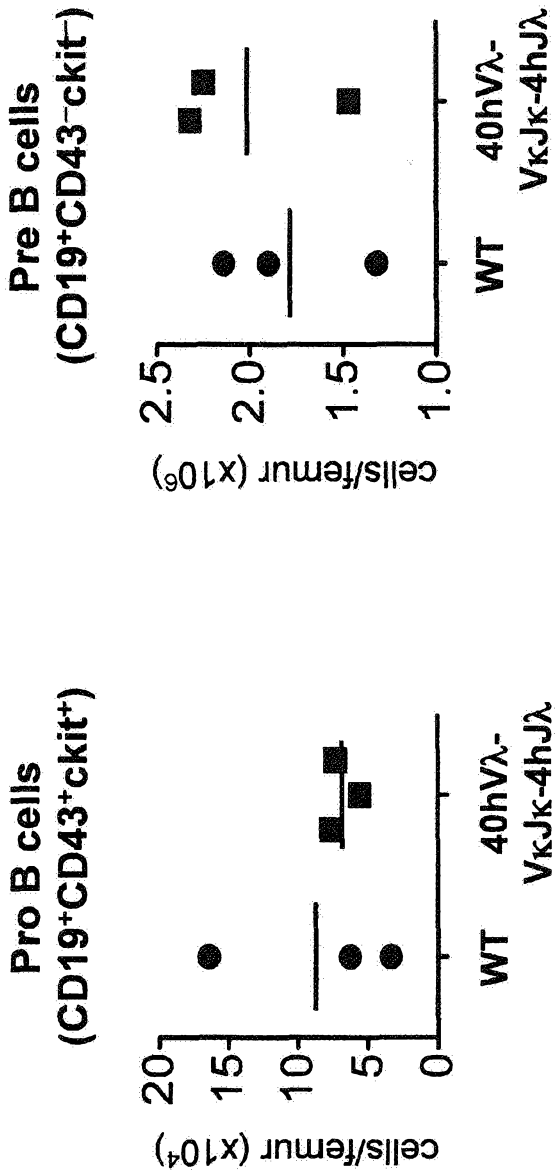
Pro B cells
(CD19+CD43+ckit+)
cells/femur (x10^4)
20
15
10
5
0
WT
40hVλ-VκJκ-4hJλ
Pre B cells
(CD19+CD43−ckit−)
cells/femur (x10^6)
2.5
2.0
1.5
1.0
WT
40hVλ-VκJκ-4hJλ

FIG. 28B

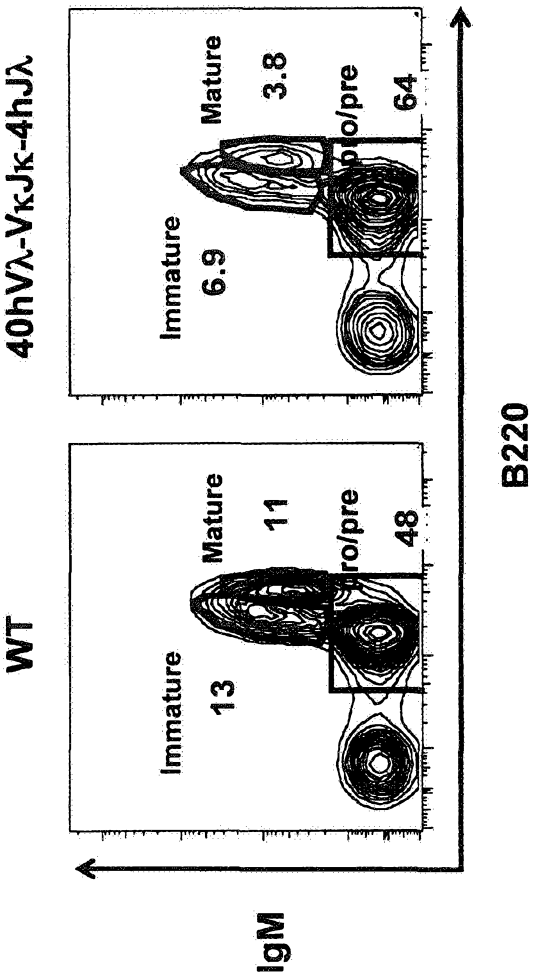
WT
40hVλ-VκJκ-4hJλ
Immature
13
Mature
11
Pro/pre
48
Immature
6.9
Mature
3.8
Pro/pre
64
IgM
B220

FIG. 28C

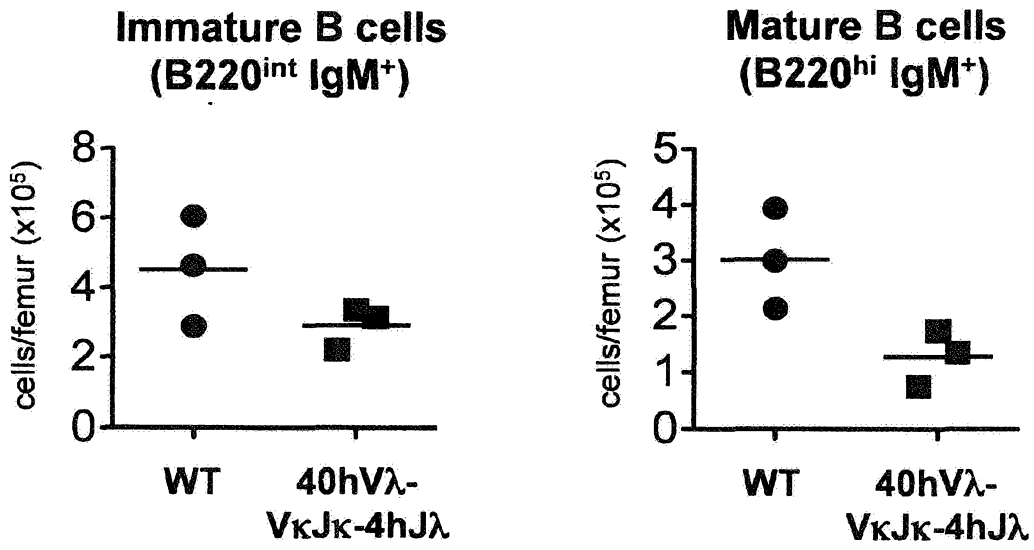

FIG. 28D

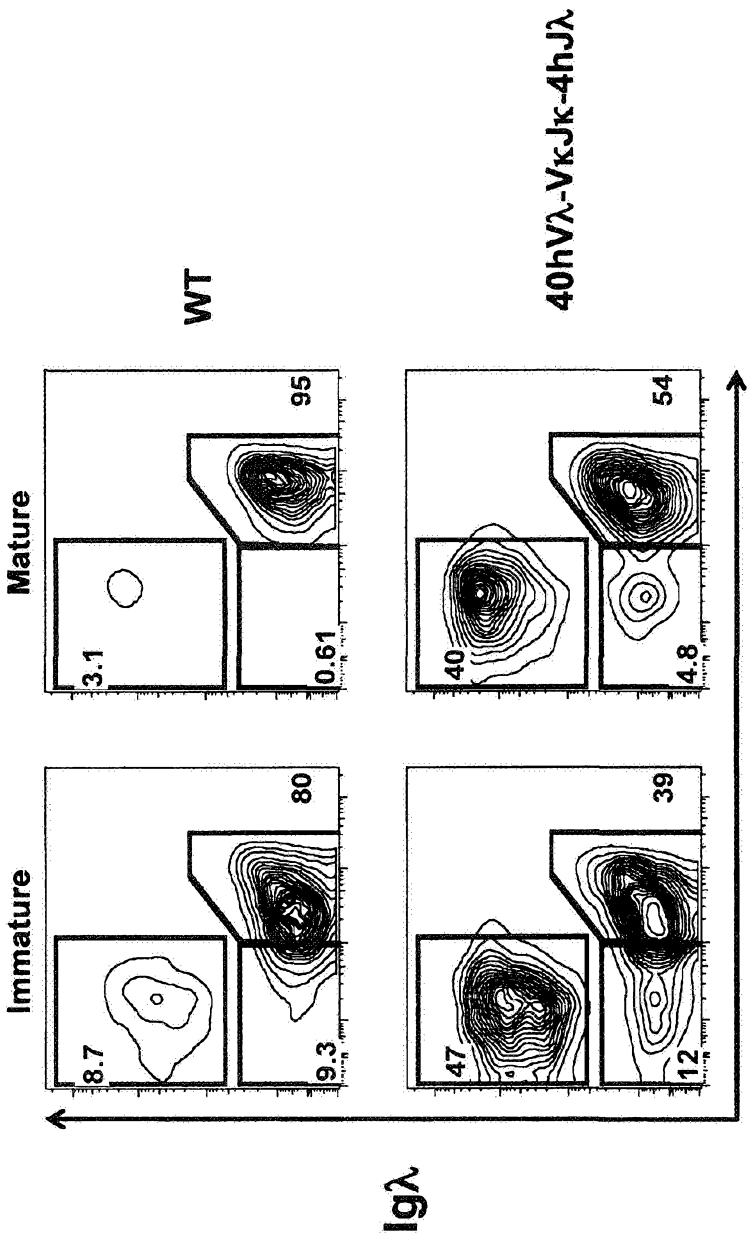
WT
40hVλ-VκJκ-4hJλ
Mature
Immature
3.1
95
0.61
40
54
4.8
8.7
80
9.3
47
39
12
Igλ
Igκ

FIG. 28E

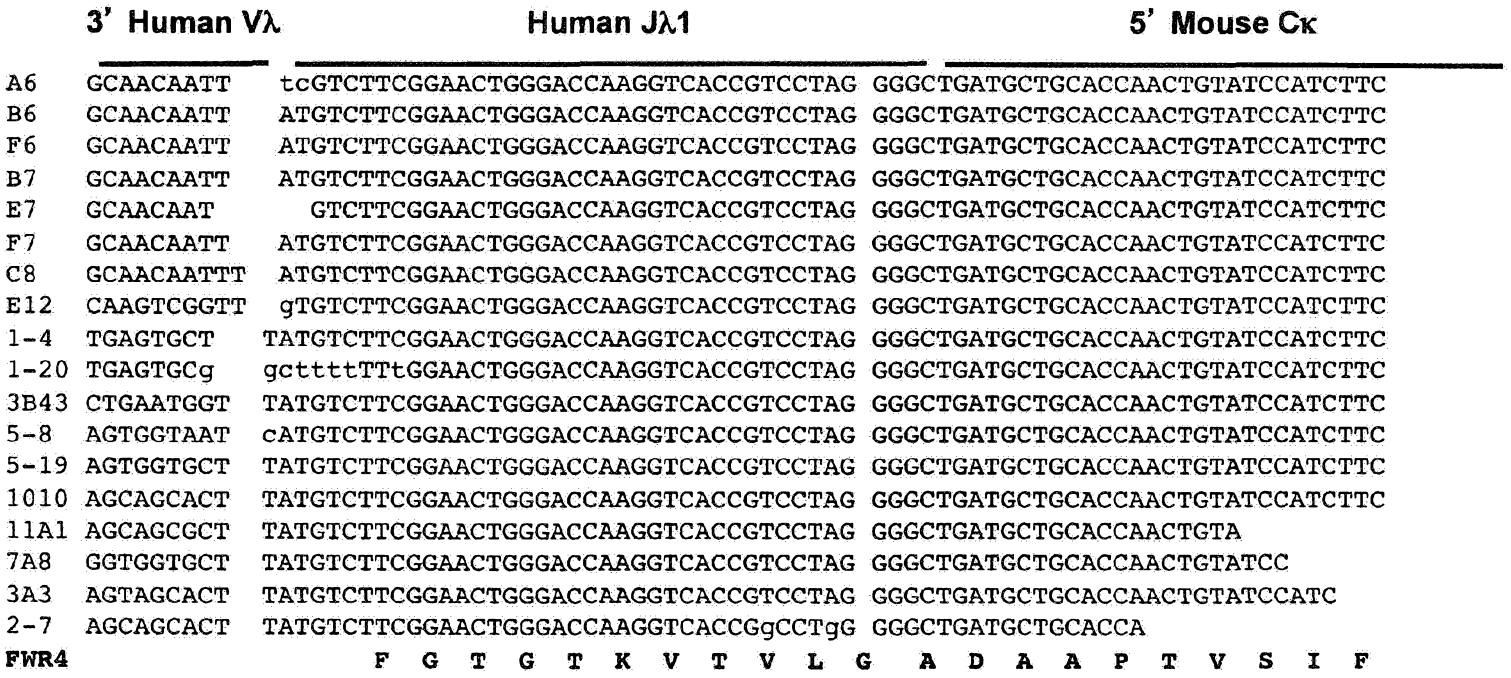

FIG. 29

DK/EP 2793567 T3

	3' Human Vλ	Human Jλ	5' Mouse Cκ
5-2	CAGCCTGAGTGGTTC	TGTGTTCGGAGGAGGCACCCGGCTGACCGCCCTCG	GGGCTGATGCTGCACCAACTGTATCCATC
2-5	CAGCCTGAGTGGTT	ATGTCTTCGGAACTGGGACCAAGGTCACCGTCCTAG	GGGCTGATGCTGCACCAACTGTATCCATC
1-3	CAGCCTGAATGGT	GCTGTGTTCGGAGGAGGCACCCAGCTGACCGCCCTCG	GGGCTGATGCTGCACCAACTGTATCCATC
4B-1	CAGCCTGAGTGGTC	GGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG	GGGCTGATGCTGCACCAACTGTATCCATC
3B-5	CAGCAGCACTGC	TGTGTTCGGAGGAGGCACCCAGCTGACCGCCCTCG	GGGCTGATGCTGCACCAACTGTATCCATC
7A-1	CAGCAGTGGTAAT	GCTGTGTTCGGAGGAGGCACCCAGCTGACCGCCCTCG	GGGCTGATGCTGCACCAACTGTATCCATC
5-1	CAGCAGTGGTAATCATAG	GGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG	GGGCTGATGCTGCACCAACTGTATCCATC
4A-1	CAGCCTGAGTGGTT	ATGTCTTCGGAACTGGGACCAAGGTCACCGTCCTAG	GGGCTGATGCTGCACCAACTGTATCCATC
11A-1	CAGCAGCGCT	GTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAG	GGGCTGATGCTGCACCAACTGTATCCATC
5-7	CTACTATGGTGGTGCTC	GGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTAG	GGGCTGATGCTGCACCAACTGTATCCATC
5-4	CTCCTATAGTGGTGCTCGa	GTATTCGGCGGAGGGACCAAGCTGACCGTCCTAG	GGGCTGATGCTGCACCAACTGTATCCATC
2-3	GAGCAACTTCGTGT	CTGTGTTCGGAGGAGGCACCCAGCTGACCGCCCTCG	GGGCTGATGCTGCACCAACTGTATCCATC
FWR4		**F G G G T K L T V L G A D A A P T V S I**	

FIG. 30

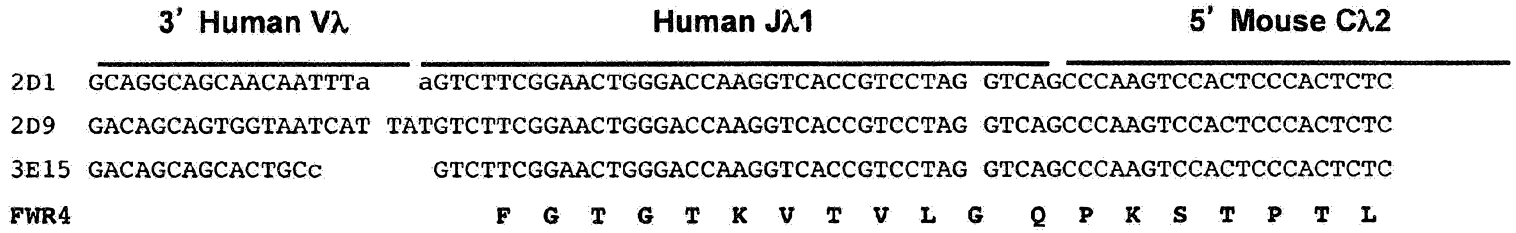

FIG. 31