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(54) Benævnelse: **Mus med en konstrueret immunglobulin-lambda-letkæde og anvendelser deraf**

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DESCRIPTION

Description

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Application No. 62/594,944, filed December 5, 2017; United States Provisional Application No. 62/594,946, filed December 5, 2017; United States Provisional Application No. 62/609,241, filed December 21, 2017; and United States Provisional Application No. 62/609,251, filed December 21, 2017.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing.

BACKGROUND

[0003] Human antibodies are the most rapidly growing class of therapeutics. Of the technologies that are currently used for their production, the development of genetically engineered animals (e.g., mice) engineered with genetic material encoding human antibodies, in whole or in part, has revolutionized the field of human therapeutic monoclonal antibodies for the treatment of various diseases. Still, development of improved *in vivo* systems for generating human monoclonal antibodies that maximize human antibody repertoires in host genetically engineered animals is needed.

SUMMARY

[0004] A method of producing an antibody in a genetically modified mouse is provided, the method comprising the steps of:

1. (a) immunizing the genetically modified mouse with an antigen of interest,
   wherein the genetically modified mouse has a germline genome comprising:

   a first engineered endogenous immunoglobulin κ light chain locus comprising:

   1. (i) one or more human Vλ gene segments,
   2. (ii) one or more human Jλ gene segments, and
   3. (iii) one mouse Cλ gene,

   wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are operably linked to the one mouse Cλ gene of (iii);

   wherein the one mouse Cλ gene (iii) is in the place of a mouse Cκ gene at the first engineered endogenous immunoglobulin κ light chain locus;

   wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are in place of one or more endogenous mouse Vκ gene segments and one or more endogenous mouse Jκ gene segments;

   wherein the genetically modified mouse lacks a mouse Cκ gene at the first engineered endogenous immunoglobulin k light chain locus;

2. (b) maintaining the genetically modified mouse under conditions sufficient for the genetically modified mouse to produce an immune response to the antigen of interest; and
3. (c) recovering from the genetically modified mouse:
   1. (i) an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain,
   2. (ii) a nucleotide that encodes a human light chain variable domain or human heavy chain variable domain, a light chain, or a heavy chain of an antibody that binds the antigen of interest, wherein the antibody that binds the antigen of interest comprises a light chain comprising a human λ variable domain and a mouse λ constant domain, or
   3. (iii) a cell that expresses an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain.

[0005] In some embodiments, a mouse Cλ gene has a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to a mouse Cλ1, mouse Cλ2 or a mouse Cλ3 gene. In some embodiments, a mouse Cλ gene is or includes a mouse Cλ1 gene.

[0006] In some embodiments, one or more human Vλ gene segments and one or more human Jλ gene segments replace one or more mouse Vκ gene segments, and one or more mouse Jκ gene segments. In some embodiments, one or more human Vλ gene segments and one or more human Jλ gene segments replace all functional mouse Vκ gene segments and/or all functional mouse Jκ gene segments.

[0007] In some embodiments, one or more human Vλ gene segments include Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1, or any combination thereof. In some embodiments, one or more human Vλ gene segments include Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1, or any combination thereof. In some embodiments, one or more human Vλ gene segments include Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, and Vλ3-1.

[0008] In some embodiments, one or more human Jλ gene segments include Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof. In some embodiments, one or more human Jλ gene segments include Jλ1, Jλ2, Jλ3, Jλ6, and Jλ7.

[0009] In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Vλ non-coding sequences, each of which is adjacent to at least one of the one or more human Vλ gene segments, where the one or more human Vλ non-coding sequences naturally appears adjacent to a human Vλ gene segment in an endogenous human immunoglobulin λ light chain locus. For example, referring to Figure 20, a first exemplary endogenous human Vλ non-coding sequence naturally appears adjacent (and 3') to a Vλ3-12 gene segment in an endogenous human immunoglobulin λ light chain locus. An engineered endogenous immunoglobulin κ light chain locus including the first exemplary endogenous human Vλ non-coding sequence could include that non-coding sequence at a position that is adjacent (and preferably 3') to a Vλ3-12 gene segment in the engineered endogenous immunoglobulin κ light chain locus. An engineered endogenous immunoglobulin κ light chain locus including the first exemplary endogenous human Vλ non-coding sequence could also include that non-coding sequence at a position that is adjacent (and preferably 5') to a Vλ2-11 gene segment in the

engineered endogenous immunoglobulin κ light chain locus. In some instances, an engineered endogenous immunoglobulin κ light chain locus including the first exemplary endogenous human Vλ non-coding sequence could also include that non-coding sequence at a position that is adjacent (and preferably 3') to a Vλ3-12 gene segment and adjacent (and preferably 5') to a Vλ2-11 gene segment in the engineered endogenous immunoglobulin κ light chain locus. In some embodiments, each of the one or more human Vλ non-coding sequences is or includes an intron.

[0010] In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jλ non-coding sequences, each of which is adjacent to at least one of the one or more human Jλ gene segments, where the one or more human Jλ non-coding sequences naturally appears adjacent to a human Jλ gene segment in an endogenous human immunoglobulin λ light chain locus. In some embodiments, each of the one or more human Jλ non-coding sequences is or includes an intron. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jκ non-coding sequences, each of which is adjacent to at least one of the one or more human Jλ gene segment, where the one or more human Jκ non-coding sequences naturally appears adjacent to a human Jκ gene segment in an endogenous human immunoglobulin κ light chain locus. For example, referring to Figure 21, a first exemplary endogenous human Jκ non-coding sequence naturally appears in an endogenous human immunoglobulin κ light chain locus. An engineered endogenous immunoglobulin κ light chain locus including the first exemplary endogenous human Jκ non-coding sequence could be a non-coding sequence at a position that is adjacent to a Jλ gene segment (e.g., Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7) in the engineered endogenous immunoglobulin κ light chain locus. In some embodiments, each of the one or more human Jκ non-coding sequences is or includes an intron.

[0011] In some embodimenets, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Vλ non-coding sequences, where each of the one or more human Vλ non-coding sequences is adjacent to the Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, or Vλ3-1 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Vλ non-coding sequences naturally appear adj acent to a Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, or Vλ3-1 of an endogenous human immunoglobulin λ light chain locus. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jλ non-coding sequences, where each of the one or more human Jλ non-coding sequences is adjacent to the Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Jλ non-coding sequences naturally appear adjacent to a Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 of an endogenous human immunoglobulin λ light chain locus. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jκ non-coding sequences, where each of the one or more human Jκ non-coding sequences is adjacent to the Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Jκ non-coding sequences naturally appear adjacent to a Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 of an endogenous human immunoglobulin κ light chain locus.

[0012] In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes a κ light chain non-coding sequence between the one or more human Vλ gene segments and the one or more human Jλ gene segments. In some embodiments, a κ light chain non-coding sequence is a human κ light chain non-coding sequence. In some embodiments, a human κ light chain non-coding sequence has a sequence that naturally appears between a human Vκ4-1 gene segment and a human Jκ1 gene segment in an endogenous human immunoglobulin κ light chain locus.

[0013] In some embodiments, a mouse described herein is homozygous for an engineered endogenous immunoglobulin κ light chain locus. In some embodiments, a mouse described herein is heterozygous for an engineered endogenous immunoglobulin κ light chain locus. In some embodiments, the germline genome of a mouse includes a second engineered endogenous immunoglobulin κ light chain locus that includes:

1. (a) one or more human Vκ gene segments, and
2. (b) one or more human Jκ gene segments,

where the one or more human Vκ gene segments and the one or more human Jκ gene segments are operably linked to a Cκ gene.

[0014] In some embodiments, the genome of the mouse further includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element. In some embodiments, the transcriptional control element includes a RAG1 transcriptional control element, a RAG2 transcriptional control element, an immunoglobulin heavy chain transcriptional control element, an immunoglobulin κ light chain transcriptional control element, an immunoglobulin λ light chain transcriptional control element, or any combination thereof. In some embodiments, the nucleic acid sequence encoding an exogenous TdT is located at an immunoglobulin κ light chain locus, an immunoglobulin λ light chain locus, an immunoglobulin heavy chain locus, a RAG1 locus, or a RAG2 locus. In some embodiments, a TdT is a human TdT. In some embodiments, a TdT is a short isoform of TdT (TdTS).

[0015] In some embodiments, a mouse described herein includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome and exhibits light chains (e.g., expresses light chain variable domains including) with at least a 1.2-fold, at least a 1.5-fold, at least a 1.75-fold, at least a 2-fold, at least a 3-fold, at least a 4- fold, or a least a 5-fold increase in junctional diversity over a comparable mouse (e.g., littermate) that does not include an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome. In some embodiments, junctional diversity is measured by number of unique CDR3/ 10,000 reads.

[0016] In some embodiments, a mouse described herein includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome and at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65% of light chains (e.g., lambda and/or kappa light chains) produced by the mouse exhibit non-template additions.

[0017] In some embodiments, the germline genome of the mouse employed further comprises:
an engineered endogenous immunoglobulin heavy chain locus, comprising:

1. (a) one or more human V_H gene segments,
2. (b) one or more human D_H gene segments, and
3. (c) one or more human J_H gene segments,

wherein the one or more human V_H gene segments of (a), the one or more human D_H gene segments of (b), and the one or more human J_H gene segments of (c) are operably linked to one or more mouse immunoglobulin heavy chain constant region genes at the engineered endogenous immunoglobulin heavy chain locus.

[0018] In some embodiments, 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 in place of one or more mouse V_H gene segments, one or more mouse D_H gene segments, one or more mouse J_H gene segments, or a combination thereof. In some embodiment, 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 replace one or more mouse V_H gene segments, one or more mouse D_H gene segments, one or more mouse J_H gene segments, or any combination thereof.

[0019] In some embodiments, one or more human V_H gene segments include V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, V_H6-1, or any combination thereof. In some embodiments, one or more human V_H gene segments include V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, and V_H6-1.

[0020] In some embodiments, one or more human D_H gene segments include D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26, D_H7-27, or any combination thereof. In some embodiments, one or more human D_H gene segments include D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26,

and D_H7-27.

[0021] In some embodiments, one or more human J_H gene segments include J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, or any combination thereof. In some embodiments, one or more human J_H gene segments include J_H1, J_H2, J_H3, J_H4, J_H5, and J_H6.

[0022] In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human V_H non-coding sequences, each of which is adjacent to at least one of the one or more human V_H gene segments, where each of the one or more V_H non-coding sequences naturally appears adjacent to a human V_H gene segment in an endogenous human immunoglobulin heavy chain locus. In some embodiments, each of the one or more human V_H non-coding sequences is or includes an intron. In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human D_H non-coding sequences, each of which is adjacent to at least one of the one or more human D_H gene segments, where each of the one or more D_H non-coding sequences naturally appears adjacent to a human D_H gene segment in an endogenous human immunoglobulin heavy chain locus. In some embodiments, each of the one or more human D_H non-coding sequences is or includes an intron. In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human J_H non-coding sequences, each of which is adjacent to at least one of the one or more human J_H gene segments, where each of the one or more J_H non-coding sequences naturally appears adjacent to a human J_H gene segment in an endogenous human immunoglobulin heavy chain locus. In some embodiments, each of the one or more human J_H non-coding sequences is or includes an intron.

[0023] In some embodiments, a mouse described herein is homozygous for an engineered endogenous immunoglobulin heavy chain locus.

[0024] In some embodiments, a mouse immunoglobulin heavy chain constant region is an endogenous mouse immunoglobulin heavy chain constant region.

[0025] In some embodiments, endogenous Vλ gene segments, endogenous Jλ gene segments, and the endogenous Cλ genes are deleted in whole or in part. In some embodiments, a mouse described herein does not detectably express endogenous immunoglobulin λ light chain variable domains. In some embodiments, a mouse described herein does not detectably express endogenous immunoglobulin κ light chain variable domains.

[0026] In some embodiments, an engineered endogenous immunoglobulin heavy chain locus lacks a functional endogenous mouse Adam6 gene. In some embodiments, a germline genome of a mouse includes one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof. In some embodiments, one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are expressed (e.g., in a cell of the male reproductive system, e.g., a testes cell).

[0027] In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are included on the same chromosome as the engineered endogenous immunoglobulin heavy chain locus. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are included in the engineered endogenous immunoglobulin heavy chain locus. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are between a first human V_H gene segment and a second human V_H gene segment. In some embodiments, a first human V_H gene segment is V_H1-2 and a second human V_H gene segment is V_H6-1. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are in place of a human Adam6 pseudogene. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof replace a human Adam6 pseudogene. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are between a human V_H gene segment and a human D_H gene segment.

[0028] In some embodiments, a mouse described herein includes a population of B cells that express antibodies, including immunoglobulin λ light chains that each include a human immunoglobulin λ light chain variable domain. In some embodiments, a human immunoglobulin λ light chain variable domain is encoded by a rearranged human immunoglobulin λ light chain variable region sequence including (i) one of the one or more human Vλ gene segments or a somatically hypermutated variant thereof, and (ii) one of the one or more human Jλ gene segments or a somatically hypermutated variant thereof.

[0029] In some embodiments, a mouse described herein includes a population of B cells that express antibodies, including immunoglobulin heavy chains that each include a human immunoglobulin heavy chain variable domain. In some embodiments, a human immunoglobulin heavy chain variable domain is encoded by a rearranged human immunoglobulin heavy chain variable region sequence including (i) one of the one or more human V_H gene segments or a somatically hypermutated variant thereof, (ii) one of the one or more human D_H gene segments or a somatically hypermutated variant thereof, and (ii) one of the one or more human J_H gene segments or a somatically hypermutated variant thereof.

[0030] In some embodiments, a mouse described herein produces a population of B cells in response to immunization with an antigen that includes one or more epitopes. In some embodiments, a mouse produces a population of B cells that express antibodies that bind (e.g., specifically bind) to one or more epitopes of antigen of interest. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence and/or a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence and/or a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein.

[0031] In some embodiments, a mouse produces a population of B cells that express antibodies that bind to one or more epitopes of antigen of interest, where antibodies expressed by the population of B cells produced in response to an antigen include: (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein, or (iv) any combination thereof.

[0032] In some embodiments, a human heavy chain variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence as described herein is somatically hypermutated. In some embodiments, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% of the B cells in a population of B cells produced in response to an antigen include a human heavy chain variable region sequence, λ light chain variable region sequence, and/or κ light chain variable region sequence that is somatically hypermutated.

[0033] In some embodiments, cells and/or tissues (e.g., isolated cells and/or tissues) from a mouse are described herein. In some embodiments, cells and tissues include, for example, lymphoid tissue, splenocytes, B cells, stem cells and/or germ cells. In some embodiments, a cell is isolated. In some embodiments, an isolated cell is or includes a pro B-cell, a pre-B cell, an immature B cell, a mature naive B cell, an activated B cell, a memory B cell, a B lineage lymphocyte, and/or a plasma cell. In some embodiments, an isolated cell includes a stem cell (e.g., an embryonic stem cell) and/or a germ cell (e.g., sperm, oocyte).

[0034] Also disclosed is an isolated rodent cell, whose germline genome includes:

an engineered endogenous immunoglobulin κ light chain locus including:

1. (a) one or more human Vλ gene segments,
2. (b) one or more human Jλ gene segments, and
3. (c) a Cλ gene,

where the one or more human Vλ gene segments and the one or more human Jλ gene segments are operably linked to the Cλ gene.

[0035] In some embodiments, an isolated mouse cell described herein is a mouseembryonic stem (ES) cell.

[0036] In some embodiments, the present disclosure includes a mouse embryo generated from a mouse ES cell described herein.

[0037] In some embodiments, the present disclosure includes an immortalized cell generated from an isolated mouse cell described herein.

[0038] Also disclosed is a method of making a mouse whose germline genome includes an engineered endogenous immunoglobulin κ light chain locus, the method including the steps of:

1. (a) introducing one or more DNA fragments into the germline genome of a mouse ES cell, where the one or more DNA fragments comprise:
    1. (i) one or more human Vλ gene segments,
    2. (ii) one or more human Jλ gene segments, and
    3. (iii) one mouse Cλ gene,
    where the one or more human Vλ gene segments, the one or more human Jλ gene segments, and the one mouse Cλ gene are introduced into the germline genome of the mouseES cell at the endogenous immunoglobulin κ light chain locus, and where the one or more human Vλ gene segments, the one or more human Jλ gene segments, and the one mouse Cλ gene are operably linked; and
2. (b) generating a mouse using the mouse ES cell generated in (a).

[0039] Also disclosed is a method of making a mouse whose germline genome includes an engineered endogenous immunoglobulin κ light chain locus, includes the step of introducing a κ light chain non-coding sequence into the germline genome of the mouseES cell so that the κ light chain non-coding sequence is between the one or more human Vλ gene segments and the one or more human Jλ gene segments in the germline genome of the mouse ES cell.

[0040] Also disclosed is a method of making a mouse whose germline genome includes an engineered endogenous immunoglobulin κ light chain locus, the method including the steps of:

engineering the endogenous immunoglobulin κ light chain locus in the germline genome to include:

1. (a) one or more human Vλ gene segments,
2. (b) one or more human Jλ gene segments, and
3. (c) one mouse Cλ gene,

where the one or more human Vλ gene segments and the one or more human Jλ gene segments are operably linked to the one mouse Cλ gene, and

where the one mouse Cλ gene is inserted in place of a mouse Cκ gene at the endogenous immunoglobulin κ locus.

[0041] In some embodiments, a mouse Cλ gene replaces a mouse Cκ gene at the endogenous immunoglobulin κ locus.

[0042] In some embodiments, one or more human Vλ gene segments include Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1, or any combination thereof. In some embodiments, one or more human Vλ gene segments include Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, and Vλ3-1. In some embodiments, one or more human Vλ gene segments include Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, and Vλ3-1.

[0043] In some embodiments, one or more human Jλ gene segments includes Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof. In some embodiments, one or more human Jλ gene segments includes Jλ1, Jλ2, Jλ3, Jλ6, and Jλ7.

[0044] In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Vλ non-coding sequences, each of which is adjacent to at least one of the one or more human Vλ gene segments, where the one or more human Vλ non-coding sequences naturally appears adjacent to a human Vλ gene segment in an endogenous human immunoglobulin λ light chain locus. In some embodiments, each of the one or more human Vλ non-coding sequences is or includes an intron. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jλ non-coding sequences, each of which is adjacent to at least one of the one or more human Jλ gene segment, where the one or more human Jλ non-coding sequences naturally appears adjacent to a human Jλ gene segment in an endogenous human immunoglobulin λ light chain locus. In some embodiments, each of the one or more human Jλ non-coding sequences is or includes an intron. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jκ non-coding sequences, each of which is adjacent to at least one of the one or more human Jλ gene segment, where the one or more human Jκ non-coding sequences naturally appears adjacent to a human Jκ gene segment in an endogenous human immunoglobulin κ light chain locus. In some embodiments, each of the one or more human Jκ non-coding sequences is or includes an intron.

[0045] In some embodiments, a mouse Cλ gene has a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to a mouse Cλ1, mouse Cλ2 or a mouse Cλ3 gene. In some embodiments, a mouse Cλ gene is or includes a mouse Cλ1 gene.

[0046] In some embodiments, one or more DNA fragments include at least one selection marker. In some embodiments, one or more DNA fragments include at least one site-specific recombination site.

[0047] In some embodiments, the germline genome of a mouse includes:

an engineered endogenous immunoglobulin heavy chain locus, including:

1. (a) one or more human V_H gene segments,
2. (b) one or more human D_H gene segments, and
3. (c) one or more human J_H gene segments,

where the one or more human V_H gene segments, the one or more human D_H gene segments, and the one or more human J_H gene segments are operably linked to a mouse immunoglobulin heavy chain constant region.

[0048] In some embodiments, the step of engineering the endogenous immunoglobulin κ light chain locus in the germline genome is carried out in a mouse ES cell whose germline genome includes an engineered endogenous immunoglobulin heavy chain locus including 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 a mouse immunoglobulin heavy chain constant region.

[0049] In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human V_H non-coding sequences, each of which is adjacent to at least one of the one or more human V_H gene segments, where each of the one or more human V_H non-coding sequences naturally appears adjacent to a human V_H gene segment in an endogenous human immunoglobulin heavy chain locus. In some embodiments, each of the one or more human V_H non-coding sequences is or includes an intron. In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human D_H non-coding sequences, each of which is adjacent to at least one of the one or more human D_H gene segments, where each of the one or more D_H non-coding sequences naturally appears adjacent to a human D_H gene segment in an endogenous human immunoglobulin heavy chain locus. In some embodiments, each of the one or more human D_H non-coding sequences is or includes an intron. In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human J_H non-coding sequences, each of which is adjacent to at least one of the one or more human J_H gene segments, where each of the one or more J_H non-coding sequences naturally appears adjacent to a human J_H gene segment in an endogenous human immunoglobulin heavy chain locus. In some embodiments, each of the one or more human J_H non-coding sequences is or includes an intron.

[0050] The present invention provides a method of producing an antibody in a mouse, the method including the steps of:

1. (a) immunizing the genetically modified mouse with an antigen of interest,

   wherein the genetically modified mouse has a germline genome comprising:

   a first engineered endogenous immunoglobulin κ light chain locus comprising:

   1. (i) one or more human Vλ gene segments,
   2. (ii) one or more human Jλ gene segments, and
   3. (iii) one mouse Cλ gene,

   wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are operably linked to the one mouse Cλ gene of (iii);

   wherein the one mouse Cλ gene (iii) is in the place of a mouse Cκ gene at the first engineered endogenous immunoglobulin κ light chain locus;

   wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are in place of one or more endogenous mouse Vκ gene segments and one or more endogenous mouse Jκ gene segments;

   wherein the genetically modified mouse lacks a mouse Cκ gene at the first engineered endogenous immunoglobulin k light chain locus;

2. (b) maintaining the genetically modified mouse under conditions sufficient for the genetically modified mouse to produce an immune response to the antigen of interest; and
3. (c) recovering from the genetically modified mouse:
   1. (i) an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain,
   2. (ii) a nucleotide that encodes a human light chain variable domain or human heavy chain variable domain, a light chain, or a heavy chain of an antibody that binds the antigen of interest, wherein the antibody that binds the antigen of interest comprises a light chain comprising a human λ variable domain and a mouse λ constant domain, or
   3. (iii) a cell that expresses an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain.

[0051] In some embodiments, in response to the step of immunizing, a mouse produces a B cell that expresses an antibody that binds the antigen of interest. In some embodiments, an antibody expressed by a B cell includes a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence and/or a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein. In some embodiments, an antibody expressed by a B cell includes (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein, or (iv) any combination thereof.

[0052] In some embodiments, in response to the step of immunizing, the mouse produces a population of B cells that expresses antibodies that bind an antigen of interest. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence and/or a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein, or (iv) any combination thereof.

[0053] In some embodiments, in response to the step of immunizing, a mouse produces a population of B cells that express antibodies that bind to one or more epitopes of antigen of interest, where antibodies expressed by the population of B cells produced in response to an antigen include: (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein, or (iv) any combination thereof.

[0054] In some embodiments, a human heavy chain variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence as described herein is somatically hypermutated. In some embodiments, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% of the B cells in a population of B cells produced in response to an antigen include a human heavy chain variable region sequence, λ light chain variable region sequence, and/or κ light chain variable region sequence that is somatically hypermutated.

[0055] In some embodiments, an antibody that binds the antigen of interest is isolated from, recovered from, or identified from a B cell of the mouse. In some embodiments, an antibody that binds an antigen of interest is isolated from, recovered from, or identified from a hybridoma made with a B cell of the mouse.

[0056] In some embodiments, an antigen includes one or more epitopes and an antibody that binds an antigen of interest binds to an epitope of the one or more epitopes.

[0057] In some embodiments, a mouse Cλ gene has a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to a mouse Cλ1, mouse Cλ2 or a mouse Cλ3 gene. In some embodiments, a mouse Cλ gene is or includes a mouse Cλ1 gene.

[0058] In some embodiments, one or more human Vλ gene segments comprise Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1, or any combination thereof. In some embodiments, one or more human Vλ gene segments comprise Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, and Vλ3-1. In some embodiments, one or more human Vλ gene segments comprise Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, and Vλ3-1.

[0059] In some embodiments, one or more human Jλ gene segments comprise Jλ1, Jλ2, Jλ3, Jλ6, Jλ7 or any combination thereof. In some embodiments, one or more human Jλ gene segments includes Jλ1, Jλ2, Jλ3, Jλ6, and Jλ7.

[0060] In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Vλ non-coding sequences, where each of the one or more human Vλ non-coding sequences is adjacent to the Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3 or Vλ3-1 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Vλ non-coding sequences naturally appear adjacent to a Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3 or Vλ3-1 of an endogenous human immunoglobulin λ light chain locus. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jλ non-coding sequences, where each of the one or more human Jλ non-coding sequences is adjacent to the Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Jλ non-coding sequences naturally appear adjacent to a Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 of an endogenous human immunoglobulin λ light chain locus. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes one or more human Jκ non-coding sequences, where each of the one or more human Jκ non-coding sequences is adjacent to the Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Jκ non-coding sequences naturally appear adjacent to a Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 of an endogenous human immunoglobulin κ light chain locus.

[0061] In some embodiments, a mouse has a germline genome including an engineered endogenous immunoglobulin heavy chain locus including:

1. (a) one or more human V_H gene segments,
2. (b) one or more human D_H gene segments, and
3. (c) one or more human J_H gene segments,

where the one or more human V_H gene segments, the one or more human D_H gene segments, and the one or more human J_H gene segments are operably linked to a mouse immunoglobulin heavy chain constant region.

[0062] In some embodiments, one or more human V_H gene segments comprise V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, V_H6-1 or any combination thereof. In some embodiments, one or more human V_H gene segments comprise V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, and V_H6-1.

[0063] In some embodiments, one or more human D_H gene segments comprise D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26, D_H7-27, or any combination thereof. In some embodiments, one or more human D_H gene segments comprise D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26, and D_H7-27.

[0064] In some embodiments, one or more human J_H gene segments comprise J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, or any combination thereof. In some embodiments, one or more human J_H gene segments comprise J_H1, J_H2, J_H3, J_H4, J_H5, and J_H6.

[0065] In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human V_H non-coding sequences, where each of the one or more human V_H non-coding sequences is adjacent to the V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2 or V_H6-1 in the engineered endogenous immunoglobulin heavy chain locus, and where each of the one or more human V_H non-coding sequences naturally appear adjacent to a V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2 or V_H6-1 of an endogenous human immunoglobulin heavy chain locus. In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human D_H non-coding sequences, where each of the one or more human D_H non-coding sequences is adjacent to the D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26 or D_H7-27 in the engineered endogenous immunoglobulin heavy chain locus, and where each of the one or more human D_H non-coding sequences naturally appear adjacent to a D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26 or D_H7-27 of an endogenous human immunoglobulin heavy chain locus. In some embodiments, an engineered endogenous immunoglobulin heavy chain locus includes one or more human J_H non-coding sequences, where each of the one or more human J_H non-coding sequences is adjacent to the J_H1, J_H2, J_H3, J_H4, J_H5 or J_H6 in the engineered endogenous immunoglobulin heavy chain locus, and where each of the one or more human J_H non-coding sequences naturally appear adjacent to a J_H1, J_H2, J_H3, J_H4, J_H5 or J_H6 of an endogenous human immunoglobulin heavy chain locus.In some embodiments, a cell of the mouse that is recovered is a B cell. In some embodiments, a cell derived from a cell of the mouse is a hybridoma.

[0066] In some embodiments, a nucleotide sequence that encodes a human heavy chain variable region sequence, a human lambda light chain variable region sequence, and/or a human kappa light chain variable region sequence is obtained from a B cell.

[0067] In some embodiments, an engineered endogenous immunoglobulin heavy chain locus lacks a functional endogenous mouse Adam6 gene. In some embodiments, a germline genome of a mouse includes one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof. In some embodiments, one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are expressed (e.g., in a cell of the male reproductive system, e.g., a testes cell).

[0068] In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are included on the same chromosome as the engineered endogenous immunoglobulin heavy chain locus. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are included in the engineered endogenous immunoglobulin heavy chain locus. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are between a first human V_H gene segment and a second human V_H gene segment. In some embodiments, a first human V_H gene segment is V_H1-2 and a second human V_H gene segment is V_H6-1. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are in place of a human Adam6 pseudogene. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof replace a human Adam6 pseudogene. In some embodiments, one or more nucleotide sequences encoding one or more mouse ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are between a human V_H gene segment and a human D_H gene segment.

[0069] In some embodiments, the mouse employed is one whose germline genome includes a homozygous engineered endogenous immunoglobulin κ light chain locus including:

1. (i) one or more human Vλ gene segments, where the one or more human Vλ gene segments comprise Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1, or any combination thereof,
2. (ii) one or more human Jλ gene segments, where the one or more human Jλ gene segments compriseJλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof, and
3. (iii) a mouse Cλ gene;

where the one or more human Vλ gene segments, the one or more human Jλ gene segments, and the mouse Cλ gene are operably linked to each other,

where the mouse Cλ gene is in place of a mouse Cκ gene of the endogenous immunoglobulin κ light chain locus,

where the engineered endogenous immunoglobulin κ light chain locus includes:

1. (a) one or more human Vλ non-coding sequences, where each of the one or more human Vλ non-coding sequences is adjacent to the Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3 or Vλ3-1 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Vλ non-coding sequences naturally appear adjacent to a Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3 or Vλ3-1 of an endogenous human immunoglobulin λ light chain locus, and
2. (b) one or more human Jκ non-coding sequences, where each of the one or more human Jκ non-coding sequences is adjacent to the Jλ1, Jλ2, Jλ3, Jλ6, or Jλ7 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Jκ non-coding sequences naturally appear adjacent to a Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 of an endogenous human immunoglobulin κ light chain locus, and

where the immunoglobulin κ light chain locus includes a human κ light chain non-coding sequence between the one or more human Vλ gene segments and the one or more human Jλ gene segments that has a sequence that naturally appears between a human Vκ4-1 gene segment and a human Jκ1 gene segment in an endogenous human immunoglobulin κ light chain locus.

[0070] In some embodiments, a mouse Cλ gene is a mouse Cλ1 gene.

[0071] In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes mouse immunoglobulin κ light chain enhancers $E\kappa_i$ and Eκ3'.

[0072] In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes a deletion of one or more mouse Vκ gene segments and/or one or more Jκ gene segments. In some embodiments, an engineered endogenous immunoglobulin κ light chain locus includes a deletion of all functional mouse Vκ and/or Jκ gene segments.

[0073] In some embodiments, the mouse employed is one whose germline genome includes:

1. (a) a homozygous endogenous immunoglobulin heavy chain locus including 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 endogenous immunoglobulin heavy chain constant region genes such that the mouse expresses immunoglobulin heavy chains that each comprise a human heavy chain variable domain sequence and a mouse heavy chain constant domain sequence,
2. (b) a first engineered endogenous immunoglobulin κ light chain locus including one or more human Vκ gene segments and one or more Jκ gene segments operably linked to an endogenous mouse Cκ region gene such that the mouse expresses immunoglobulin light chains that each includes a human κ light chain variable domain sequence and a mouse κ light chain constant domain sequence, and
3. (c) a second engineered endogenous immunoglobulin κ light chain locus including:
    1. (i) one or more human Vλ gene segments, where the one or more human Vλ gene segments comprise Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1, or any combination thereof,
    2. (ii) one or more human Jλ gene segments, where the one or more human Jλ gene segments comprise Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof, and
    3. (iii) a mouse Cλ gene;

    where the one or more human Vλ gene segments, the one or more human Jλ gene segments, and the mouse Cλ gene are operably linked to each other,
    where the mouse Cλ gene is in place of a mouse Cκ gene of the endogenous immunoglobulin κ light chain locus,

    where the engineered endogenous immunoglobulin κ light chain locus includes:

    1. (a) one or more human Vλ non-coding sequences, where each of the one or more human Vλ non-coding sequences is adjacent to the Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3 or Vλ3-1 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Vλ non-coding sequences naturally appear adjacent to a Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3 or Vλ3-1 of an endogenous human immunoglobulin λ light chain locus, and
    2. (b) one or more human Jκ non-coding sequences, where each of the one or more human Jκ non-coding sequences is adjacent to the Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 in the engineered endogenous immunoglobulin κ light chain locus, and where each of the one or more human Jκ non-coding sequences naturally appear adjacent to a Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 of an endogenous human immunoglobulin κ light chain locus, and

    where the immunoglobulin κ light chain locus includes a human κ light chain non-coding sequence between the one or more human Vλ gene segments and the one or more human Jλ gene segments that has a sequence that naturally appears between a human Vκ4-1 gene segment and a human Jκ1 gene segment in an endogenous human immunoglobulin κ light chain locus;

    such that the mouse expresses immunoglobulin light chains that each comprise a human λ light chain variable domain sequence and a mouse λ light chain constant domain sequence.

[0074] In some embodiments, a mouse described herein includes an inactivated endogenous immunoglobulin λ light chain locus. In some embodiments, a mouse described herein is heterozygous for the inactivated endogenous immunoglobulin λ light chain locus. In some embodiments, a mouse described herein is homozygous for the inactivated endogenous immunoglobulin λ light chain locus.

[0075] In some embodiments, the genome of the mouse further includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element. In some embodiments, the transcriptional control element includes a RAG1 transcriptional control element, a RAG2 transcriptional control element, an immunoglobulin heavy chain transcriptional control element, an immunoglobulin κ light chain transcriptional control element, an immunoglobulin λ light chain transcriptional control element, or any combination thereof. In some embodiments, the nucleic acid sequence encoding an exogenous TdT is located at an immunoglobulin κ light chain locus, an immunoglobulin λ light chain locus, an immunoglobulin heavy chain locus, a RAG1 locus, or a RAG2 locus. In some embodiments, a TdT is a human TdT. In some embodiments, a TdT is a short isoform of TdT (TdTS).

[0076] In some embodiments, a mouse described herein includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome and exhibits light chains (e.g., expresses light chain variable domains including) with at least a 1.2-fold, at least a 1.5-fold, at least a 1.75-fold, at least a 2-fold, at least a 3-fold, at least a 4- fold, or a least a 5-fold increase in junctional diversity over a comparable mouse (e.g., littermate) that does not include an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome. In some embodiments, junctional diversity is measured by number of unique CDR3/ 10,000 reads. In some embodiments, junctional diversity is measured by number of unique CDR3/ 10,000 reads.

[0077] In some embodiments, a mouse described herein includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome and at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65% of light chains (e.g., lambda and/or kappa light chains) produced by the mouse exhibit non-template additions.

[0078] Also disclosed is an antibody prepared by a method including the steps of:

1. (a) providing a mouse described herein;
2. (b) immunizing the mouse with an antigen of interest;
3. (c) maintaining the mouse under conditions sufficient for the mouse to produce an immune response to the antigen of interest; and
4. (d) recovering an antibody that binds the antigen of interest from the mouse, or a cell of the mouse, or a cell derived from a cell of the mouse,

where the antibody of (d) includes human heavy chain variable and human λ light chain variable domains.

[0079] Also disclosed is an antibody prepared by a method including the steps of:

1. (a) immunizing a mouse described herein with an antigen of interest;
2. (b) maintaining the mouse under conditions sufficient for the mouse to produce an immune response to the antigen of interest; and
3. (c) recovering an antibody that binds the antigen of interest from the mouse, or a cell of the mouse, or a cell derived from a cell of the mouse,

where the antibody of (c) includes human heavy chain variable and human λ light chain variable domains.

[0080] In some embodiments, a mouse does not detectably express endogenous immunoglobulin κ light chain variable domains. In some embodiments, a mouse does not detectably express endogenous immunoglobulin λ light chain variable domains.

[0081] In some embodiments, a mouse described herein produces a population of B cells in response to immunization with an antigen that includes one or more epitopes. In some embodiments, a mouse produces a population of B cells that express antibodies that bind (e.g., specifically bind) to one or more epitopes of antigen of interest. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence and/or a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein, or (iv) any combination thereof.

[0082] In some embodiments, a mouse produces a population of B cells that express antibodies that bind to one or more epitopes of antigen of interest, where antibodies expressed by the population of B cells produced in response to an antigen include: (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, and/or (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein.

[0083] In some embodiments, a human heavy chain variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence as described herein is somatically hypermutated. In some embodiments, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% of the B cells in a population of B cells produced in response to an antigen include a human heavy chain variable region sequence, λ light chain variable region sequence, and/or κ light chain variable region sequence that is somatically hypermutated.

[0084] In some embodiments, the method of making an antibody, may be one that further includes:

1. (i) expressing a first nucleotide sequence that encodes an immunoglobulin heavy chain in a host cell, where the first nucleotide sequence includes a human heavy chain variable region sequence;
2. (ii) expressing a second nucleotide sequence that encodes an immunoglobulin λ light chain in a host cell, where the second nucleotide sequence includes a human λ light chain variable region sequence that was identified (e.g., expressed and/or isolated) from a mouse whose germline genome comprises a first engineered endogenous immunoglobulin κ light chain locus comprising:
    1. (i) one or more human Vλ gene segments,
    2. (ii) one or more human Jλ gene segments, and
    3. (iii) one mouse Cλ gene,

    wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are operably linked to the one mouse Cλ gene of (iii);

    wherein the one mouse Cλ gene (iii) is in the place of a mouse Cκ gene at the first engineered endogenous immunoglobulin κ light chain locus;
    wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are in place of one or more endogenous mouse Vκ gene segments and one or more endogenous mouse Jκ gene segments;

    wherein the genetically modified mouse lacks a mouse Cκ gene at the first engineered endogenous immunoglobulin k light chain locus.:
3. (iii) culturing the host cell so that immunoglobulin light chains and immunoglobulin heavy chains are expressed and form an antibody; and
4. (iv) obtaining the antibody from the host cell and/or host cell culture.

[0085] In some embodiments, a first nucleotide sequence includes a human heavy chain constant region. In some embodiments, an antibody is a fully human antibody.

[0086] In some embodiments, a second nucleotide includes a human λ light chain constant region sequence.

[0087] In some embodiments, an antibody is a reverse chimeric antibody. In some embodiments, a first nucleotide sequence includes a mouse heavy chain constant region. In some embodiments, a second nucleotide sequence includes a mouse λ light chain constant region sequence.

[0088] In some embodiments, the mouse employed is one whose germline genome includes:

1. (a) a first engineered endogenous immunoglobulin κ light chain locus comprising:

    a first engineered endogenous immunoglobulin κ light chain locus comprising:

    1. (i) one or more human Vλ gene segments,
    2. (ii) one or more human Jλ gene segments, and
    3. (iii) one mouse Cλ gene,

    wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are operably linked to the one mouse Cλ gene of (iii);

    wherein the one mouse Cλ gene (iii) is in the place of a mouse Cκ gene at the first engineered endogenous immunoglobulin κ light chain locus;

    wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are in place of one or more endogenous mouse Vκ gene segments and one or more endogenous mouse Jκ gene segments;

    wherein the genetically modified mouse lacks a mouse Cκ gene at the first engineered endogenous immunoglobulin k light chain locus; and

2. (b) a second engineered endogenous immunoglobulin κ light chain locus further includes:
    1. (i) one or more human Vκ gene segments, and
    2. (ii) one or more human Jκ gene segments,

where the one or more human Vκ gene segments and the one or more human Jκ gene segments are operably linked to a Cκ gene.

[0089] In some embodiments, a Cκ gene is an endogenous mouse Cκ gene.

[0090] In some embodiments, the genome of the mouse further includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element. In some embodiments, the transcriptional control element includes a RAG1 transcriptional control element, a RAG2 transcriptional control element, an immunoglobulin heavy chain transcriptional control element, an immunoglobulin κ light chain transcriptional control element, an immunoglobulin λ light chain transcriptional control element, or any combination thereof. In some embodiments, the nucleic acid sequence encoding an exogenous TdT is located at an immunoglobulin κ light chain locus, an immunoglobulin λ light chain locus, an immunoglobulin heavy chain locus, a RAG1 locus, or a RAG2 locus. In some embodiments, a TdT is a human TdT. In some embodiments, a TdT is a short isoform of TdT (TdTS).

[0091] In some embodiments, the genome of the mouse further includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element. In some embodiments, the transcriptional control element includes a RAG1 transcriptional control element, a RAG2 transcriptional control element, an immunoglobulin heavy chain transcriptional control element, an immunoglobulin κ light chain transcriptional control element, an immunoglobulin λ light chain transcriptional control element, or any combination thereof. In some embodiments, the nucleic acid sequence encoding an exogenous TdT is located at an immunoglobulin κ light chain locus, an immunoglobulin λ light chain locus, an immunoglobulin heavy chain locus, a RAG1 locus, or a RAG2 locus. In some embodiments, a TdT is a human TdT. In some embodiments, a TdT is a short isoform of TdT (TdTS).

[0092] In some embodiments, a mouse described herein includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome and exhibits light chains (e.g., expresses light chain variable domains including) with at least a 1.2-fold, at least a 1.5-fold, at least a 1.75-fold, at least a 2-fold, at least a 3-fold, at least a 4- fold, or a least a 5-fold increase in junctional diversity over a comparable mouse (e.g., littermate) that does not include an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome. In some embodiments, junctional diversity is measured by number of unique CDR3/ 10,000 reads. In some embodiments, junctional diversity is measured by number of unique CDR3/ 10,000 reads.

[0093] In some embodiments, a mouse described herein includes a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element in its germline genome and at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65% of light chains (e.g., lambda and/or kappa light chains) produced by the mouse exhibit non-template additions.

[0094] In some embodiments, a mouse, mouse cell or mouse tissue as described herein comprises a genetic background that includes a 129 strain, a BALB/c strain, a C57BL/6 strain, a mixed 129xC57BL/6 strain, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0095] The Drawings included herein, which are composed of the following Figures, is for illustration purposes only and not for limitation.

Figure 1A and 1B show illustrations of an exemplary embodiment, not to scale, of a strategy for constructing a targeting vector (described in Example 1.1) used in generating an embodiment of the mouse according to the present disclosure.

Figure 2A shows an illustration of an exemplary embodiment, not to scale, of the insertion of a targeting vector (described in Example 1.1) into an engineered Igκ light chain locus of a mouse embryonic stem (ES) cell clone, which ES cell clone was used in generating an embodiment according to the present disclosure.

Figure 2B shows an illustration of an exemplary embodiment, not to scale, of recombinase-mediated removal of selection cassette(s) in an engineered Igκ light chain locus resulting from the insertion of a targeting vector (described in Example 1.1) used in generating an embodiment of the mouse according to the present disclosure.

Figure 3 shows an illustration of an exemplary embodiment, not to scale, of a strategy for constructing a targeting vector (described in Example 1.2) used in generating an embodiment of the mouse according to the present disclosure.

Figure 4A shows an illustration, not to scale, of the insertion of a targeting vector (described in Example 1.2) into an engineered Igκ light chain locus of a mouse embryonic stem (ES) cell clone, which ES cell clone was used in generating an embodiment of the mouse according to the present disclosure.

Figure 4B shows an illustration of an exemplary embodiment, not to scale, of recombinase-mediated removal of selection cassette(s) in an engineered Igκ light chain locus resulting from the insertion of a targeting vector (described in Example 1.2) used in generating an embodiment of the mouse according to the present disclosure..

Figure 5 shows results derived from a representative embodiment according to the present disclosure, showing single cell-gated splenocytes harvested from wild-type (WT) and 6558 HO (LiK, homozygous) mice, the top row illustrating expression of CD19 (y-axis) and CD3 (x-axis), and the bottom row illustrating $CD19^+$-gated splenocytes expressing immunoglobulin D (IgD, y-axis) and immunoglobulin M (IgM, x-axis).

Figure 6 shows results derived from a representative embodiment according to the present disclosure, including representative single cell-gated bone marrow harvested from wild-type (WT) and 6558HO (LiK, homozygous) mice, the top row illustrating expression of CD19 (y-axis) and CD3 (x-axis), and the bottom row illustrating expression of immunoglobulin M (IgD, y-axis) and B220 (x-axis).

Figure 7 shows results derived from a representative embodiment according to the present disclosure, including representative $CD19^+$-gated splenocytes harvested from wild-type (WT) and 6558HO (LiK, homozygous) mice illustrating expression of immunoglobulin light chains containing mouse Igλ (y-axis) or mouse Igκ (x-axis) constant regions.

Figure 8 shows results derived from a representative embodiment according to the present disclosure, including representative single cell-gated splenocytes harvested from various indicated humanized mice illustrating expression of CD19 (y-axis) and CD3 (x-axis). HOH/LiK/$\lambda^{-/-}$ mice - mice homozygous for humanized immunoglobulin heavy chain (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940), homozygous for LiK locus and homozygous for an inactivated endogenous immunoglobulin λ light chain locus; HOH/KoK/LiK/$\lambda^{-/-}$ mice - mice homozygous for humanized immunoglobulin heavy chain (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940), hemizygous for one kappa locus comprising LiK locus and a second kappa locus comprising humanized immunoglobulin kappa light chain locus, and homozygous for an inactivated endogenous immunoglobulin λ light chain locus; HOH/KoK mice - control mice homozygous for humanized immunoglobulin heavy chain and homozygous for humanized immunoglobulin kappa light chain.

Figure 9 shows results derived from a representative embodiment according to the present disclosure, including representative $CD19^+$-gated splenocytes harvested from various indicated humanized mice illustrating expression of immunoglobulin light chains containing mouse Igλ (y-axis) or mouse Igκ (x-axis) constant regions.

Figure 10 shows results derived from a representative embodiment according to the present disclosure, including representative single cell-gated bone marrow harvested from various indicated humanized mice illustrating expression of immunoglobulin M (IgD, y-axis) and B220 (x-axis).

Figure 11 shows results derived from a representative embodiment according to the present disclosure, including representative single cell-gated bone marrow harvested from various indicated humanized mice illustrating expression of immunoglobulin light chains containing mouse Igλ (y-axis) or mouse Igκ (x-axis) constant regions in immature (top row) and mature (bottom row) B cells.

Figure 12 shows a schematic illustration of an exemplary embodiment, according to the present disclosure, not to scale, of an engineered immunoglobulin κ light chain locus as described herein and the rearrangement of the locus to form an mRNA molecule.

Figure 13 shows results derived from a representative embodiment according to the present disclosure, including representative protein immunoblots (Western blots) of SDS-PAGE using serum isolated from wild-type (WT) and 6558 homozygous (LiK HO) mice as described in Example 3.3.

Figure 14 shows results of testing an embodiment according to the present disclosure, showing representative single cell-gated splenocytes harvested from humanized mice illustrating expression of CD19 (y-axis) and CD3 (x-axis). HOH/LiK/λ$^{-/-}$/TdT mice - mice homozygous for humanized immunoglobulin heavy chain (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940), homozygous for LiK locus and homozygous for an inactivated endogenous immunoglobulin λ light chain locus that include a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT); and HOH/KoK/LiK/λ$^{-/-}$/TdT mice - mice homozygous for humanized immunoglobulin heavy chain (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940), hemizygous for one kappa locus comprising an LiK locus and a second kappa locus comprising humanized immunoglobulin kappa light chain locus, and homozygous for an inactivated endogenous immunoglobulin λ light chain locus that include a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT).

Figure 15 shows results of testing an embodiment according to the present disclosure, showing representative CD19^{+}-gated splenocytes harvested from various indicated humanized mice illustrating expression of immunoglobulin light chains containing mouse Igλ (y-axis) or mouse Igκ (x-axis) constant regions.

Figure 16 shows results of testing an embodiment according to the present disclosure, showing representative single cell-gated bone marrow harvested from various indicated humanized mice illustrating expression of immunoglobulin M (IgM, y-axis) and B220 (x-axis).

Figure 17 shows results of testing an embodiment according to the present disclosure, showing representative single cell-gated bone marrow harvested from various indicated humanized mice illustrating expression of immunoglobulin light chains containing mouse Igλ (y-axis) or mouse Igκ (x-axis) constant regions in immature (top row) and mature (bottom row) B cells.

Figure 18 shows results of testing an embodiment according to the present disclosure, showing a graph comparing immune responses in LiK/VI-3, LiK/VI-3/TdT and VI-3/TdT mice strains following immunization with a protein immunogen.

Figure 19 shows results of testing an embodiment according to the present disclosure, showing a graph comparing immune responses against His tag in LiK/VI-3, LiK/VI-3/TdT and VI-3/TdT mice strains following immunization with an irrelevant protein antigen fused to a HIS tag.

Figure 20 shows an illustration, not to scale, of a portion of an endogenous human immunoglobulin λ light chain locus. Figure 20 includes a first arrow pointing to a representation of a first exemplary endogenous human Vλ non-coding sequence in the endogenous human immunoglobulin λ light chain locus. As illustrated, the first exemplary endogenous human Vλ non-coding sequence (represented by a line) in the endogenous human immunoglobulin λ light chain locus naturally appears adjacent to a human Vλ3-12 gene segment (represented by a dark grey square) and a human Vλ2-11 gene segment (represented by a dark grey square) in the endogenous human immunoglobulin Igλ light chain locus. Figure 20 also includes a second arrow pointing to a representation of a second exemplary endogenous human Vλ non-coding sequence in the endogenous human immunoglobulin λ light chain locus. As illustrated, the second exemplary endogenous human Vλ non-coding sequence (represented by a line) in the endogenous human immunoglobulin λ light chain locus naturally appears adjacent to a human Vλ2-11 gene segment (represented by a dark grey square) and a human Vλ3-10 gene segment (represented by a dark grey square) in the endogenous human immunoglobulin λ light chain locus.

Figure 21 shows an illustration, not to scale, of a portion of an endogenous human immunoglobulin κ light chain locus. Figure 21 includes a first arrow pointing to a representation of a first exemplary endogenous human Jκ non-coding sequence in the endogenous human immunoglobulin κ light chain locus. As illustrated, the first exemplary endogenous human Jκ non-coding sequence (represented by a line) in the endogenous human immunoglobulin κ light chain locus naturally appears adjacent to a human Jκ1 gene segment (represented by a dark grey square) and a human Jκ2 gene segment (represented by a dark grey square) in the endogenous human immunoglobulin κ light chain locus. Figure 21 also includes a second arrow pointing to a representation of a second exemplary endogenous human Jκ non-coding sequence in the endogenous human immunoglobulin κ light chain locus. As illustrated, the second exemplary endogenous human Jκ non-coding sequence (represented by a line) in the endogenous human immunoglobulin κ light chain locus naturally appears adjacent to a human Jκ2 gene segment (represented by a dark grey square) and a human Jκ3 gene segment (represented by a dark grey square) in the endogenous human immunoglobulin κ light chain locus.

BRIEF DESCRIPTION OF SELECTED SEQUENCES IN THE SEQUENCE LISTING

[0096] The following are representative nucleic acid and amino acid sequence of various immunoglobulin constant regions of the mouse, rat, or human lambda genes. Nucleic acid and amino acid sequences of immunoglobulin genes and polypeptides are available from the International Immunogenetics Information System website, www.imgt.org.

Mouse CΛ1 DNA (SEQ ID NO:1):
GCCAGCCCAAGTCTTCGCCATCAGTCACCCTGTTTCCACCTTCCTCTGAAGAGCTCGAG
ACTAACAAGGCCACACTGGTGTGTACGATCACTGATTTCTACCCAGGTGTGGTGACAGT
GGACTGGAAGGTAGATGGTACCCCTGTCACTCAGGGTATGGAGACAACCCAGCCTTCC
AAACAGAGCAACAACAAGTACATGGCTAGCAGCTACCTGACCCTGACAGCAAGAGCA
TGGGAAAGGCATAGCAGTTACAGCTGCCAGGTCACTCATGAAGGTCACACTGTGGAGA
AGAGTTTGTCCCGTGCTGACTGTTCC

Mouse CΛ1 amino acid (SEQ ID NO:2):
GQPKSSPSVTLFPPSSEELETNKATLVCTITDFYPGVVTVDWKVDGTPVTQGMETTQPSKQS
NNKYMASSYLTLTARAWERHSSYSCQVTHEGHTVEKSLSRADCS

Mouse CΛ2 DNA (SEQ ID NO:3):
GTCAGCCCAAGTCCACTCCCACTCTCACCGTGTTTCCACCTTCCTCTGAGGAGCTCAAG
GAAAACAAAGCCACACTGGTGTGTCTGATTTCCAACTTTTCCCCGAGTGGTGTGACAGT
GGCCTGGAAGGCAAATGGTACACCTATCACCCAGGGTGTGGACACTTCAAATCCCACC
AAAGAGGGCAACAAGTTCATGGCCAGCAGCTTCCTACATTTGACATCGGACCAGTGGA
GATCTCACAACAGTTTTACCTGTCAAGTTACACATGAAGGGGACACTGTGGAGAAGAG
TCTGTCTCCTGCAGAATGTCTC

Mouse CΛ2 amino acid (SEQ ID NO:4):
GQPKSTPTLTVFPPSSEELKENKATLVCLISNFSPSGVTVAWKANGTPITQGVDTSNPTKEG
NKFMASSFLHLTSDQWRSHNSFTCQVTHEGDTVEKSLSPAECL

Mouse CΛ3 DNA (SEQ ID NO:5):
GTCAGCCCAAGTCCACTCCCACACTCACCATGTTTCCACCTTCCCCTGAGGAGCTCCAG
GAAAACAAAGCCACACTCGTGTGTCTGATTTCCAATTTTTCCCCAAGTGGTGTGACAGT
GGCCTGGAAGGCAAATGGTACACCTATCACCCAGGGTGTGGACACTTCAAATCCCACC
AAAGAGGACAACAAGTACATGGCCAGCAGCTTCTTACATTTGACATCGGACCAGTGGA
GATCTCACAACAGTTTTACCTGCCAAGTTACACATGAAGGGGACACTGTGGAGAAGAG
TCTGTCTC CTGCAGAATG TCTC

Mouse CΛ3 amino acid (SEQ ID NO:6):
GQPKSTPTLTMFPPSSEELQENKATLVCLISNFSPSGVTVAWKANGTPITQGVDTSNPTKED

NKYMASSFLHLTSDQWRSHNSFTCQVTHEGDTVEKSLSPAECL

Rat CΛ1 DNA (SEQ ID NO:7):
GTCAGCCCAAGTCCACTCCCACACTCACAGTATTTCCACCTTCAACTGAGGAGCTCCAG
GGAAACAAAGCCACACTGGTGTGTCTGATTTCTGATTTCTACCCGAGTGATGTGGAAGT
GGCCTGGAAGGCAAATGGTGCACCTATCTCCCAGGGTGTGGACACTGCAAATCCCACC
AAACAGGGCAACAAATACATCGCCAGCAGCTTCTTACGTTTGACAGCAGAACAGTGGA
GATCTCGCAACAGTTTTACCTGCCAAGTTACACATGAAGGGAACACTGTGGAGAAGAG
TCTGTCTCCTGCAGAATGTGTC

Rat CΛ1 amino acid (SEQ ID NO:8):
GQPKSTPTLTVFPPSTEELQGNKATLVCLISDFYPSDVEVAWKANGAPISQGVDTANPTKQ
GNKYIASSFLRLTAEQWRSRNSFTCQVTHEGNTVEKSLSPAECV

Rat CΛ2 DNA (SEQ ID NO:9):
ACCAACCCAAGGCTACGCCCTCAGTCACCCTGTTCCCACCTTCCTCTGAAGAGCTCAAG
ACTGACAAGGCTACACTGGTGTGTATGGTGACAGATTTCTACCCTGGTGTTATGACAGT

GGTCTGGAAGGCAGATGGTACCCCTATCACTCAGGGTGTGGAGACTACCCAGCCTTTC
AAACAGAACAACAAGTACATGGCTACCAGCTACCTGCTTTTGACAGCAAAAGCATGGG
AGACTCATAGCAATTACAGCTGCCAGGTCACTCACGAAGAGAACACTGTGGAGAAGAG
TTTGTCCCGTGCTGAGTGTTCC

Rat CΛ2 amino acid (SEQ ID NO:10):
DQPKATPSVTLFPPSSEELKTDKATLVCMVTDFYPGVMTVVWKADGTPITQGVETTQPFK
QNNKYMATSYLLLTAKAWETHSNYSCQVTHEENTVEKSLSRAECS

Rat CΛ3 DNA (SEQ ID NO:11):
GTCAGCCCAAGTCCACTCCCACACTCACAGTATTTCCACCTTCAACTGAGGAGCTCCAG
GGAAACAAAGCCACACTGGTGTGTCTGATTTCTGATTTCTACCCGAGTGATGTGGAAGT
GGCCTGGAAGGCAAATGGTGCACCTATCTCCCAGGGTGTGGACACTGCAAATCCCACC
AAACAGGGCAACAAATACATCGCCAGCAGCTTCTTACGTTTGACAGCAGAACAGTGGA
GATCTCGCAACAGTTTTACCTGCCAAGTTACACATGAAGGGAACACTGTGGAAAAGAG
TCTGTCTCCTGCAGAGTGTGTC

Rat CΛ3 amino acid (SEQ ID NO:12):
GQPKSTPTLTVFPPSTEELQGNKATLVCLISDFYPSDVEVAWKANGAPISQGVDTANPTKQ
GNKYIASSFLRLTAEQWRSRNSFTCQVTHEGNTVEKSLSPAECV

Rat CΛ4 DNA (SEQ ID NO:13):
ACCAACCCAAGGCTACGCCCTCAGTCACCCTGTTCCCACCTTCCTCTGAAGAGCTCAAG
ACTGACAAGGCTACACTGGTGTGTATGGTGACAGATTTCTACCCTGGTGTTATGACAGT
GGTCTGGAAGGCAGATGGTACCCCTATCACTCAGGGTGTGGAGACTACCCAGCCTTTC
AAACAGAACAACAAGTACATGGCTACCAGCTACCTGCTTTTGACAGCAAAAGCATGGG
AGACTCATAGCAATTACAGCTGCCAGGTCACTCACGAAGAGAACACTGTGGAGAAGAG
TTTGTCCCGTGCTGAGTGTTCC

Rat CΛ4 amino acid (SEQ ID NO:14):
DQPKATPSVTLFPPSSEELKTDKATLVCMVTDFYPGVMTVVWKADGTPITQGVETTQPFK
QNNKYMATSYLLLTAKAWETHSNYSCQVTHEENTVEKSLSRAECS

Human CΛ1 DNA (SEQ ID NO:15):
CCCAAGGCCAACCCCACGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGCCAA
CAAGGCCACACTAGTGTGTCTGATCAGTGACTTCTACCCGGGAGCTGTGACAGTGGCTT
GGAAGGCAGATGGCAGCCCCGTCAAGGCGGGAGTGGAGACGACCAAACCCTCCAAAC
AGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCGAGCAGTGGA

AGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGA
CAGTGGCCCCTACAGAATGTTCATAG

Human CΛ1 amino acid (SEQ ID NO:16):
PKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSN
NKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Human CΛ2 DNA (SEQ ID NO:17):
GTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAA
GCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAG
TGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTC
CAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACGCCTGAGCA
GTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGA
GAAGACAGTGGCCCCTACAGAATGTTCA

Human CΛ2 amino acid (SEQ ID NO:18):
QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQS
NNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Human CΛ3 DNA (SEQ ID NO:19):
CCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCACCCTCCTCTGAGGAGCTTCAAGCCAA
CAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTTGCCT
GGAAGGCAGATAGCAGCCCCGTCAAGGCGGGGGTGGAGACCACCACACCCTCCAAAC
AAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCTGAGCAGTGGA
AGTCCCACAAAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGA
CAGTTGCCCCTACGGAATGTTCATAG

Human CΛ3 amino acid (SEQ ID NO:20):
PKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSN
NKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS

Human CΛ6 DNA (SEQ ID NO:21):
GGTCAGCCCAAGGCTGCCCCATCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCA
AGCCAACAAGGCCACACTGGTGTGCCTGATCAGTGACTTCTACCCGGGAGCTGTGAAA
GTGGCCTGGAAGGCAGATGGCAGCCCCGTCAACACGGGAGTGGAGACCACCACACCCT
CCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCTGAGC

AGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCGTGG
AGAAGACAGTGGCCCCTGCAGAATGTTCATAG

Human CA6 amino acid (SEQ ID NO:22):
QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVKVAWKADGSPVNTGVETTTPSKQS
NNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECS

Human CA7 DNA (SEQ ID NO:23):
GTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCACCCTCCTCTGAGGAGCTTCAA
GCCAACAAGGCCACACTGGTGTGTCTCGTAAGTGACTTCTACCCGGGAGCCGTGACAG
TGGCCTGGAAGGCAGATGGCAGCCCCGTCAAGGTGGGAGTGGAGACCACCAAACCCTC
CAAACAAAGCAACAACAAGTATGCGGCCAGCAGCTACCTGAGCCTGACGCCCGAGCA
GTGGAAGTCCCACAGAAGCTACAGCTGCCGGGTCACGCATGAAGGGAGCACCGTGGA
GAAGACAGTGGCCCCTGCAGAATGCTCT

Human CA7 amino acid (SEQ ID NO:24):
QPKAAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAWKADGSPVKVGVETTKPSKQ
SNNKYAASSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAPAECS

DEFINITIONS

[0097] The scope of the present invention is defined by the claims appended hereto and is not limited by certain embodiments described herein. Those skilled in the art, reading the present specification, will be aware of various modifications that may be equivalent to such described embodiments, or otherwise within the scope of the claims. In general, terms used herein are in accordance with their understood meaning in the art, unless clearly indicated otherwise. Explicit definitions of certain terms are provided below; meanings of these and other terms in particular instances throughout this specification will be clear to those skilled in the art from context. Additional definitions for the following and other terms are set forth throughout the specification. Patent and non-patent literature references cited within this specification, or relevant portions thereof.

[0098] Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[0099] As used in this application, the terms "*about*" and "*approximately*" are used as equivalents. Any numerals used in this application with or without about or approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0100] The articles "a" and "an" in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification.

[0101] ***Administration:*** as used herein, includes the administration of a composition to a subject or system (e.g., to a cell, organ, tissue, organism, or relevant component or set of components thereof). The skilled artisan will appreciate that route of administration may vary depending, for example, on the subject or system to which the composition is being administered, the nature of the composition, the purpose of the administration, etc. For example, in certain embodiments, administration to an animal subject (e.g., to a human or a mouse) may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and/or vitreal. In some embodiments, administration may involve intermittent dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time.

[0102] ***Amelioration*****:** as used herein, includes the prevention, reduction or palliation of a state, or improvement of the state of a subject. Amelioration includes but does not require complete recovery or complete prevention of a disease, disorder or condition.

[0103] ***Approximately***: as applied to one or more values of interest, includes to a value that is similar to a stated reference value. In certain embodiments, the term "*approximately*" or "*about*" refers to a range of values that fall within ± 10% (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0104] ***Biologically active***: as used herein, refers to a characteristic of any agent that has activity in a biological system, *in vitro* or *in vivo* (e.g., in an organism). For instance, an agent that, when present in an organism, has a biological effect within that organism is considered to be biologically active. In particular embodiments, where a protein or polypeptide is biologically active, a portion of that protein or polypeptide that shares at least one biological activity of the protein or polypeptide is typically referred to as a "*biologically active*" portion.

[0105] ***Comparable***: as used herein, refers to two or more agents, entities, situations, sets of conditions, etc. that may not be identical to one another but that are sufficiently similar to permit comparison there between so that conclusions may reasonably be drawn based on differences or similarities observed. Persons of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc. to be considered comparable.

[0106] ***Conservative***: as used herein, refers to instances when describing a conservative amino acid substitution, including a substitution of an amino acid residue by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of interest of a protein, for example, the ability of a receptor to bind to a ligand. Examples of groups of amino acids that have side chains with similar chemical properties include: aliphatic side chains such as glycine (Gly, G), alanine (Ala, A), valine (Val, V), leucine (Leu, L), and isoleucine (Ile, I); aliphatic-hydroxyl side chains such as serine (Ser, S) and threonine (Thr, T); amide-containing side chains such as asparagine (Asn, N) and glutamine (Gln, Q); aromatic side chains such as phenylalanine (Phe, F), tyrosine (Tyr, Y), and tryptophan (Trp, W); basic side chains such as lysine (Lys, K), arginine (Arg, R), and histidine (His, H); acidic side chains such as aspartic acid (Asp, D) and glutamic acid (Glu, E); and sulfur-containing side chains such as cysteine (Cys, C) and methionine (Met, M). Conservative amino acids substitution groups include, for example, valine/leucine/isoleucine (Val/Leu/Ile, V/L/I), phenylalanine/tyrosine (Phe/Tyr, F/Y), lysine/arginine (Lys/Arg, K/R), alanine/valine (Ala/Val, A/V), glutamate/aspartate (Glu/Asp, E/D), and asparagine/glutamine (Asn/Gln, N/Q). In some embodiments, a conservative amino acid substitution can be a substitution of any native residue in a protein with alanine, as used in, for example, alanine scanning mutagenesis. In some embodiments, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet, G.H. et al., 1992, Science 256:1443-1445. In some embodiments, a substitution is a moderately conservative substitution wherein the substitution has a nonnegative value in the PAM250 log-likelihood matrix.

[0107] ***Control***: as used herein, refers to the art-understood meaning of a "*control*" being a standard against which results are compared. Typically, controls are used to augment integrity in experiments by isolating variables in order to make a conclusion about such variables. In some embodiments, a control is a reaction or assay that is performed simultaneously with a test reaction or assay to provide a comparator. A "*control*" also includes a "*control animal*." A "*control animal*" may have a modification as described herein, a modification that is different as described herein, or no modification (i.e., a wild-type animal). In one experiment, a "*test*" (i.e., a variable being tested) is applied. In a second experiment, the "*control*," the variable being tested is not applied. In some embodiments, a control is a historical control (i.e., of a test or assay performed previously, or an amount or result that is previously known). In some embodiments, a control is or comprises a printed or otherwise saved record. A control may be a positive control or a negative control.

[0108] ***Disruption***: as used herein, refers to the result of a homologous recombination event with a DNA molecule (e.g., with an endogenous homologous sequence such as a gene or gene locus). In some embodiments, a disruption may achieve or represent an insertion, deletion, substitution, replacement, missense mutation, or a frame-shift of a DNA sequence(s), or any combination thereof. Insertions may include the insertion of entire genes or gene fragments, e.g., exons, which may be of an origin other than the endogenous sequence (e.g., a heterologous sequence). In some embodiments, a disruption may increase expression and/or activity of a gene or gene product (e.g., of a polypeptide encoded by a gene). In some embodiments, a disruption may decrease expression and/or activity of a gene or gene product. In some embodiments, a disruption may alter sequence of a gene or an encoded gene product (e.g., an encoded polypeptide). In some embodiments, a disruption may truncate or fragment a gene or an encoded gene product (e.g., an encoded polypeptide). In some embodiments, a disruption may extend a gene or an encoded gene product. In some such embodiments, a disruption may achieve assembly of a fusion polypeptide. In some embodiments, a disruption may affect level, but not activity, of a gene or gene product. In some embodiments, a disruption may affect activity, but not level, of a gene or gene product. In some embodiments, a disruption may have no significant effect on level of a gene or gene product. In some embodiments, a disruption may have no significant effect on activity of a gene or gene product. In some embodiments, a disruption may have no significant effect on either level or activity of a gene or gene product.

[0109] ***Determining, measuring, evaluating, assessing, assaying*** and ***analyzing***: are used interchangeably herein to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assaying may be relative or absolute. "*Assaying for the presence of*" can be determining the amount of something present and/or determining whether or not it is present or absent.

[0110] ***Endogenous promoter***: as used herein, refers to a promoter that is naturally associated, e.g., in a wild-type organism, with an endogenous gene.

[0111] ***Engineered***: as used herein refers, in general, to the aspect of having been manipulated by the hand of man. For example, in some embodiments, a polynucleotide may be considered to be "*engineered*" when two or more sequences that are not linked together in that order in nature are manipulated by the hand of man to be directly linked to one another in the engineered polynucleotide. In some embodiments, an engineered polynucleotide may comprise a regulatory sequence that is found in nature in operative association with a first coding sequence but not in operative association with a second coding sequence, is linked by the hand of man so that it is operatively associated with the second coding sequence. Alternatively, or additionally, in some embodiments, first and second nucleic acid sequences that each encode polypeptide elements or domains that in nature are not linked to one another may be linked to one another in a single engineered polynucleotide. Comparably, in some embodiments, a cell or organism may be considered to be "*engineered*" if it has been manipulated so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, or previously present genetic material has been altered or removed). As is common practice and is understood by persons of skill in the art, progeny of an engineered polynucleotide or cell are typically still referred to as "*engineered*" even though the actual manipulation was performed on a prior entity. Furthermore, as will be appreciated by persons of skill in the art, a variety of methodologies are available through which "*engineering*" as described herein may be achieved. For example, in some embodiments, "*engineering*" may involve selection or design (e.g., of nucleic acid sequences, polypeptide sequences, cells, tissues, and/or organisms) through use of computer systems programmed to perform analysis or comparison, or otherwise to analyze, recommend, and/or select sequences, alterations, etc.). Alternatively, or additionally, in some embodiments, "*engineering*" may involve use of *in vitro* chemical synthesis methodologies and/or recombinant nucleic acid technologies such as, for example, nucleic acid amplification (e.g., via the polymerase chain reaction) hybridization, mutation, transformation, transfection, etc., and/or any of a variety of controlled mating methodologies. As will be appreciated by those skilled in the art, a variety of established such techniques (e.g., for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection, etc.) are well known in the art and described in various general and more specific references that are cited and/or discussed throughout the present specification. See e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989 and Principles of Gene Manipulation: An Introduction to Genetic Manipulation, 5th Ed., ed. By Old, R.W. and S.B. Primrose, Blackwell Science, Inc., 1994.

[0112] ***Functional***: as used herein, refers to a form or fragment of an entity (e.g., a gene or gene segment) that exhibits a particular property (e.g., forms part of a coding sequence) and/or activity. For example, in the context of immunoglobulins, variable regions are encoded by unique gene segments (i.e., V, D and/or J) that are assembled (or recombined) to form functional coding sequences. When present in the genome, gene segments are organized in clusters, although variations do occur. A "*functional*" gene segment is a gene segment represented in an expressed sequence (i.e., a variable region) for which the corresponding genomic DNA has been isolated (i.e., cloned) and identified by sequence. Some immunoglobulin gene segment sequences contain open reading frames and are considered functional although not represented in an expressed repertoire, while other immunoglobulin gene segment sequences contain mutations (e.g., point mutations, insertions, deletions, etc.) resulting in a stop codon and/or truncated sequence which subsequently render(s) such gene segment sequences unable to perform the property/ies and/or activity/ies associated with a non-mutated sequence(s). Such sequences are not represented in expressed sequences and, therefore, categorized as pseudogenes.

[0113] ***Gene***: as used herein, refers to a DNA sequence in a chromosome that codes for a product (e.g., an RNA product and/or a polypeptide product). In some embodiments, a gene includes coding sequence (i.e., sequence that encodes a particular product). In some embodiments, a gene includes non-coding sequence. In some particular embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequence. In some embodiments, a gene may include one or more regulatory sequences (e.g., promoters, enhancers, etc.) and/or intron sequences that, for example, may control or impact one or more aspects of gene expression (e.g., cell-type-specific expression, inducible expression, etc.). For the purpose of clarity, we note that, as used in the present disclosure, the term "*gene*" generally refers to a portion of a nucleic acid that encodes a polypeptide or fragment thereof; the term may optionally encompass regulatory sequences, as will be clear from context to those of ordinary skill in the art. This definition is not intended to exclude application of the term "*gene*" to non-protein-coding expression units but rather to clarify that, in most cases, the term as used in this document refers to a polypeptide-coding nucleic acid.

[0114] ***Heterologous***: as used herein, refers to an agent or entity from a different source. For example, when used in reference to a polypeptide, gene, or gene product present in a particular cell or organism, the term clarifies that the relevant polypeptide, gene, or gene product: 1) was engineered by the hand of man; 2) was introduced into the cell or organism (or a precursor thereof) through the hand of man (e.g., via genetic engineering); and/or 3) is not naturally produced by or present in the relevant cell or organism (e.g., the relevant cell type or organism type). "*Heterologous*" also includes a polypeptide, gene or gene product that is normally present in a particular native cell or organism, but has been altered or modified, for example, by mutation or placement under the control of non-naturally associated and, in some embodiments, non-endogenous regulatory elements (e.g., a promoter).

[0115] ***Host cell***: as used herein, refers to a cell into which a nucleic acid or protein has been introduced. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but also is used to refer to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the phrase "*host cell.*" In some embodiments, a host cell is or comprises a prokaryotic or eukaryotic cell. In general, a host cell is any cell that is suitable for receiving and/or producing a heterologous nucleic acid or protein, regardless of the Kingdom of life to which the cell is designated. Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of *Escherichia coli, Bacillus spp., Streptomyces spp.*, etc.), mycobacteria cells, fungal cells, yeast cells (e.g., *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Pichia 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, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, a cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, a 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, a cell comprises one or more viral genes, e.g., a retinal cell that expresses a viral gene (e.g., a PER.C6® cell). In some embodiments, a host cell is or comprises an isolated cell. In some embodiments, a host cell is part of a tissue. In some embodiments, a host cell is part of an organism.

[0116] ***Identity*****:** as used herein in connection with a comparison of sequences, refers to identity as determined by a number of different algorithms known in the art that can be used to measure nucleotide and/or amino acid sequence identity. In some embodiments, identities as described herein are determined using a ClustalW v. 1.83 (slow) alignment employing an open gap penalty of 10.0, an extend gap penalty of 0.1, and using a Gonnet similarity matrix (MACVECTOR™ 10.0.2, MacVector Inc., 2008).

[0117] ***In place of*****:** as used herein, refers to a positional substitution in which a first nucleic acid sequence is located at the position of a second nucleic acid sequence in a chromosome (e.g., where the second nucleic acid sequence was previously (e.g., originally) located in a chromosome, e.g., at the endogenous locus of the second nucleic acid sequence). The phrase "in place of" does not require that the second nucleic acid sequence be removed from, e.g., a locus or chromosome. In some embodiments, the second nucleic acid sequence and the first nucleic acid sequence are comparable to one another in that, for example, the first and second sequences are homologous to one another, contain corresponding elements (e.g., protein-coding elements, regulatory elements, etc.), and/or have similar or identical sequences. In some embodiments, a first and/or second nucleic acid sequence includes one or more of a promoter, an enhancer, a splice donor site, a splice acceptor site, an intron, an exon, an untranslated region (UTR); in some embodiments, a first and/or second nucleic acid sequence includes one or more coding sequences. In some embodiments, a first nucleic acid sequence is a homolog or variant (e.g., mutant) of the second nucleic acid sequence. In some embodiments, a first nucleic acid sequence is an ortholog or homolog of the second sequence. In some embodiments, a first nucleic acid sequence is or comprises a human nucleic acid sequence. In some embodiments, including where the first nucleic acid sequence is or comprises a human nucleic acid sequence, the second nucleic acid sequence is or comprises a rodent sequence (e.g., a mouse or rat sequence). In some embodiments, including where the first nucleic acid sequence is or comprises a human nucleic acid sequence, the second nucleic acid sequence is or comprises a human sequence. In some embodiments, a first nucleic acid sequence is a variant or mutant (i.e., a sequence that contains one or more sequence differences, e.g., substitutions, as compared to the second sequence) of the second sequence. The nucleic acid sequence so placed may include one or more regulatory sequences that are part of source nucleic acid sequence used to obtain the sequence so placed (e.g., promoters, enhancers, 5'- or 3'-untranslated regions, etc.). For example, in various embodiments, a first nucleic acid sequence is a substitution of an endogenous sequence with a heterologous sequence that results in the production of a gene product from the nucleic acid sequence so placed (comprising the heterologous sequence), but not expression of the endogenous sequence; a first nucleic acid sequence is of an endogenous genomic sequence with a nucleic acid sequence that encodes a polypeptide that has a similar function as a polypeptide encoded by the endogenous sequence (e.g., the endogenous genomic sequence encodes a non-human variable region polypeptide, in whole or in part, and the DNA fragment encodes one or more human variable region polypeptides, in whole or in part). In various embodiments, a human immunoglobulin gene segment or fragment thereof is in place of an endogenous non-human immunoglobulin gene segment or fragment.

[0118] ***In vitro*****:** as used herein refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

[0119] ***In vivo*****:** as used herein refers to events that occur within a multi-cellular organism, such as a human and/or a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0120] ***Isolated*****:** as used herein, refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are separated from 10% to 100%, 15%-100%, 20%-100%, 25%-100%, 30%-100%, 35%-100%, 40%-100%, 45%-100%, 50%-100%, 55%-100%, 60%-100%, 65%-100%, 70%-100%, 75%-100%, 80%-100%, 85%-100%, 90%-100%, 95%-100%, 96%-100%, 97%-100%, 98%-100%, or 99%-100% of the other components with which they were initially associated. In some embodiments, isolated agents are separated from 10% to 100%, 10%-99%, 10%-98%, 10%-97%, 10%-96%, 10%-95%, 10%-90%, 10%-85%, 10%-80%, 10%-75%, 10%-70%, 10%-65%, 10%-60%, 10%-55%, 10%-50%, 10%-45%, 10%-40%, 10%-35%, 10%-30%, 10%-25%, 10%-20%, or 10%-15% of the other components with which they were initially associated. In some embodiments, isolated agents are separated from 11% to 99%, 12%-98%, 13%-97%, 14%-96%, 15%-95%, 20%-90%, 25%-85%, 30%-80%, 35%-75%, 40%-70%, 45%-65%, 50%-60%, or 55%-60% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. In some embodiments, isolated agents are 80%-99%, 85%-99%, 90%-99%, 95%-99%, 96%-99%, 97%-99%, or 98%-99% pure. In some embodiments, isolated agents are 80%-99%, 80%-98%, 80%-97%, 80%-96%, 80%-95%, 80%-90%, or 80%-85% pure. In some embodiments, isolated agents are 85%-98%, 90%-97%, or 95%-96% pure. In some embodiments, a substance is "*pure*" if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered *"isolated"* or even "*pure*", after having been combined with certain other components such as, for example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be *"isolated"* when: a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; or c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide that is chemically synthesized, or is synthesized in a cellular system different from that which produces it in nature, is considered to be an *"isolated"* polypeptide. Alternatively, or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an *"isolated"* polypeptide to the extent that it has been separated from other components: a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0121] ***Locus*** or ***loci***: as used herein, refers to a location(s) of a gene (or significant sequence), DNA sequence, polypeptide-encoding sequence, or position on a chromosome of the genome of an organism. For example, an "*immunoglobulin locus*" may refer to the location of an immunoglobulin gene segment (e.g., V, D, J or C), immunoglobulin gene segment DNA sequence, immunoglobulin gene segment-encoding sequence, or immunoglobulin gene segment position on a chromosome of the genome of an organism that has been identified as to where such a sequence resides. An "*immunoglobulin locus*" may comprise a regulatory element of an immunoglobulin gene segment, including, but not limited to, an enhancer, a promoter, 5' and/or 3' regulatory sequence or region, or a combination thereof. An "*immunoglobulin locus*" may comprise intergenic DNA, e.g., DNA that normally resides or appears between gene segments in a wild-type locus. Persons of ordinary skill in the art will appreciate that chromosomes may, in some embodiments, contain hundreds or even thousands of genes and demonstrate physical co-localization of similar genetic loci when comparing between different species. Such genetic loci can be described as having shared synteny.

[0122] ***Naturally appears***: as used herein in reference to a biological element (e.g., a nucleic acid sequence) means that the biological element can be found in a specified context and/or location, absent engineering (e.g., genetic engineering), in a cell or organism (e.g., an animal). In other words, a sequence that naturally appears in a specified context and/or location is not in the specified context and/or location as the result of engineering (e.g., genetic engineering). For example, a sequence that naturally appears adjacent to a human Jκ1 gene segment in an endogenous human immunoglobulin kappa light chain locus is a sequence that can be found adjacent to a human Jκ1 gene segment in an endogenous human immunoglobulin kappa light chain locus, absent genetic engineering, in a human. In some embodiments, a sequence can be obtained, derived, and/or isolated from where it naturally appears in a cell or organism. In some embodiments, a cell or organism is not a direct source of a sequence that naturally appears in the cell or organism. For example, a corresponding sequence in a cell or organism could be identified and then produced or replicated by mechanisms known in the art.

[0123] ***Non-human animal***: as used herein, refers to any vertebrate organism that is not a human. In some instances, a non-human animal is a cyclostome, a bony fish, a cartilaginous fish (e.g., a shark or a ray), an amphibian, a reptile, a mammal, and a bird. In some embodiments, a non-human animal is a mammal. In some instances, a non-human mammal is a primate, a goat, a sheep, a pig, a dog, a cow, or a mouse. In some instances, a non-human animal is a mouse. The non-human animal employed in a method of the present invention is a mouse.

[0124] ***Nucleic acid***: as used herein, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a "*nucleic acid*" is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some embodiments, "*nucleic acid*" refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides); in some embodiments, "*nucleic acid*" refers to an oligonucleotide chain comprising individual nucleic acid residues. In some embodiments, a "nucleic acid" is or comprises RNA; in some embodiments, a "*nucleic acid*" is or comprises DNA. In some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a *"nucleic acid*" is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a nucleic acid analog differs from a "*nucleic acid*" in that it does not utilize a phosphodiester backbone. For example, in some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more "*peptide nucleic acids*", which are known in the art and have peptide bonds instead of phosphodiester bonds in the

backbone. Alternatively, or additionally, in some embodiments, a "*nucleic acid*" has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine). In some embodiments, a "*nucleic acid*" is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a "*nucleic acid*" comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some embodiments, a "*nucleic acid*" has a nucleotide sequence that encodes a functional gene product such as an RNA or polypeptide. In some embodiments, a "*nucleic acid*" includes one or more introns. In some embodiments, a "*nucleic acid*" includes one or more exons. In some embodiments, a "*nucleic acid*" is prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a "*nucleic acid*" is at least, e.g., but not limited to, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 20, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some embodiments, a "*nucleic acid*" is single stranded; in some embodiments, a "*nucleic acid*" is double stranded. In some embodiments, a "*nucleic acid*" has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some embodiments, a "*nucleic acid*" has enzymatic activity.

[0125] ***Operably linked***: as used herein, refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "*operably linked*" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "*Operably linked*" sequences include both expression control sequences that are contiguous with a gene of interest and expression control sequences that act in trans or at a distance to control a gene of interest (or sequence of interest). The term "*expression control sequence*" includes polynucleotide sequences, which are necessary to affect the expression and processing of coding sequences to which they are ligated. "*Expression control sequences*" include: appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance polypeptide stability; and when desired, sequences that enhance polypeptide secretion. The nature of such control sequences differs depending upon the host organism. For example, in prokaryotes, such control sequences generally include promoter, ribosomal binding site and transcription termination sequence, while in eukaryotes typically such control sequences include promoters and transcription termination sequence. The term "*control sequences*" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0126] ***Physiological conditions:*** as used herein, refers to its art-understood meaning referencing conditions under which cells or organisms live and/or reproduce. In some embodiments, the term includes conditions of the external or internal milieu that may occur in nature for an organism or cell system. In some embodiments, physiological conditions are those conditions present within the body of a human or non-human animal, especially those conditions present at and/or within a surgical site. Physiological conditions typically include, e.g., a temperature range of 20-40°C, atmospheric pressure of 1, pH of 6-8, glucose concentration of 1-20mM, oxygen concentration at atmospheric levels, and gravity as it is encountered on earth. In some embodiments, conditions in a laboratory are manipulated and/or maintained at physiologic conditions. In some embodiments, physiological conditions are encountered in an organism.

[0127] ***Polypeptide***: as used herein, refers to any polymeric chain of amino acids. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that contains portions that occur in nature separately from one another (i.e., from two or more different organisms, for example, human and non-human portions). In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some embodiments, a polypeptide has an amino acid sequence encoded by a sequence that does not occur in nature (e.g., a sequence that is engineered in that it is designed and/or produced through action of the hand of man to encode said polypeptide).

[0128] ***Recombinant***: as used herein, refers to polypeptides that are designed, engineered, prepared, expressed, created or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell, polypeptides isolated from a recombinant, combinatorial human polypeptide library (Hoogenboom, H. R., 1997, TIB Tech. 15:62-70; Azzazy, H. and W.E. Highsmith, 2002, Clin. Biochem. 35:425-45; Gavilondo, J. V. and J.W. Larrick, 2002, BioTechniques 29:128-45; Hoogenboom H., and P. Chames, 2000, Immunol. Today 21:371-8), antibodies isolated from an animal (e.g., a mouse) that has been genetically engineered to include human immunoglobulin genes (see e.g., Taylor, L. D. et al., 1992, Nucl. Acids Res. 20:6287-95; Kellermann, S-A. and L.L. Green, 2002, Curr. Opin. Biotechnol. 13:593-7; Little, M. et al., 2000, Immunol. Today 21:364-70; Osborn, M.J. et al., 2013, J. Immunol. 190:1481-90; Lee, E-C. et al., 2014, Nat. Biotech. 32(4):356-63; Macdonald, L.E. et al., 2014, Proc. Natl. Acad. Sci. U.S.A. 111(14):5147-52; Murphy, A.J. et al., 2014, Proc. Natl. Acad. Sci. U.S.A. 111(14):5153-8) or polypeptides prepared, expressed, created or isolated by any other means that involves splicing selected sequence elements to one another. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed *in silico*. In some embodiments, one or more such selected sequence elements result from mutagenesis (e.g., *in vivo* or *in vitro*) of a known sequence element, e.g., from a natural or synthetic (e.g., man-made) source. For example, in some embodiments, a recombinant polypeptide is comprised of sequences found in the genome of a source organism of interest (e.g., human, mouse, etc.). In some embodiments, a recombinant polypeptide has an amino acid sequence that resulted from mutagenesis (e.g., *in vitro* or *in vivo*, for example, in a non-human animal), so that the amino acid sequences of the recombinant polypeptides are sequences that, while originating from and related to polypeptides sequences, may not naturally exist within the genome of a non-human animal *in vivo*.

[0129] ***Reference***: as used herein, refers to a standard or control agent, animal, cohort, individual, population, sample, sequence or value against which an agent, animal, cohort, individual, population, sample, sequence or value of interest is compared. In some embodiments, a reference agent, animal, cohort, individual, population, sample, sequence or value is tested and/or determined substantially simultaneously with the testing or determination of an agent, animal, cohort, individual, population, sample, sequence or value of interest. In some embodiments, a reference agent, animal, cohort, individual, population, sample, sequence or value is a historical reference, optionally embodied in a tangible medium. In some embodiments, a reference may refer to a control. A "*reference*" also includes a "*reference animal.*" A "*reference animal*" may have a modification as described herein, a modification that is different as described herein or no modification (i.e., a wild-type animal). Typically, as would be understood by persons of skill in the art, a reference agent, animal, cohort, individual, population, sample, sequence or value is determined or characterized under conditions comparable to those utilized to determine or characterize an agent, animal (e.g., a mammal), cohort, individual, population, sample, sequence or value of interest.

[0130] ***Replacement***: as used herein, refers to a process through which a "*replaced*" nucleic acid sequence (e.g., a gene) found in a host locus (e.g., in a genome) is removed from that locus, and a different, "*replacement*" nucleic acid is located in its place. In some embodiments, the replaced nucleic acid sequence and the replacement nucleic acid sequences are comparable to one another in that, for example, they are homologous to one another, contain corresponding elements (e.g., protein-coding elements, regulatory elements, etc.), and/or have similar or identical sequences. In some embodiments, a replaced nucleic acid sequence includes one or more of a promoter, an enhancer, a splice donor site, a splice acceptor site, an intron, an exon, an untranslated region (UTR); in some embodiments, a replacement nucleic acid sequence includes one or more coding sequences. In some embodiments, a replacement nucleic acid sequence is a homolog or variant (e.g., mutant) of the replaced nucleic acid sequence. In some embodiments, a replacement nucleic acid sequence is an ortholog or homolog of the replaced sequence. In some embodiments, a replacement nucleic acid sequence is or comprises a human nucleic acid sequence. In some embodiments, including where the replacement nucleic acid sequence is or comprises a human nucleic acid sequence, the replaced nucleic acid sequence is or comprises a mouse sequence. In some embodiments, including where the replacement nucleic acid sequence is or comprises a human nucleic acid sequence, the replaced nucleic acid sequence is or comprises a human sequence. In some embodiments, a replacement nucleic acid sequence is a variant or mutant (i.e., a sequence that contains one or more sequence differences, e.g., substitutions, as compared to the replaced sequence) of the replaced sequence. The nucleic acid sequence so placed may include one or more regulatory sequences that are part of source nucleic acid sequence used to obtain the sequence so placed (e.g., promoters, enhancers, 5'- or 3'-untranslated regions, etc.). For example, in various embodiments, a replacement is a substitution of an endogenous sequence with a heterologous sequence that results in the production of a gene product from the nucleic acid sequence so placed (comprising the heterologous sequence), but not expression of the endogenous sequence; a replacement is of an endogenous genomic sequence with a nucleic acid sequence that encodes a polypeptide that has a similar function as a polypeptide encoded by the endogenous sequence (e.g., the endogenous genomic sequence encodes a non-human variable region polypeptide, in whole or in part, and the DNA fragment encodes one or more human variable region polypeptides, in whole or in part). In various embodiments, an endogenous non-human immunoglobulin gene segment or fragment thereof is replaced with a human immunoglobulin gene segment or fragment thereof.

[0131] ***Substantially***: as used herein, refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "*substantially*" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0132] ***Substantial similarity***: as used herein, refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "*substantially similar*" if they contain similar residues (e.g., amino acids or nucleotides) in corresponding positions. As is understood in the art, while similar residues may be identical residues (see also ***Substantial Identity,*** below), similar residues may also be non-identical residues with appropriately comparable structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as "*hydrophobic*" or "*hydrophilic*" amino acids, and/or as having "*polar*" or "*non-polar*" side chains. Substitution of one amino acid for another of the same type may often be considered a "*conservative*" substitution. Typical amino acid categorizations are summarized in the table below.

Alanine	Ala	A	Nonpolar	Neutral	1.8
Arginine	Arg	R	Polar	Positive	-4.5
Asparagine	Asn	N	Polar	Neutral	-3.5
Aspartic acid	Asp	D	Polar	Negative	-3.5
Cysteine	Cys	C	Nonpolar	Neutral	2.5
Glutamic acid	Glu	E	Polar	Negative	-3.5
Glutamine	Gln	Q	Polar	Neutral	-3.5
Glycine	Gly	G	Nonpolar	Neutral	-0.4
Histidine	His	H	Polar	Positive	-3.2
Isoleucine	Ile	I	Nonpolar	Neutral	4.5
Leucine	Leu	L	Nonpolar	Neutral	3.8
Lysine	Lys	K	Polar	Positive	-3.9
Methionine	Met	M	Nonpolar	Neutral	1.9
Phenylalanine	Phe	F	Nonpolar	Neutral	2.8
Proline	Pro	P	Nonpolar	Neutral	-1.6
Serine	Ser	S	Polar	Neutral	-0.8
Threonine	Thr	T	Polar	Neutral	-0.7
Tryptophan	Trp	W	Nonpolar	Neutral	-0.9
Tyrosine	Tyr	Y	Polar	Neutral	-1.3
Valine	Val	V	Nonpolar	Neutral	4.2

Ambiguous Amino Acids	3 -Letter	1-Letter
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z
Leucine or Isoleucine	Xle	J
Unspecified or unknown amino acid	Xaa	X

[0133] As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, S. F. et al., 1990, J. Mol. Biol., 215(3): 403-10; Altschul, S.F. et al., 1996, Meth. Enzymol. 266:460-80; Altschul, S.F. et al., 1997, Nucleic Acids Res., 25:3389-402; Baxevanis, A.D. and B.F.F. Ouellette (eds.) Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener et al. (eds.) Bioinformatics Methods and Protocols, Methods in Molecular Biology, Vol. 132, Humana Press, 1998. In addition to identifying similar sequences, the programs mentioned above typically provide an indication of the degree of similarity. In some embodiments, two sequences are considered to be substantially similar if at least, e.g., but not limited to, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are similar (e.g., identical or include a conservative substitution) over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence (e.g. a sequence of a gene, a gene segment, a sequence encoding a domain, a polypeptide, or a domain). In some embodiments, the relevant stretch is at least 9, 10, 11, 12, 13, 14, 15, 16, 17 or more residues. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues. In some embodiments, the relevant stretch includes contiguous residues along a complete sequence. In some embodiments, the relevant stretch includes discontinuous residues along a complete sequence, for example, noncontiguous residues brought together by the folded conformation of a polypeptide or a portion thereof.

[0134] ***Substantial identity***: as used herein, refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be "*substantially identical*" if they contain identical residues (e.g., amino acids or nucleotides) in corresponding positions. As is well-known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul, S. F. et al., 1990, J. Mol. Biol., 215(3): 403-10; Altschul, S.F. et al., 1996, Meth. Enzymol. 266:460-80; Altschul, S.F. et al., 1997, Nucleic Acids Res., 25:3389-402; Baxevanis, A.D. and B.F.F. Ouellette (eds.) Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener et al. (eds.) Bioinformatics Methods and Protocols, Methods in Molecular Biology, Vol. 132, Humana Press, 1998. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some embodiments, two sequences are considered to be substantially identical if at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, a relevant stretch of residues is a complete sequence. In some embodiments, a relevant stretch of residues is, e.g., but not limited to, at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues.

[0135] ***Targeting construct*** or ***targeting vector***: as used herein, refers to a polynucleotide molecule that comprises a targeting region. A targeting region comprises a sequence that is identical or substantially identical to a sequence in a target cell, tissue or animal and provides for integration of the targeting construct into a position within the genome of the cell, tissue or animal via homologous recombination. Targeting regions that target using site-specific recombinase recognition sites (e.g., *lox*P or Frt sites) are also included and described herein. In some embodiments, a targeting construct as described herein further comprises a nucleic acid sequence or gene of particular interest, a selectable marker, control and/or regulatory sequences, and other nucleic acid sequences that allow for recombination mediated through exogenous addition of proteins that aid in or facilitate recombination involving such sequences. In some embodiments, a targeting construct as described herein further comprises a gene of interest in whole or in part, wherein the gene of interest is a heterologous gene that encodes a polypeptide, in whole or in part, that has a similar function as a protein encoded by an endogenous sequence. In some embodiments, a targeting construct as described herein further comprises a humanized gene of interest, in whole or in part, wherein the humanized gene of interest encodes a polypeptide, in whole or in part, that has a similar function as a polypeptide encoded by an endogenous sequence. In some embodiments, a targeting construct (or targeting vector) may comprise a nucleic acid sequence manipulated by the hand of man. For example, in some embodiments, a targeting construct (or targeting vector) may be constructed to contain an engineered or recombinant polynucleotide that contains two or more sequences that are not linked together in that order in nature yet manipulated by the hand of man to be directly linked to one another in the engineered or recombinant polynucleotide.

[0136] ***Transgene*** or ***transgene construct:*** as used herein, refers to a nucleic acid sequence (encoding e.g., a polypeptide of interest, in whole or in part) that has been introduced into a cell by the hand of man such as by the methods described herein. A transgene could be partly or entirely heterologous, i.e., foreign, to the genetically engineered animal or cell into which it is introduced. A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns or promoters, which may be necessary for expression of a selected nucleic acid sequence.

[0137] ***Genetically modified non-human animal or genetically engineered non-human animal***: are used interchangeably herein and refer to any non-naturally occurring non-human animal in which one or more of the cells of the non-human animal contain heterologous nucleic acid and/or gene encoding a polypeptide of interest, in whole or in part. For example, in some embodiments, a "*genetically modified non-human animal*" or "*genetically engineered non-human animal*" refers to non-human animal that contains a transgene or transgene construct as described herein. In some embodiments, a heterologous nucleic acid and/or gene is introduced into the cell, directly or indirectly by introduction into a precursor cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classic breeding techniques, but rather is directed to introduction of recombinant DNA molecule(s). This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. The phrases "*genetically modified non-human animal*" or "*genetically engineered non-human animal*" refers to animals that are heterozygous or homozygous for a heterologous nucleic acid and/or gene, and/or animals that have single or multi-copies of a heterologous nucleic acid and/or gene. The genetically modified non-human animal employed in the method provided is a mouse.

[0138] ***Vector***: as used herein, refers to a nucleic acid molecule capable of transporting another nucleic acid to which it is associated. In some embodiment, vectors are capable of extra-chromosomal replication and/or expression of nucleic acids to which they are linked in a host cell such as a eukaryotic and/or prokaryotic cell. Vectors capable of directing the expression of operably linked genes are referred to herein as "*expression vectors*."

[0139] ***Wild-type***: as used herein, refers to an entity having a structure and/or activity as found in nature in a "*normal*" (as contrasted with mutant, diseased, altered, etc.) state or context. Those of ordinary skill in the art will appreciate that wild-type genes and polypeptides often exist in multiple different forms (e.g., alleles).

DETAILED DESCRIPTION

[0140] A method of producing an antibody in a genetically modified mouse is provided, the method comprising the steps of:

1. (a) immunizing the genetically modified mouse with an antigen of interest,
   wherein the genetically modified mouse has a germline genome comprising:

   a first engineered endogenous immunoglobulin κ light chain locus comprising:

   1. (i) one or more human Vλ gene segments,
   2. (ii) one or more human Jλ gene segments, and
   3. (iii) one mouse Cλ gene,

      wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are operably linked to the one mouse Cλ gene of (iii);

      wherein the one mouse Cλ gene (iii) is in the place of a mouse Cκ gene at the first engineered endogenous immunoglobulin κ light chain locus;

      wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are in place of one or more endogenous mouse Vκ gene segments and one or more endogenous mouse Jκ gene segments;

      wherein the genetically modified mouse lacks a mouse Cκ gene at the first engineered endogenous immunoglobulin κ light chain locus;

2. (b) maintaining the genetically modified mouse under conditions sufficient for the genetically modified mouse to produce an immune response to the antigen of interest; and
3. (c) recovering from the genetically modified mouse:
   1. (i) an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain,
   2. (ii) a nucleotide that encodes a human light chain variable domain or human heavy chain variable domain, a light chain, or a heavy chain of an antibody that binds the antigen of interest, wherein the antibody that binds the antigen of interest comprises a light chain comprising a human λ variable domain and a mouse λ constant domain, or
   3. (iii) a cell that expresses an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain. The present disclosure also describes, among other things, engineered mice having heterologous genetic material encoding human Vλ domains, which heterologous genetic material comprises human Vλ and Jλ gene sequences (i.e., gene segments) and other human sequences that provide for proper rearrangement (e.g., recombination signal sequence (RSS)) and expression of antibodies having Igλ light chains that include a human portion and a non-human portion, or antibodies having Igλ light chains that are fully human. For example, in various embodiments, when a human gene segment is present in a genome of an engineered mouse, the corresponding recombination signal sequence(s) can also be present (e.g., Vλ RSS with Vλ gene segment, Jλ RSS with Jλ gene segment, Vκ RSS with Vκ gene segment, Jκ RSS with Jκ gene segment, etc.). Also described are engineered micecontaining heterologous genetic material that is inserted in such a way so that antibodies containing light chains that have a human Vλ domain and a mouse Cλ domain are expressed in the antibody repertoire of the mouse. Further, described are engineered mice that contain heterologous genetic material that is inserted in such a way so that antibodies containing light chains that have a human Vλ domain and a mouse Cλ domain are expressed from engineered Igκ light chain loci that include human and non-human Igλ gene sequences (e.g., gene segments) and, in some instances, human Igκ light chain sequences, in the germline genome of the mouse.

[0141] Without wishing to be bound by any particular theory, it is contemplated that mice as described herein provide an improved *in vivo* system that exploits the expression of antibodies containing human Vλ domains for the production of therapeutic antibodies. It is also contemplated that mice as described herein, in some embodiments, provide alternate engineered forms of light chain loci (e.g., Igκ light chain loci) that contain heterologous genetic material for the development of human antibody-based therapeutics (e.g., human monoclonal antibodies, multi-specific binding agents, scFvs, fusion polypeptides, etc.) to disease targets that are associated with biased antibody responses (e.g., antibody responses characterized by an overwhelming proportion of either κ or λ light chains). Thus, the employed mice are particularly useful for the development of human antibodies and human antibody-based molecules (e.g., multi-specific binding agents, scFvs, fusion polypeptides, etc.) against targets associated with poor immunogenicity (e.g., viruses) due, in part, to skewed antibody repertoires and/or responses.

[0142] The present disclosure describes, among other things, an immunoglobulin κ light chain locus that includes one or more human Vλ gene segments, one or more human Jλ gene segments, and a mouse Cλ gene. Such a locus is referred to as a "lambda in kappa" locus or "LiK".

[0143] In particular, the present disclosure describes the production of a mouse having a germline genome that contains an engineered Igκ light chain locus that is, in some embodiments, characterized by the introduction of a plurality of human Vλ and Jλ gene segments and introduction of a mouse Cλ gene in the place of a mouse Cκ gene, so that said plurality of human Vλ and Jλ gene segments are operably linked to said mouse Cλ gene. As described herein, the production of such an engineered Igκ light chain locus results in the expression of antibodies that contain light chains that include a human Vλ domain and a mouse Cλ domain from said engineered Igκ light chain locus in the germline genome of the mouse. The germline genome of employed mice comprises an Igκ light chain locus including human Igλ light chain sequences. In some embodiments, the germline genome of employed mice comprises (i) an Igκ light chain locus including human Igλ light chain sequences, and (ii)(a) an Igκ light chain locus including human Igλ light chain sequences or (ii)(b) an Igκ light chain locus including human Igκ light chain sequences. The germline genome of employed mice, in some embodiments, comprises an Igκ light chain locus as described herein and further comprises (i) a humanized IgH locus or (ii) a humanized IgH locus and functionally silenced or otherwise rendered non-functional endogenous Igλ light chain locus. Employed mice, as described herein, express antibody repertoires that contain Igλ light chains that include human Vλ domains.

[0144] The mice employed contain human Igλ light chain sequences within an Igκ light chain locus. Mice as described herein contain human and mouse Igλ light chain sequences within an Igκ light chain locus. In some embodiments, non-human animals as described herein contain human Igλ and human Igκ light chain sequences within an Igκ light chain locus.

[0145] In some embodiments, Igκ and/or Igλ light chain sequences include intergenic DNA that is of human and/or murine origin. In some embodiments, Igκ and/or Igλ light chain sequences include intergenic DNA that is engineered and based on a source sequence that is of human or murine origin. In some embodiments, said intergenic DNA is of the same immunoglobulin locus in which the intergenic DNA is so placed, inserted, positioned or engineered (e.g., Igκ intergenic DNA in an Igκ light chain locus). In some embodiments, said intergenic DNA is of a different immunoglobulin locus in which the intergenic DNA is so placed, inserted, positioned or engineered (e.g., Igλ intergenic DNA in an Igκ light chain locus). In some certain embodiments, miceas described herein contain an engineered Igκ light chain locus that contains intergenic DNA that includes Igκ light chain sequence(s), Igλ light chain sequence(s) and/or combinations thereof.

[0146] In various embodiments, a humanized immunoglobulin heavy chain locus contains at least one human V_H, at least one human D_H and at least one human J_H gene segment operably linked to to a non-human immunoglobulin heavy chain constant region (e.g., an endogenous mouse immunoglobulin heavy chain constant region that includes one or more immunoglobulin heavy chain constant region genes such as, for example, IgM, IgD, IgG, IgE, IgA, etc.), e.g., a plurality of human V_H, D_H and J_H gene segments operably linked to a non-human immunoglobulin heavy chain constant region. In some embodiments, employedmice have a germline genome that includes one or more immunoglobulin loci depicted in the Drawings. Such engineered mice provide a source of human antibodies and human antibody fragments, and provide an improved *in vivo* system suitable for exploiting human Vλ sequences for the production of human therapeutic antibodies.

[0147] As described in the Examples section below, mice are employed that have a genome that contains at least one of each human heavy (i.e., V_H, D_H and J_H) and light chain (e.g., Vλ and Jλ at the endogenous kappa locus) variable region gene segments, e.g., a plurality of human heavy (i.e., V_H, D_H and J_H) and light chain (e.g., Vλ and Jλ at the endogenous kappa locus) variable region gene segments, in the place of mouse variable region gene segments at endogenous immunoglobulin loci, and include human non-coding intergenic DNA between the human variable region gene segments. Such intergenic DNA includes, for example, promoters, leader sequences and recombination signal sequences that allow for proper recombination and expression of the human gene segments in the context of variable domains of antibodies. Persons of skill understand that mouse immunoglobulin loci also contain such non-coding intergenic DNA. Upon reading this disclosure, persons of skill will understand that other human or non-human intergenic DNA can be employed in constructing such loci resulting in the same expression of human variable domains in the context of antibodies in the mouse. Such similar loci need only contain the human coding sequences (i.e., exons) of the desired human gene segments to achieve expression of antibodies that contain human variable domains.

[0148] Various aspects of certain embodiments are described in detail in the following sections, each of which can apply to any aspect or embodiment as described herein. The use of sections is not for limitation.

Antibody repertoires in mice

[0149] Immunoglobulins (also called antibodies) are large (~150 kD), Y-shaped glycoproteins that are produced by B cells of a host immune system to neutralize pathogens (e.g., viruses, bacteria, etc.). Each immunoglobulin (Ig) is composed of two identical heavy chains and two identical light chains, each of which has two structural components: a variable domain and a constant domain. The heavy and light chain variable regions differ in antibodies produced by different B cells, but are the same for all antibodies produced by a single B cell or B cell clone. The heavy and light chain variable regions of each antibody together comprise the antigen-binding region (or antigen-binding site). Immunoglobulins can exist in different varieties that are referred to as isotypes or classes based on the heavy chain constant regions (or domains) that they contain. The heavy chain constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes. The table below summarizes the nine antibody isotypes in mouse and human.

Mouse	Human
IgM	IgM
IgD	IgD
IgG1	IgG1
IgG2a	IgG2
IgG2b	IgG3
IgG2c	IgG4
IgG3	IgE
IgE	IgA1
IgA	IgA2

[0150] Additional isotypes have been identified in other species. Isotypes confer specialized biological properties on the antibody due to the different structural characteristics among the different isotypes and are found in different locations (cells, tissues, etc.) within an animal body. Initially, B cells produce IgM and IgD with identical antigen-binding regions. Upon activation, B cells switch to different isotypes by a process referred to as class switching, which involves a change of the constant region of the antibody produced by the B cell while the variable regions remain the same, thereby preserving antigen specificity of the original antibody (B cell).

[0151] Two separate loci (Igκ and Igλ) contain the gene segments that, upon rearrangement, encode the light chains of antibodies, and exhibit both allelic and isotypic exclusion. The expression ratios of κ^+ to λ^+ B cells vary among species. For example, humans demonstrate a ratio of about 60:40 (κ:λ). In mice and rats, a ratio of 95:5 (κ:λ) is observed. Interestingly, the κ:λ ratio observed in cats (5:95) is opposite of mice and rats. Several studies have been conducted to elucidate the possible reasons behind these observed ratios, and both the complexity of the locus (i.e., number of gene segments, in particular, V gene segments) and the efficiency of gene segment rearrangement have been proposed as rationale. The human Igλ light chain locus extends over 1,000 kb and contains approximately 70 Vλ gene segments (29 to 33 functional) and seven Jλ-Cλ gene segment pairs (four to five functional) organized into three clusters (see, e.g., Fig. 1 of U.S. Patent No. 9,006,511). The majority of the observed Vλ regions in the expressed antibody repertoire are encoded by gene segments contained within the most proximal cluster (referred to as cluster A). The mouse Igλ light chain locus is strikingly different than the human locus and, depending on the strain, contains only a few Vλ and Jλ gene segments organized in two distinct gene clusters (see, e.g., Fig. 2 of U.S. Patent No. 9,006,511).

[0152] Development of therapeutic antibodies for the treatment of various human diseases has largely been centered on the creation of engineered non-human animal lines, in particular, engineered rodent lines, harboring varying amounts of genetic material in their genomes corresponding to human immunoglobulin genes (reviewed in, e.g., Brüggemann, M. et al., 2015, Arch. Immunol. Ther. Exp. 63:101-8). Initial efforts in creating such genetically engineered rodent lines focused on integration of portions of human immunoglobulin loci that could, by themselves, support recombination of gene segments and production of heavy and/or light chains that were entirely human while having endogenous immunoglobulin loci inactivated (see e.g., Brüggemann, M. et al., 1989, Proc. Nat. Acad. Sci. U.S.A. 86:67-09-13; Brüggemann, M. et al., 1991, Eur. J. Immunol. 21:1323-6; Taylor, L.D. et al., 1992, Nucl. Acids Res. 20:6287-6295; Davies, N.P. et al., 1993, Biotechnol. 11:911-4; Green, L.L. et al., 1994, Nat. Genet. 7:13-21; Lonberg, N. et al., 1994, Nature 368:856-9; Taylor, L.D. et al., 1994, Int. Immunol. 6:579-91; Wagner, S.D. et al., 1994, Eur. J. Immunol. 24:2672-81; Fishwild, D.M. et al., 1996, Nat. Biotechnol. 14:845-51; Wagner, S.D. et al., 1996, Genomics 35:405-14; Mendez, M.J. et al., 1997, Nat. Genet. 15:146-56; Green, L.L. et al., 1998, J. Exp. Med. 188:483-95; Xian, J. et al., 1998, Transgenics 2:333-43; Little, M. et al., 2000, Immunol. Today 21:364-70; Kellermann, S.A. and L.L. Green, 2002, Cur. Opin. Biotechnol. 13:593-7). In particular, some efforts have included integration of human Igλ light chain sequences (see, e.g., U.S. Patent Application Publication Nos. 2002/0088016 A1, 2003/0217373 A1 and 2011/0236378 A1; U.S. Patent Nos. 6,998,514 and 7,435,871; Nicholson, I.C. et al., 1999, J. Immunol. 163:6898-906; Popov, A.V et al., 1999, J. Exp. Med. 189(10):1611-19). Such efforts have focused on the random integration of yeast artificial chromosomes containing human Vλ, Jλ and Cλ sequences thereby creating mouse strains that express fully human Igλ light chains (i.e., human Vλ and Cλ domains). More recent efforts have employed similar strategies using constructs that also contain human Vλ, Jλ and Cλ sequences (Osborn, M.J. et al., 2013, J. Immunol. 190:1481-90; Lee, E-C. et al., 2014, Nat. Biotech. 32(4):356-63).

[0153] Yet other efforts have included the specific insertion of human Vλ and Jλ gene segments into endogenous rodent Ig light chain loci (κ and λ) so that said human Vλ and Jλ gene segments are operably linked to endogenous Ig light chain constant region genes (see, e.g., U.S. Patent Nos. 9,006,511, 9,012,717, 9,029,628, 9,035,128, 9,066,502, 9,150,662 and 9,163,092). In such animals, all of the human Vλ gene segments from clusters A and B and either one or four human Jλ gene segments were inserted into endogenous Igκ and Igλ light chain loci. As a result, several different human Vλ and Jλ gene segments demonstrated proper rearrangement at both engineered rodent Ig light

chain loci to form functional light chains expressed in the rodent antibody repertoire, which light chains included human Vλ domains in the context of either endogenous Cκ and Cλ regions (see, e.g., Table 7 and Figures 11-13 of U.S. Patent No. 9,006,511). In particular, mice having engineered Igκ light chain loci harboring human Vλ and Jλ gene segments demonstrated a human lambda to endogenous lambda ratio (as measured by IgCκ to IgCλ ratio) of about 1:1 in the splenic compartment (see, e.g., Table 4 of U.S. Patent No. 9,006,511). Indeed, both engineered mouse strains (i.e., engineered Igκ or engineered Igλ light chain loci) demonstrated that human Vλ domains could be expressed from endogenous Ig light chain loci in rodents, which normally display a large bias in light chain expression (see above). The present disclosure provides the recognition that alternate engineered Ig light chain locus structures can be produced to maximize usage of human Vλ and Jλ gene segments in antibody repertoires to therapeutic targets in non-human animals, in particular, as compared to non-human animals that contain an Igλ light chain locus that lacks the complexity and robust quality (e.g., mice and rats) that is normally associated with a human Igλ light chain locus (i.e., such a locus that appears in a human cell). Such alternate engineered Ig light chain locus structures provide the capacity for unique antibody repertoires resulting from their design.

[0154] The present disclosure exemplifies the successful production of a mouse whose germline genome contains an engineered endogenous Igκ light chain locus comprising a plurality of human Vλ and Jλ gene segments in operable linkage to a mouse Igλ light chain constant region gene, which mouse Igλ light chain constant region gene is inserted in the place of a non-human Igκ light chain constant region gene of the endogenous Igκ light chain locus. In particular, the present disclosure specifically demonstrates the successful production of (1) an engineered mouse that expresses antibodies having human variable regions and mouse constant regions, which antibodies include light chains that contain a human Vλ domain and a mouse Cλ domain, and (2) an engineered mouse animal that expresses antibodies having human variable regions and human constant regions, which antibodies include light chains that contain human Vλ and Cλ domains. As specifically exemplified herein, expression of such light chains is achieved by insertion of said plurality of human Vλ and Jλ gene segments into an endogenous Igκ light chain locus (or allele). In some embodiments, employed mice are engineered so that expression of endogenous Igλ light chain variable regions is inactivated (e.g., by gene deletion).

[0155] In some embodiments, employed mice are engineered so that expression of endogenous Igκ light chain variable regions is inactivated (e.g., by replacement or substitution). In some embodiments, employed mice are engineered so that the mice express human Igλ light chain variable regions from an engineered endogenous Igκ light chain locus and human Igκ light chain variable regions from an engineered endogenous Igκ light chain locus. Thus, the present disclosure, in at least some embodiments, embraces the development of an improved *in vivo* system for the production of human antibodies by providing an engineered mouse containing an alternatively engineered Igκ light chain locus that results in an expressed antibody repertoire containing human Vλ domains and mouse Cλ domains.

Nucleic Acid Constructs

[0156] Typically, a polynucleotide molecule containing human Igλ light chain sequences (e.g., human Vλ and Jλ gene segments) or portion(s) thereof linked with (e.g., is inserted into) a vector, preferably a DNA vector, in order to replicate the polynucleotide molecule in a host cell.

[0157] Human Igλ light chain sequences can be cloned directly from known sequences or sources (e.g., libraries) or synthesized from germline sequences designed *in silico* based on published sequences available from GenBank or other publically available databases (e.g., IMGT). Alternatively, bacterial artificial chromosome (BAC) libraries can provide immunoglobulin DNA sequences of interest (e.g., human Vλ and Jλ sequences and combinations thereof). BAC libraries can contain an insert size of 100-150kb and are capable of harboring inserts as large as 300kb (Shizuya, et al., 1992, Proc. Natl. Acad. Sci., USA 89:8794-8797; Swiatek, et al., 1993, Genes and Development 7:2071-2084; Kim, et al., 1996, Genomics 34 213-218). For example, a human BAC library harboring average insert sizes of 164-196kb has been described (Osoegawa, K. et al., 2001, Genome Res. 11(3):483-96; Osoegawa, K. et al., 1998, Genomics 52:1-8, Article No. GE985423). Human and mouse genomic BAC libraries have been constructed and are commercially available (e.g., ThermoFisher). Genomic BAC libraries can also serve as a source of immunoglobulin DNA sequences as well as transcriptional control regions.

[0158] Alternatively, immunoglobulin DNA sequences may be isolated, cloned and/or transferred from yeast artificial chromosomes (YACs). For example, the nucleotide sequence of the human Igλ light chain locus has been determined (see, e.g., Dunham, I. et al., 1999, Nature 402:489-95). Further, YACs have previously been employed to assemble a human Igλ light chain locus transgene (see, e.g., Popov, A.V. et al., 1996, Gene 177:195-201; Popov, A.V. et al., 1999, J. Exp. Med. 189(10):1611-19). An entire Igλ light chain locus (human or mouse) can be cloned and contained within several YACs. If multiple YACs are employed and contain regions of overlapping similarity, they can be recombined within yeast host strains to produce a single construct representing the entire locus or desired portions of the locus (e.g., a region to targeted with a targeting vector). YAC arms can be additionally modified with mammalian selection cassettes by retrofitting to assist in introducing the constructs into embryonic stems cells or embryos by methods known in the art and/or described herein.

[0159] DNA and amino acid sequences of human Igλ light chain gene segments for use in constructing an engineered Igκ light chain locus as described herein may be obtained from published databases (e.g., GenBank, IMGT, etc.) and/or published antibody sequences. In some embodiments, nucleic acid constructs containing human Igλ light chain gene segments comprise a J region (i.e., a genomic sequence that includes a plurality of light chain J gene segments), where the J region comprises coding sequences of human Jλ gene segments with their corresponding 12RSS, where the 12RSS have been positioned among non-coding intergenic DNA typically associated with coding sequences of human Jκ gene segments with their corresponding 23RSS.

[0160] In some embodiments, such a sequence may be referred to as an engineered light chain J region. In some certain embodiments, nucleic acid constructs containing human Igλ light chain gene segments comprise human Vλ and Jλ sequences operably linked to a mouse Igλ light chain constant region (Cλ) gene. In some certain embodiments, nucleic acid constructs containing human Igλ light chain gene segments comprise human Vλ and Jλ sequences operably linked to one or more mouse Igκ light chain enhancer regions (or enhancer sequences). In some certain embodiments, nucleic acid constructs containing human Igλ light chain gene segments comprise human Vλ and Jλ sequences operably linked to a mouse Cλ region gene and mouse Igκ light chain enhancer regions (or enhancer sequences).

[0161] In some embodiments, nucleic acid constructs containing human Vλ and Jλ sequences further comprises intergenic DNA that is of human and/or murine origin. In some embodiments, intergenic DNA is or comprises non-coding murine Igκ light chain sequence, non-coding human Igκ light chain sequence, non-coding murine Igλ light chain sequence, non-coding human Igλ light chain sequence, or combinations thereof.

[0162] Nucleic acid constructs can be prepared using methods known in the art. For example, a nucleic acid construct can be prepared as part of a larger plasmid. Such preparation allows the cloning and selection of the correct constructions in an efficient manner as is known in the art. Nucleic acid constructs containing human Igλ light chain sequences, in whole or in part, as described herein can be located between restriction sites on the plasmid so that they can be isolated from the remaining plasmid sequences for incorporation into a desired non-human animal.

[0163] Various methods employed in preparation of nucleic acid constructs (e.g., plasmids) and transformation of host organisms are known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see Principles of Gene Manipulation: An Introduction to Genetic Manipulation, 5th Ed., ed. By Old, R.W. and S.B. Primrose, Blackwell Science, Inc., 1994 and Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. by Sambrook, J. et al., Cold Spring Harbor Laboratory Press: 1989.

Targeting Vectors

[0164] Targeting vectors can be employed to introduce a nucleic acid construct into a genomic target locus and comprise a nucleic acid construct and homology arms that flank said nucleic acid construct; those skilled in the art will be aware of a variety of options and features generally applicable to the design, structure, and/or use of targeting vectors. For example, targeting vectors can be in linear form or in circular form, and they can be single-stranded or double-stranded. Targeting vectors can be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). For ease of reference, homology arms are referred to herein as 5' and 3' (i.e., upstream and downstream) homology arms. This terminology relates to the relative position of the homology arms to a nucleic acid construct within a targeting vector. 5' and 3' homology arms correspond to regions within a targeted locus or to a region within another targeting vector, which are referred to herein as "*5' target sequence*" and "*3' target sequence*," respectively. In some embodiments, homology arms can also function as a 5' or a 3' target sequence.

[0165] In some embodiments, methods described herein employ two, three or more targeting vectors that are capable of recombining with each other. In various embodiments, targeting vectors are large targeting vectors (LTVEC) as described elsewhere herein. In such embodiments, first, second, and third targeting vectors each comprise a 5' and a 3' homology arm. The 3' homology arm of the first targeting vector comprises a sequence that overlaps with the 5' homology arm of the second targeting vector (i.e., overlapping sequences), which allows for homologous recombination between first and second LTVECs.

[0166] In the case of double targeting methods, a 5' homology arm of a first targeting vector and a 3' homology arm of a second targeting vector can be similar to corresponding segments within a target genomic locus (i.e., a target sequence), which can promote homologous recombination of the first and the second targeting vectors with corresponding genomic segments and modifies the target genomic locus.

[0167] In the case of triple targeting methods, a 3' homology arm of a second targeting vector can comprise a sequence that overlaps with a 5' homology arm of a third targeting vector (i.e., overlapping sequences), which can allow for homologous recombination between the second and the third LTVEC. The 5' homology arm of the first targeting vector and the 3' homology arm of the third targeting vector are similar to corresponding segments within the target genomic locus (i.e., the target sequence), which can promote homologous recombination of the first and the third targeting vectors with the corresponding genomic segments and modifies the target genomic locus.

[0168] A homology arm and a target sequence or two homology arms "*correspond*" or are "*corresponding*" to one another when the two regions share a sufficient level of sequence identity to one another to act as substrates for a homologous recombination reaction. The sequence identity between a given target sequence and the corresponding homology arm found on a targeting vector (i.e., overlapping sequence) or between two homology arms can be any degree of sequence identity that allows for homologous recombination to occur. To give but one example, an amount of sequence identity shared by a homology arm of a targeting vector (or a fragment thereof) and a target sequence of another targeting vector or a target sequence of a target genomic locus (or a fragment thereof) can be, e.g., but not limited to, at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity, such that the sequences undergo homologous recombination.

[0169] Moreover, a corresponding region of similarity (e.g., identity) between a homology arm and a corresponding target sequence can be of any length that is sufficient to promote homologous recombination at the target genomic locus. For example, a given homology arm and/or corresponding target sequence can comprise corresponding regions of similarity that are, e.g., but not limited to, about 5-10kb, 5-15kb, 5-20kb, 5-25kb, 5-30kb, 5-35kb, 5-40kb, 5-45kb, 5-50kb, 5-55kb, 5-60kb, 5-65kb, 5-70kb, 5-75kb, 5-80kb, 5-85kb, 5-90kb, 5-95kb, 5-100kb, 100-200kb, or 200-300kb in length (such as described elsewhere herein) such that a homology arm has sufficient similarity to undergo homologous recombination with a corresponding target sequence(s) within a target genomic locus of the cell or within another targeting vector. In some embodiments, a given homology arm and/or corresponding target sequence comprise corresponding regions of similarity that are, e.g., but not limited to, about 10-100kb, 15-100kb, 20-100kb, 25-100kb, 30-100kb, 35-100kb, 40-100kb, 45-100kb, 50-100kb, 55-100kb, 60-100kb, 65-100kb, 70-100kb, 75-100kb, 80-100kb, 85-100kb, 90-100kb, or 95-100kb in length (such as described elsewhere herein) such that a homology arm has sufficient similarity to undergo homologous recombination with a corresponding target sequence(s) within a target genomic locus of the cell or within another targeting vector.

[0170] Overlapping sequences of a 3' homology arm of a first targeting vector and a 5' homology arm of a second targeting vector or of a 3' homology arm of a second targeting vector and a 5' homology arm of a third targeting vector can be of any length that is sufficient to promote homologous recombination between said targeting vectors. For example, a given overlapping sequence of a homology arm can comprise corresponding overlapping regions that are about 1-5kb, 5-10kb, 5-15kb, 5-20kb, 5-25kb, 5-30kb, 5-35kb, 5-40kb, 5-45kb, 5-50kb, 5-55kb, 5-60kb, 5-65kb, 5-70kb, 5-75kb, 5-80kb, 5-85kb, 5-90kb, 5-95kb, 5-100kb, 100-200kb, or 200-300kb in length such that an overlapping sequence of a homology arm has sufficient similarity to undergo homologous recombination with a corresponding overlapping sequence within another targeting vector. In some embodiments, a given overlapping sequence of a homology arm comprises an overlapping region that is about 1-100kb, 5-100kb, 10-100kb, 15-100kb, 20-100kb, 25-100kb, 30-100kb, 35-100kb, 40-100kb, 45-100kb, 50-100kb, 55-100kb, 60-100kb, 65-100kb, 70-100kb, 75-100kb, 80-100kb, 85-100kb, 90-100kb, or 95-100kb in length such that an overlapping sequence of a homology arm has sufficient similarity to undergo homologous recombination with a corresponding overlapping sequence within another targeting vector. In some embodiments, an overlapping sequence is from 1-5kb, inclusive. In some embodiments, an overlapping sequence is from about 1kb to about 70kb, inclusive. In some embodiments, an overlapping sequence is from about 10kb to about 70kb, inclusive. In some embodiments, an overlapping sequence is from about 10kb to about 50kb, inclusive. In some embodiments, an overlapping sequence is at least 10kb. In some embodiments, an overlapping sequence is at least 20kb. For example, an overlapping sequence can be from about 1kb to about 5kb, inclusive, from about 5kb to about 10kb, inclusive, from about 10kb to about 15kb, inclusive, from about 15kb to about 20kb, inclusive, from about 20kb to about 25kb, inclusive, from about 25kb to about 30 kb, inclusive, from about 30kb to about 35kb, inclusive, from about 35kb to about 40kb, inclusive, from about 40kb to about 45kb, inclusive, from about 45kb to about 50kb, inclusive, from about 50kb to about 60kb, inclusive, from about 60kb to about 70kb, inclusive, from about 70kb to about 80kb, inclusive, from about 80kb to about 90kb, inclusive, from about 90kb to about 100kb, inclusive, from about 100kb to about 120kb, inclusive, from about 120kb to about 140kb, inclusive, from about 140kb to about 160kb, inclusive, from about 160kb to about 180kb, inclusive, from about 180kb to about 200kb, inclusive, from about 200kb to about 220kb, inclusive, from about 220kb to about 240kb, inclusive, from about 240kb to about 260kb, inclusive, from about 260kb to about 280kb, inclusive, or about 280kb to about 300 kb, inclusive. To give but one example, an overlapping sequence can be from about 20kb to about 60kb, inclusive. Alternatively, an overlapping sequence can be at least 1kb, at least 5kb, at least 10kb, at least 15kb, at least 20kb, at least 25kb, at least 30kb, at least 35kb, at least 40kb, at least 45kb, at least 50kb, at least 60kb, at least 70kb, at least 80kb, at least 90kb, at least 100kb, at least 120kb, at least 140kb, at least 160kb, at least 180kb, at least 200kb, at least 220kb, at least 240kb, at least 260kb, at least 280kb, or at least 300kb. In some embodiments, an overlapping sequence can be at most 400kb, at most 350kb, at most 300kb, at most 280kb, at most 260kb, at most 240kb, at most 220kb, at most 200kb, at most 180kb, at most 160kb, at most 140kb, at most 120kb, at most 100kb, at most 90kb, at most 80 kb, at most 70kb, at most 60 kb or at most 50kb.

[0171] Homology arms can, in some embodiments, correspond to a locus that is native to a cell (e.g., a targeted locus), or alternatively they can correspond to a region of a heterologous or exogenous segment of DNA that was integrated into the genome of the cell, including, for example, transgenes, expression cassettes, or heterologous or exogenous regions of DNA. In some embodiments, homology arms can, in some embodiments, correspond to a region on a targeting vector in a cell. In some embodiments, homology arms of a targeting vector may correspond to a region of a yeast artificial chromosome (YAC), a bacterial artificial chromosome (BAC), a human artificial chromosome, or any other engineered region contained in an appropriate host cell. Still further, homology arms of a targeting vector may correspond to or be derived from a region of a BAC library, a cosmid library, or a P1 phage library. In some certain embodiments, homology arms of a targeting vector correspond to a locus that is native, heterologous, or exogenous to a prokaryote, a yeast, a bird (e.g., chicken), a non-human mammal, a mouse, a human, a rat, a mouse, a hamster a rabbit, a pig, a bovine, a deer, a sheep, a goat, a cat, a dog, a ferret, a primate (e.g., marmoset, rhesus monkey), a domesticated mammal, an agricultural mammal, or any other organism of interest. In some embodiments, homology arms correspond to a locus of the cell that shows limited susceptibility to targeting using a conventional method or that has shown relatively low levels of successful integration at a targeted site, and/or significant levels of off-target integration, in the absence of a nick or double-strand break induced by a nuclease agent (e.g., a Cas protein). In some embodiments, homology arms are designed to include engineered DNA.

[0172] In some embodiments, 5' and 3' homology arms of a targeting vector(s) correspond to a targeted genome. Alternatively, homology arms correspond to a related genome. For example, a targeted genome is a mouse genome of a first strain, and targeting arms correspond to a mouse genome of a second strain, wherein the first strain and the second strain are different. In certain embodiments, homology arms correspond to the genome of the same animal or are from the genome of the same strain, e.g., the targeted genome is a mouse genome of a first strain, and the targeting arms correspond to a mouse genome from the same mouse or from the same strain.

[0173] A homology arm of a targeting vector can be of any length that is sufficient to promote a homologous recombination event with a corresponding target sequence, including, for example, 1-5kb, inclusive, 5-10kb, inclusive, 5-15kb, inclusive, 5-20kb, inclusive, 5-25kb, inclusive, 5-30kb, inclusive, 5-35kb, inclusive, 5-40kb, inclusive, 5-45kb, inclusive, 5-50kb, inclusive, 5-55kb, inclusive, 5-60kb, inclusive, 5-65kb, inclusive, 5-70kb, inclusive, 5-75kb, inclusive, 5-80kb, inclusive, 5-85kb, inclusive, 5-90kb, inclusive, 5-95kb, inclusive, 5-100kb, inclusive, 100-200kb, inclusive, or 200-300kb, inclusive, in length. In some embodiments, a homology arm of a targeting vector has a length that is sufficient to promote a homologous recombination event with a corresponding target sequence that is 1-100kb, inclusive, 5-100kb, inclusive, 10-100kb, inclusive, 15-100kb, inclusive, 20-100kb, inclusive, 25-100kb, inclusive, 30-100kb, inclusive, 35-100kb, inclusive, 40-100kb, inclusive, 45-100kb, inclusive, 50-100kb, inclusive, 55-100kb, inclusive, 60-100kb, inclusive, 65-100kb, inclusive, 70-100kb, inclusive, 75-100kb, inclusive, 80-100kb, inclusive, 85-100kb, inclusive, 90-100kb, inclusive, or 95-100kb, inclusive, in length. As described herein, large targeting vectors can employ targeting arms of greater length.

[0174] Nuclease agents (e.g., CRISPR/Cas systems) can be employed in combination with targeting vectors to facilitate the modification of a target locus (e.g., modification of an Igκ light chain locus, or modification of a previously modified or engineered Igκ light chain locus). Such nuclease agents may promote homologous recombination between a

targeting vector and a target locus. When nuclease agents are employed in combination with a targeting vector, the targeting vector can comprise 5' and 3' homology arms corresponding to 5' and 3' target sequences located in sufficient proximity to a nuclease cleavage site so as to promote the occurrence of a homologous recombination event between target sequences and homology arms upon a nick or double-strand break at the nuclease cleavage site. The term *"nuclease cleavage site"* includes a DNA sequence at which a nick or double-strand break is created by a nuclease agent (e.g., a Cas9 cleavage site). Target sequences within a targeted locus that correspond to 5' and 3' homology arms of a targeting vector are *"located in sufficient proximity"* to a nuclease cleavage site if the distance is such as to promote the occurrence of a homologous recombination event between 5' and 3' target sequences and homology arms upon a nick or double-strand break at the recognition site. Thus, in certain embodiments, target sequences corresponding to 5' and/or 3' homology arms of a targeting vector are within at least one nucleotide of a given recognition site or are within at least 10 nucleotides to about 14kb of a given recognition site. In some embodiments, a nuclease cleavage site is immediately adjacent to at least one or both of the target sequences.

[0175] The spatial relationship of target sequences that correspond to homology arms of a targeting vector and a nuclease cleavage site can vary. For example, target sequences can be located 5' to a nuclease cleavage site, target sequences can be located 3' to a recognition site, or target sequences can flank a nuclease cleavage site.

[0176] Combined use of a targeting vector (including, for example, a large targeting vector) with a nuclease agent can result in an increased targeting efficiency compared to use of a targeting vector alone. For example, when a targeting vector is used in conjunction with a nuclease agent, targeting efficiency of a targeting vector can be increased by at least two-fold, at least three-fold, at least four-fold, at least five-fold, at least six-fold, at least seven-fold, at least eight-fold, at least nine-fold, at least ten-fold or within a range formed from these integers, such as 2-10-fold when compared to use of a targeting vector alone.

[0177] Some targeting vectors are *"large targeting vectors"* or "LTVECs," which includes targeting vectors that comprise homology arms that correspond to and are derived from nucleic acid sequences larger than those typically used by other approaches intended to perform homologous recombination in cells. A LTVEC can be, for example, at least 10kb in length, or the sum total of a 5' homology arm and a 3' homology arm can be, for example, at least 10kb. LTVECs also include targeting vectors comprising nucleic acid constructs larger than those typically used by other approaches intended to perform homologous recombination in cells. For example, LTVECs make possible the modification of large loci that cannot be accommodated by traditional plasmid-based targeting vectors because of their size limitations. For example, a targeted locus can be (i.e., 5' and 3' homology arms can correspond to) a locus of a cell that is not targetable using a conventional method or that can be targeted only incorrectly or only with significantly low efficiency in the absence of a nick or double-strand break induced by a nuclease agent (e.g., a Cas protein).

[0178] In some embodiments, methods described herein employ two or three LTVECs that are capable of recombining with each other and with a target genomic locus in a three-way or a four-way recombination event. Such methods make possible the modification of large loci that cannot be achieved using a single LTVEC.

[0179] Examples of LTVECs include vectors derived from a bacterial artificial chromosome (BAC), a human artificial chromosome, or a yeast artificial chromosome (YAC). LTVECs can be in linear form or in circular form. Examples of LTVECs and methods for making them are described, e.g., in U.S. Patent Nos. 6,586,251, 6,596,541 and No. 7,105,348; and International Patent Application Publication No. WO 2002/036789.

Mice, cells and tissues

[0180] Mice are employed that express (e.g., whose B cells express) antibodies that contain light chains that include a human Vλ domain resulting from integration of genetic material that corresponds to at least a portion of a human Igλ light chain locus (i.e., at least a portion of human Vλ and Jλ gene segments), and which encodes a human Vλ domain (i.e., a rearranged human Vλ-Jλ sequence), in the place of corresponding non-human Igκ light chain variable region sequences in the germline genome of the mouse.

[0181] The present disclosure employs improved *in vivo* systems for identifying and developing new antibodies, antibody components (e.g., antigen-binding portions and/or compositions or formats that include them), and/or antibody-based therapeutics that can be used, for example, in the treatment of a variety of diseases that affect humans. Further, the present disclosure also encompasses the recognition that mice having engineered immunoglobulin loci, such as engineered immunoglobulin (Ig) kappa (κ) light chain loci and/or otherwise expressing, producing or containing antibody repertoires characterized by light chains having human V lambda (λ) regions are useful. For example, in some embodiments, such mice may be used for exploiting the diversity of human Vλ sequences in the identification and development of new antibody-based therapeutics. In some embodiments, mice described herein provide improved *in vivo* systems for development of antibodies and/or antibody-based therapeutics for administration to humans. In some embodiments, mice described herein provide improved *in vivo* systems for development of antibodies and/or antibody-based therapeutics that contain human Vλ domains characterized by improved performance (e.g., expression and/or representation in an antigen-specific antibody repertoire) as compared to antibodies and/or antibody-based therapeutics obtained from existing *in vivo* systems that contain human Vλ region sequences.

[0182] The present disclosure provides, among other things, a mice having an Igκ light chain locus that contains an engineered immunoglobulin light chain variable region and an engineered immunoglobulin light chain constant region gene. As described herein, employed mice, contain in their germline genome an immunoglobulin κ light chain locus comprising an engineered immunoglobulin κ light chain variable region characterized by the presence of one or more human Vλ gene segments and one or more human Jλ gene segments, which one or more human Vλ and one or more human Jλ gene segments are operably linked to a mouse immunoglobulin λ light chain constant region (Cλ) gene, which mouse immunoglobulin λ light chain constant region (Cλ) gene is positioned in the place of a non-human immunoglobulin κ light chain constant region (Cκ) gene at the endogenous immunoglobulin κ locus of the mouse. In some embodiments, employed mice comprise an Igκ light chain locus that contains intergenic DNA that is immunoglobulin λ light chain and/or immunoglobulin κ light chain in origin, and combinations thereof.

[0183] In many embodiments, an engineered immunoglobulin κ light chain variable region further comprises an immunoglobulin κ light chain sequence positioned or inserted between said one or more human Vλ gene segments and one or more human Jλ gene segments. In some embodiments, said immunoglobulin κ light chain sequence positioned or inserted between said one or more human Vλ gene segments and one or more human Jλ gene segments is or comprises a mouse sequence. In some embodiments, said immunoglobulin κ light chain sequence positioned or inserted between said one or more human Vλ gene segments and one or more human Jλ gene segments is or comprises a human sequence. For example, in some embodiments, a human immunoglobulin κ light chain sequence is or comprises a genomic sequence that naturally appears between a human Vκ4-1 gene segment and a human Jκ1 gene segment of a human immunoglobulin κ light chain locus.

[0184] In some embodiments, employed mice comprise at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24 or at least 25 functional human Vλ gene segments. In some embodiments, employed mice comprise 5 to 25, 5 to 24, 5 to 23, 5 to 22, 5 to 21, 5 to 20, 5 to 19, 5 to 18, 5 to 17, 5 to 16, 5 to 15, 5 to 14, 5 to 13, 5 to 12, 5 to 11, 5 to 10, 5 to 9, 5 to 8, 5 to 7, or 5 to 6 functional human Vλ gene segments. In some embodiments, employed mice comprise 6 to 25, 7 to 25, 8 to 25, 9 to 25, 10 to 25, 11 to 25, 12 to 25, 13 to 25, 14 to 25, 15 to 25, 16 to 25, 17 to 25, 18 to 25, 19 to 25, 20 to 25, 21 to 25, 22 to 25, 23 to 25 or 24 to 25 functional human Vλ gene segments. In some embodiments, employedmice comprise 6 to 24, 7 to 23, 8 to 22, 9 to 21, 10 to 20, 11 to 19, 12 to 18, 13 to 17, 14 to 16, or 15 to 16 functional human Vλ gene segments. In some embodiments, employed mice comprise 6 to 24, 7 to 23, 8 to 22, 9 to 21, 10 to 20, 11 to 19, 12 to 18, 13 to 17, or 14 to 16 functional human Vλ gene segments.

[0185] In some embodiments, employed mice comprise 10 to 70, 10 to 65, 10 to 60, 10 to 55, 10 to 50, 10 to 45, 10 to 40, 10 to 35, 10 to 30, 10 to 25, 10 to 20, or 10 to 15 total human Vλ gene segments. In some embodiments, employed mice comprise 15 to 70, 20 to 70, 25 to 70, 30 to 70, 35 to 70, 40 to 70, 45 to 70, 50 to 70, 55 to 70, 60 to 70, or 65 to 70 total human Vλ gene segments. In some embodiments, employedmice comprise 15 to 65, 20 to 60, 25 to 55, 20 to 50, 25 to 45, 30 to 40, 30 to 35, or 35 to 40 total human Vλ gene segments.

[0186] In some embodiments, employed mice contain human Vλ and/or Jλ gene segments in natural or germline configuration (e.g., a DNA sequence containing a plurality of human Vλ and/or Jλ gene segment coding sequences interspersed with non-coding human immunoglobulin λ light chain sequence light chain sequence). In some embodiments, employed mice contain human Vλ and/or Jλ gene segments in configuration that departs or deviates from a natural or germline configuration (e.g., a DNA sequence containing a plurality of human Vλ and/or Jλ gene segment coding sequences interspersed with non-coding immunoglobulin κ light chain sequence (e.g., human or murine). In some embodiments, employed mice contain human Vλ and/or Jλ gene segments in a configuration that does not naturally appear in a human immunoglobulin λ light chain locus of the germline genome of a human cell.

[0187] In some embodiments, employed mice contain a DNA sequence at an endogenous non-human Igκ light chain locus that includes a plurality of human Vλ and Jλ coding sequences interspersed (or juxtaposed, associated, etc.) with non-coding human immunoglobulin light chain sequence (e.g., κ, λ and combinations thereof). In some embodiments, employed mice contain a DNA sequence at an endogenous non-human Igλ light chain locus that includes a plurality of human Vλ and Jλ coding sequences interspersed with non-coding non-human (e.g., murine) immunoglobulin λ light chain sequence.

[0188] In some embodiments, employed mice are characterized by expression of antibodies from endogenous immunoglobulin κ light chain loci in the germline genome of said mice, which antibodies contain (1) human Vλ domains and (2) mouse Cλ domains. In some embodiments, employed mice are characterized by an improved usage of human Vλ regions from engineered immunoglobulin κ light chain loci (e.g., but not limited to, about 2-fold) as compared to one or more reference engineered mices.

[0189] In some instances, a mouse, mouse cell mouse tissue is disclosed whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising: (a) one or more human Vλ gene segments, (b) one or more human Jλ gene segments, and (c) a mouse Cλ gene, wherein (a) and (b) are operably linked to (c), and wherein the mouse lacks a mouse Cκ gene at the endogenous immunoglobulin κ light chain locus.

[0190] In some instances, a mouse, mouse cell or mouse tissue is disclosed whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising insertion of one or more human Vλ gene segments, one or more human Jλ gene segments and a mouse Cλ gene, which human Vλ and Jλ gene segments are operably linked to said mouse Cλ gene, and which mouse Cλ gene is inserted in the place of a mouse Cκ gene at the endogenous immunoglobulin κ light chain locus. In many embodiments of a mouse, mouse cell or mouse tissue, a mouse Cλ gene inserted in the place of a mouse Cκ gene at an endogenous immunoglobulin κ light chain.

[0191] mouseIn some embodiments, a mouse Cλ gene comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to a mouse Cλ gene selected from the group consisting of a mouse Cλ1, mouse Cλ2 and a mouse Cλ3. In some embodiments, a mouse Cλ gene comprises a sequence that is substantially identical or identical to a mouse Cλ gene selected from the group consisting of a mouse Cλ1, mouse Cλ2 and a mouse Cλ3. In some embodiments, a mouse Cλ1 gene is or comprises SEQ ID NO:1. In some certain embodiments, a mouse Cλ2 gene is or comprises SEQ ID NO:3. In some certain embodiments, a mouse Cλ3 gene is or comprises SEQ ID NO:5. In some certain embodiments, a mouse Cλ gene comprises a sequence that is identical to a mouse Cλ1 gene.

[0192] In some embodiments, a mouse Cλ gene comprises a sequence that is 80% to 100%, 85% to 100%, 90% to 100%, 95% to 100%, or 98% to 100% identical to a mouse Cλ gene selected from the group consisting of a mouse Cλ1, mouse Cλ2 and a mouse Cλ3. In some embodiments, a mouse Cλ gene comprises a sequence that is 80% to 98%, 80% to 95%, 80% to 90%, or 80% to 85% identical to a mouse Cλ gene selected from the group consisting of a mouse Cλ1, mouse Cλ2 and a mouse Cλ3. In some embodiments, a mouse Cλ gene comprises a sequence that is 85% to 98%, 90% to 95%, or 88% to 93% identical to a mouse Cλ gene selected from the group consisting of a mouse Cλ1, mouse Cλ2 and a mouse Cλ3.

[0193] In some embodiments, a rodent Cλ gene is or comprises a rat Cλ gene. In some embodiments, a rat Cλ gene comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to a rat Cλ gene selected from the group consisting of a rat Cλ1, rat Cλ2, rat Cλ3 and a rat Cλ4 gene. In some embodiments, a rat Cλ gene comprises a sequence that is substantially identical or identical to a rat Cλ gene selected from the group consisting of a rat Cλ1, rat Cλ2, rat Cλ3 and a rat Cλ4 gene. In some certain embodiments, a rat Cλ1 gene is or comprises SEQ ID NO:7. In some certain embodiments, a rat Cλ2 gene is or comprises SEQ ID NO:9. In some certain embodiments, a rat Cλ3 gene is or comprises SEQ ID NO:11. In some certain embodiments, a rat Cλ4 gene is or comprises SEQ ID NO:13.

[0194] In some embodiments, a rat Cλ gene comprises a sequence that is 80% to 100%, 85% to 100%, 90% to 100%, 95% to 100%, or 98% to 100% identical to a rat Cλ gene selected from the group consisting of a rat Cλ1, rat Cλ2, rat Cλ3 and a rat Cλ4 gene. In some embodiments, a rat Cλ gene comprises a sequence that is 80% to 98%, 80% to 95%, 80% to 90%, or 80% to 85% identical to a rat Cλ gene selected from the group consisting of a rat Cλ1, rat Cλ2, rat Cλ3 and a rat Cλ4 gene. In some embodiments, a rat Cλ gene comprises a sequence that is 85% to 98%, 90% to 95%, or 88% to 93%, identical to a rat Cλ gene selected from the group consisting of a rat Cλ1, rat Cλ2, rat Cλ3 and a rat Cλ4 gene.

[0195] In some embodiments, a human Cλ gene comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to a human Cλ gene selected from the group consisting of a human Cλ1, human Cλ2, human Cλ3, human Cλ6 and a human Cλ7 gene. In some embodiments, a human Cλ gene comprises a sequence that is substantially identical or identical to a human Cλ gene selected from the group consisting of a human Cλ, human Cλ2, human Cλ3, human Cλ6 and a human Cλ7 gene. In some embodiments, a human Cλ gene comprises a sequence that is identical to a human Cλ gene selected from the group consisting of a human Cλ1, human Cλ2, human Cλ3, human Cλ6 and a human Cλ7 gene. In some certain embodiments, a human Cλ1 gene is or comprises SEQ ID NO:15. In some certain embodiments, a human Cλ2 gene is or comprises SEQ ID NO: 17. In some certain embodiments, a human Cλ3 gene is or comprises SEQ ID NO: 19. In some certain embodiments, a human Cλ6 gene is or comprises SEQ ID NO:21. In some certain embodiments, a human Cλ7 gene is or comprises SEQ ID NO:23. In some certain embodiments, a human Cλ gene is or comprises a human Cλ2 gene.

[0196] In some embodiments, a human Cλ gene comprises a sequence that is 80% to 100%, 85% to 100%, 90% to 100%, 95% to 100%, or 98% to 100% identical to a human Cλ gene selected from the group consisting of a human Cλ1, human Cλ2, human Cλ3, human Cλ6 and a human Cλ7 gene. In some embodiments, a human Cλ gene comprises a sequence that is 80% to 98%, 80% to 95%, 80% to 90%, or 80% to 85% identical to a human Cλ gene selected from the group consisting of a human Cλ1, human Cλ2, human Cλ3, human Cλ6 and a human Cλ7 gene. In some embodiments, a human Cλ gene comprises a sequence that is 85% to 98%, 90% to 95%, or 88% to 93%, identical to a human Cλ gene selected from the group consisting of a human Cλ1, human Cλ2, human Cλ3, human Cλ6 and a human Cλ7 gene.

[0197] In some embodiments of an employed mouse, mouse cell or mouse tissue, insertion of one or more human Vλ gene segments and one or more human Jλ gene segments replace non-human Vκ and Jκ gene segments at the endogenous immunoglobulin κ light chain locus. In some embodiments, insertion includes human non-coding DNA that naturally appears between human Vλ and Jλ gene segments, and combinations thereof. In some embodiments of an employed mouse animal, mouse cell or mouse tissue, insertion of one or more human Vλ gene segments and one or more human Jλ gene segments are in place of or replace non-human Vκ and Jκ gene segments at the endogenous immunoglobulin κ light chain locus. In some embodiments of a mouse, mouse cell or mouse tissue, an employed immunoglobulin κ light chain locus comprises insertion of at least 24, at least 34, at least 52, at least 61, or at least 70 human Vλ gene segments and at least 1, at least 2, at least 3, at least 4 or at least 5 human Jλ gene segments. In some certain embodiments of an employed mouse, mouse cell or mouse tissue, an immunoglobulin κ light chain locus comprises insertion of 39 human Vλ gene segments and at least 5 human Jλ gene segments. In some embodiments of an employed mouse, mouse cell or mouse tissue, an immunoglobulin κ light chain locus comprises insertion of human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1 or any combination thereof, and human J Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof. In some embodiments, insertion includes human non-coding DNA that naturally appears adjacent to a human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, or Vλ3-1 in an endogenous human λ light chain locus, and human non-coding DNA (in whole or in part) that naturally appears adjacent to a human Jλ1, Jλ2, Jλ3, Jλ6 or Jλ7 in an endogenous human λ light chain locus. In some certain embodiments, insertion includes human non-coding DNA that naturally appears adjacent to a human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, or Vλ3-1 in an endogenous human λ light chain locus , and human non-coding DNA that naturally appears adjacent to a human Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 in an endogenous human κ light chain locus. In some certain embodiments, insertion of human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1 or any combination thereof includes human non-coding DNA that naturally appears adjacent to a human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, or Vλ3-1 in an endogenous human λ light chain locus, and the insertion of human Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof includes human non-coding DNA (in whole or in part) that naturally appears adjacent to a human Jλ1, Jλ2, Jλ3, Jλ6, Jλ7 in an endogenous human λ light chain locus. In some certain embodiments, the insertion of human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1 or any combination thereof includes human non-coding DNA that naturally appears adjacent to a human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, or Vλ3-1 in an

endogenous human λ light chain locus, and the insertion of human Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof includes human non-coding DNA (in whole or in part) that naturally appears adjacent to a human Jκ1, Jκ2, Jκ3, Jκ4, or Jκ5 in an endogenous human κ light chain locus.

[0198] In some embodiments of an employed mouse, mouse cell or mouse tissue, an immunoglobulin κ light chain locus as described herein further comprises a human immunoglobulin κ light chain sequence between the one or more human Vλ gene segments, the one or more human Jλ gene segments, the one or more human Vλ gene segments and the one or more human Jλ gene segments, and combinations thereof. In some embodiments, a human immunoglobulin κ light chain sequence as described herein is or comprises a genomic sequence that naturally appears between a human Vκ4-1 gene segment and a human Jκ1 gene segment of a human immunoglobulin κ light chain locus.

[0199] In some embodiments of an employed mouse, mouse cell or mouse tissue, the germline genome of said mouse, mouse cell or mouse tissue further comprises an endogenous immunoglobulin heavy chain locus comprising insertion of 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, which human V_H, D_H and J_H gene segments are operably linked to a non-human immunoglobulin heavy chain constant region at the endogenous immunoglobulin heavy chain locus (see, e.g., U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and 8,791,323).

[0200] In some embodiments, insertion of 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 in place of or replace, in whole or in part, non-human V_H, D_H and J_H gene segments (e.g., positionally replace or substitute coding sequences of non-human V_H, D_H and J_H gene segments with coding sequences of human V_H, D_H and J_H gene segments). In some certain embodiments, insertion includes human non-coding DNA that naturally appears between human V_H, D_H and J_H gene segments, and combinations thereof. In some embodiments, a non-human immunoglobulin heavy chain constant region is or comprises an endogenous non-human immunoglobulin heavy chain constant region. In many embodiments, a non-human immunoglobulin heavy chain constant region (e.g., endogenous) includes one or more non-human immunoglobulin heavy chain constant region genes or gene segments (e.g., IgM, IgD, IgG, IgE, IgA, etc.). In some certain embodiments, an immunoglobulin heavy chain locus as described herein comprises insertion of the human V_H gene segments V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, V_H6-1, or any combination thereof, the human D_H gene segments D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26, D_H7-27, or any combination thereof, and the human J_H gene segments J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, or any combination thereof. In some certain embodiments, insertion includes human non-coding DNA that naturally appears adjacent to a human V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, or V_H6-1 in an endogenous heavy chain locus, human non-coding DNA that naturally appears adjacent to a human D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26, or D_H7-27, and human non-coding DNA that naturally appears adjacent to a human J_H1, J_H2, J_H3, J_H4, J_H5, or J_H in an endogenous heavy chain locus.

[0201] In some embodiments, a mouse described herein includes an Adam6 gene in its genome (e.g., its germline genome), which encodes an ADAM6 polypeptide, functional ortholog, functional homolog, or functional fragment thereof (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940). In some embodiments, an ADAM6 polypeptide, functional ortholog, functional homolog, or functional fragment thereof is expressed from an Adam6 gene. In some embodiments, an Adam6 gene is does not originate from the mouse that includes an Adam6 gene (e.g., a mouse that includes a rat Adam6 gene or a mouse Adam6 gene obtained from another strain of mouse). In some embodiments, a mouse described herein includes an ectopic Adam6 gene. An "ectopic" Adam6 gene, as used herein, refers to an Adam6 gene that is in a different context than the Adam6 gene appears in a wild-type mouse. For example, the Adam6 gene could be located on a different chromosome, located at a different locus, or positioned adjacent to different sequences. An exemplary ectopic Adam6 gene is a mouse Adam6 gene located within human immunoglobulin sequences (e.g., human heavy chain variable region gene segments). In some embodiments, a mouse described herein includes an inserted or integrated Adam6 gene.

[0202] In some embodiments, a mouse described herein includes an insertion of one or more nucleotide sequences encoding one or more non-human Adam6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof in its genome (e.g., its germline genome).

[0203] In some embodiments, a mouse described herein includes one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof in its genome (e.g., its germline genome). In some embodiments, a mouse described herein includes a mouse Adam6a gene and/or a mouse Adam6b gene in its genome (e.g. its germline genome). In some embodiments, a mouse described herein includes one or more nucleotide sequences a mouse ADAM6a, functional ortholog, functional homolog, or functional fragment thereof, and/or a mouse ADAM6b, functional ortholog, functional homolog, or functional fragment thereof.

[0204] In some embodiments, one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are inserted and/or are located on the same chromosome as the endogenous immunoglobulin heavy chain locus. In some embodiments, one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are inserted and/or are located in a position so that the one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are contiguous with human immunoglobulin heavy chain variable region gene segments. In some embodiments, one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are inserted and/or are located in a position so that the one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are adjacent to human immunoglobulin heavy chain variable region gene segments. In some embodiments, one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are inserted and/or are located in a position so that the one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are located in between human immunoglobulin heavy chain variable region gene segments. In some embodiments, one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are inserted and/or are located between a first and a second human V_H gene segment. In some embodiments, a first human V_H gene segment is human V_H1-2 and a second human V_H gene segment is human V_H6-1. In some embodiments, one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are inserted and/or are located in the place of a human Adam6 pseudogene. In some embodiments, one or more nucleotide sequences encoding one or more non-human ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof are inserted between a human V_H gene segment and a human D_H gene segment.

[0205] In some embodiments, a mouse described herein includes an Adam6 gene that restores or enhances ADAM6 activity. In some embodiments, the Adam6 gene restores ADAM6 activity to the level of a comparable mouse that includes a functional, endogenous Adam6 gene. In some embodiments, the Adam6 gene enhances ADAM6 activity to a level that is at least 2 times, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or at least 10 times the ADAM6 activity of a comparable non-human animal that does not include a functional Adam6 gene.

[0206] In some embodiments, a mouse described herein includes an Adam6 gene that restores or enhances fertility in a male mouse. In some embodiments, the Adam6 gene restores fertility in a male mouse to a level of a comparable non-human animal that includes a functional, endogenous Adam6 gene. In some embodiments, the Adam6 gene restores fertility in a male mouse so that the number of pups produced by mating the male mouse is at least 70%, at least 80%, at least 90%, at least 95% the number of pups produced from a comparable mating of a comparable, male mouse that does not include a functional Adam6 gene. In some embodiments, the Adam6 gene enhances fertility in a male non-human animal so that number of pups produced by the mating of the male non-human animal include at least 2 times, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or at least 10 times the number of pups produced from a comparable mating of a comparable, male non-human animal that does not include a functional Adam6 gene.

[0207] In some embodiments, a mouse immunoglobulin heavy chain locus as described herein lacks at least one endogenous non-human Adam6 gene. In some embodiments, the lack of the at least one endogenous mouse Adam6 gene reduces ADAM6 activity and/or fertility in a male mouse that lacks an endogenous mouse Adam6 gene. In some embodiments, a non-human immunoglobulin heavy chain locus as described herein includes a disruption of at least one endogenous mouse Adam6 gene. In some embodiments,

the disruption of at least one endogenous mouse Adam6 gene reduces ADAM6 activity and/or fertility in a male mouse that lacks an endogenous mouse Adam6 gene.

[0208] In some embodiments of a mouse, mouse cell or mouse tissue, the mouse, mouse cell or mouse tissue is homozygous or heterozygous for an endogenous immunoglobulin heavy chain locus as described herein.

[0209] In some embodiments of a mouse, mouse cell or mouse tissue, the mouse, mouse cell or mouse tissue is homozygous or heterozygous for an endogenous immunoglobulin κ light chain locus as described herein.

[0210] In some embodiments of an employed mouse, mouse cell or mouse tissue, the endogenous immunoglobulin λ light chain locus is deleted in whole or in part. In some embodiments of an employed mouse, mouse cell or mouse tissue, the endogenous immunoglobulin λ light chain locus is functionally silenced or otherwise non-functional (e.g., by gene targeting). In some certain embodiments of an employed mouse, mouse cell or mouse tissue, the mouse, mouse cell or mouse tissue is homozygous or heterozygous for a functionally silenced or otherwise non-functional endogenous immunoglobulin λ light chain locus as described herein.

[0211] In some embodiments, a mouse, mouse cell or mouse tissue as described herein does not detectably express endogenous immunoglobulin λ light chains, endogenous immunoglobulin κ light chains, or endogenous immunoglobulin λ light chains and endogenous immunoglobulin κ light chains.

[0212] In some embodiments, a mouse animal, mouse cell or mouse tissue as described herein has a genome further comprising a nucleic acid sequence encoding an exogenous terminal deoxynucleotidyltransferase (TdT) operably linked to a transcriptional control element.

[0213] In some embodiments, a transcriptional control element includes a RAG1 transcriptional control element, a RAG2 transcriptional control element, an immunoglobulin heavy chain transcriptional control element, an immunoglobulin κ light chain transcriptional control element, an immunoglobulin λ light chain transcriptional control element, or any combination thereof.

[0214] In some embodiments, a nucleic acid sequence encoding an exogenous TdT is located at an immunoglobulin κ light chain locus, an immunoglobulin λ light chain locus, an immunoglobulin heavy chain locus, a RAG1 locus, or a RAG2 locus.

[0215] In some embodiments, the TdT is a human TdT. In some embodiments, the TdT is a short isoform of TdT (TdTS).

[0216] A human Igλ light chain sequence, in some embodiments, comprises genetic material from (e.g., isolated or obtained from) or identical to a human Igλ light chain locus, wherein the human Igλ light chain sequence encodes an Ig light chain that comprises the encoded portion of the genetic material from the human Igλ light chain locus. In some embodiments, a human Igλ light chain sequence as described herein comprises at least one human Vλ gene segment and at least one human Jλ gene segment, and one or more sequences necessary to promote rearrangement (e.g., recombination signal sequence(s)) of said at least one human Vλ gene segment with said at least one human Jλ gene segment to form a functional rearranged human Vλ-Jλ sequence that encodes a human Vλ domain. In many embodiments, a human Igλ light chain sequence comprises a plurality of human Vλ and Jλ gene segments and one or more sequences necessary to promote rearrangement of said human Vλ gene segments with said human Jλ gene segments. In many embodiments, a human Igλ light chain sequence comprises at least the coding sequences (e.g., exons) of one or more human Vλ gene segments and at least the coding sequences (e.g., exons) of one or more human Jλ gene segments. In some embodiments, a human Igλ light chain sequence as described herein is a genomic sequence of a human Igλ light chain locus (e.g., isolated and/or cloned from a bacterial artificial chromosome) and contains a plurality of human Vλ gene segments in germline configuration. In some embodiments, a human Igλ light chain sequence comprises human Vλ and Jλ sequences (i.e., gene segments) in germline configuration (i.e., a plurality of human Vλ gene segments separated by intervening DNA that includes sequences necessary for and that promote recombination, and a plurality of Jλ gene segments separated by intervening DNA that incudes sequences necessary for and that promote recombination).

[0217] In some embodiments, a human Igλ light chain sequence as described herein is an engineered sequence and contains a plurality of human Jλ gene segments in a configuration that is different than that which appears in a human Igλ light chain locus in a human cell. In some embodiments, a human Igλ light chain sequence as described herein is an engineered sequence and contains a plurality of human Vλ and Jλ gene segments in a configuration that resembles or is similar to that which appears in an Igκ light chain locus of a wild-type murine or human cell. In some embodiments, a human Igλ light chain sequence comprises engineered human Jλ sequences (i.e., coding sequences of human Jλ gene segments made by *de novo* DNA synthesis that includes sequences necessary for and that promote recombination with one or more human Vλ gene segments). In some embodiments, a human Igλ light chain sequence comprises Igκ and Igλ sequences that naturally appear separately in Igκ and Igλ genomic sequences, respectively. In some certain embodiments, a human Igλ light chain sequence comprises a Igκ sequence(s), in particular, a Jκ region (i.e., a sequence that contains coding and non-coding sequences that appear in a region containing a plurality of Jκ gene segments), that naturally appears in an Igκ light chain locus except that said Igκ sequence contains coding sequences of Jλ gene segments and Jλ 12RSS in the place of corresponding coding sequences of Jκ gene segments and Jκ 23RSS, respectively. In some certain embodiments, a human Igλ light chain sequence comprises a plurality of Jλ gene segments and Jλ 12RSS in the place of Jκ gene segments and Jκ 23RSS of a Jκ region sequence. In various embodiments, intervening (or intergenic) DNA that includes sequences necessary for and that promote recombination includes human Igκ and/or human Igλ genomic sequence(s). Alternatively, and in some embodiments, intervening (or intergenic) DNA that includes sequences necessary for and that promote recombination includes murine Igκ and/or murine Igλ genomic sequence(s).

[0218] In some certain embodiments, a human Igλ light chain sequence is or comprises a sequence that appears in the Drawing. In some embodiments, a human Igλ light chain sequence encodes, or is capable of encoding (e.g., after rearrangement of human gene segments), a Vλ domain polypeptide, which Vλ domain polypeptide appears in an immunoglobulin, in particular, an immunoglobulin that is expressed by a human B cell. Mice, embryos, cells and targeting constructs for making mice, mouse embryos, and cells containing said human Igλ light chain sequence in the place of a corresponding mouse Igκ light chain sequence (e.g., an endogenous mouse Igκ light chain locus) are also employed.

[0219] In some embodiments, a human Igλ light chain sequence is inserted in the place of a corresponding mouse Igκ light chain sequence within the germline genome of a mouse. In some embodiments, a human Igλ light chain sequence is inserted upstream of a mouse Igλ light chain sequence, which mouse Igλ light chain sequence is positioned in the place of a mouse Igκ light chain sequence. In some embodiments, a human Igκ light chain sequence is inserted in the midst of said human Igλ light chain sequence (i.e., between human Vλ and Jλ gene segments) so that said human Igκ light chain sequence is juxtaposed by human Igλ light chain sequences.

[0220] In some embodiments, all or substantially all of the variable region of a mouse Igκ light chain locus is replaced or substituted with one or more human Igλ light chain sequences (as described herein), and said one or more human Igλ light chain sequences are operably linked to a mouse Igλ light chain constant region gene. In some embodiments, a mouse Igκ light chain constant region gene is deleted or replaced in a mouse that includes a human Igλ light chain sequence as described herein. In one non-limiting example, in the instance of an insertion of a human Igλ light chain sequence that is inserted into a mouse Igκ light chain locus, said insertion is made in manner to maintain the integrity of mouse Igκ light chain enhancer regions (or enhancer sequences) near the insertion point (e.g., a mouse Igκ intronic enhancer and/or a mouse Igκ 3' enhancer). Thus, such mice have wild-type Igκ light chain enhancer regions (or enhancer sequences) operably linked to human and mouse Igλ light chain sequences (e.g., human Vλ and Jλ gene segments, and a mouse Cλ region gene) or operably linked to human Igλ light chain sequences (e.g., human Vλ and Jλ gene segments, and a human Cλ region gene). In some embodiments, a mouse Igκ light chain locus that is altered, displaced, disrupted, deleted, replaced or engineered with one or more human Igλ light chain sequences as described herein is a murine Igκ light chain locus. In some embodiments, one or more human Igλ light chain sequences as described herein is inserted into one copy (i.e., allele) of a mouse Igκ light chain locus of the two copies of said mouse Igκ light chain locus, giving rise to a mouse that is heterozygous with respect to the human Igκ light chain sequence. In some embodiments of a mouse that is heterozygous with respect to the human Igκ light chain sequence, the mouse includes one or more human Igκ light chain sequences inserted into the other copy (i.e., allele) of the mouse Igκ light chain locus. In some embodiments, a mouse is employed that is homozygous for an Igκ light chain locus that includes one or more human Igλ light chain sequences as described herein.

[0221] In some embodiments, an engineered mouse Igκ light chain locus as described herein comprises human Vλ and Jλ gene segments operably linked to a mouse Igλ light chain constant region gene, wherein said mouse Igλ light chain constant region gene is located in the place of a mouse Igκ light chain constant region gene that appears in a wild-type Igκ light chain locus of a mouse of the same species.

[0222] In some embodiments, one or more endogenous mouse Igλ light chain sequences (or portions thereof) of an endogenous mouse Igλ light chain locus are not deleted. In some embodiments, one or more endogenous mouse Igλ light chain sequences (or portions thereof) of an endogenous mouse Igλ light chain locus are deleted. In some embodiments, one or more endogenous mouse Igλ light chain sequences (e.g., V, J and/or C or any combination thereof) of an endogenous mouse Igλ light chain locus is altered, displaced, disrupted, deleted or replaced so that said mouse Igλ light chain locus is functionally silenced. In some embodiments, one or more endogenous mouse Igλ light chain sequences (e.g., V, J and/or C or any combination thereof) of an endogenous mouse Igλ light chain locus is altered, displaced, disrupted, deleted or replaced with a targeting vector so that said mouse Igλ light chain locus is functionally inactivated (i.e., unable to produce a functional light chain of an antibody that is expressed and/or detectable in the antibody repertoire of themouse). Guidance for inactivation of an endogenous mouse Igλ light chain locus is provided in, e.g., U.S. Patent No. 9,006,511 (see, e.g., Fig. 2).

[0223] Also referred to is a mouse that contains an engineered Igκ light chain locus as described herein that is randomly integrated into its genome (e.g., as part of a randomly integrated human Igλ light chain sequence). Thus, such mice can be described as having a human Igλ light chain transgene containing a plurality of human Vλ and Jλ gene segments operably linked to a mouse or human Igλ light chain constant region gene and mouse Igκ light chain enhancer regions (or enhancer sequences), so that that said human Vλ and Jλ gene segments are capable of rearrangement and encoding an Ig light chain of an antibody in the expressed repertoire of the mouse animal, which Ig light chain includes a human Vλ domain and a mouse Cλ domain or which Ig light chain includes human Vλ and Cλ domains. An engineered Igκ light chain locus or transgene as described herein can be detected using a variety of methods including, for example, PCR, Western blot, Southern blot, restriction fragment length polymorphism (RFLP), or a gain or loss of allele assay. In some embodiments, a mouse as described herein is heterozygous with respect to an engineered Igκ light chain locus as described herein. In some embodiments, a mouse as described herein is hemizygous with respect to an engineered Igκ light chain locus as described herein. In some embodiments, a mouse as described herein contains one or more copies of an engineered Igκ light chain locus as described herein. In some embodiments, a mouseas described herein contains an Igκ light chain locus as depicted in the Drawing.

[0224] The present disclosure recognizes that a mouse animal as described herein will utilize human heavy chain, λ light chain, and κ light chain variable region gene segments included in its genome in its antibody selection and generation mechanisms (e.g., recombination and somatic hypermutation). As such, in various embodiments, human immunoglobulin human heavy chain, λ light chain, and κ light chain variable domains generated by mice described herein are encoded by the human heavy, λ light chain, and κ light chain variable region gene segments included in their genome or somatically hypermutated variants thereof, respectively.

[0225] In some embodiments, a mouse is employed whose genome comprises an engineered immunoglobulin κ light chain locus, where the mouse includes a B cell that includes a human heavy variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence that is somatically hypermutated. In some embodiments, a human heavy variable region sequence, a human λ light chain, and/or a human κ light chain variable region sequence present in a B cell of a mouse of the present disclosure has 1, 2, 3, 4, 5, or more somatic hypermutations. Those skilled in the art are aware of methods for identifying source gene segments in a mature antibody sequence. For example, various tools are available to aid in this analysis, such as, for example, DNAPLOT, IMGT/V-QUEST, JOINSOLVER, SoDA, and Ab-origin.

[0226] The present disclosure includes, among other things, cells and tissues from mice described herein. In some instances, disclosed are splenocytes (and/or other lymphoid tissue) from a mouse as described herein. In some instances, pdisclosed is a B cell from a mouse as described herein. In some instances, disclosed is a pro-B cell from a mouse as described herein. In some instances, disclosed is a pre-B cell from a mouse as described herein. In some instances, disclosed is an immature B cell from a mouse as described herein. In some instances, disclosed is a mature naive B cell from a mouse as described herein. In some instances, disclosed is an activated B cell from a mouse as described herein. In some instances, disclosed is a memory B cell from a mouse as described herein. In some instances, disclosed is a B lineage lymphocyte from a mouse as described herein. In some instances, disclosed is plasma or a plasma cell from a mouse as described herein. In some instances, disclosed is a stem cell from a mouse as described herein. In some instances, a stem cell is an embryonic stem cell. In some instances, provided is a germ cell from a mouse as described herein. In some embodiments, a germ cell is an oocyte. In some instances, a germ cell is a sperm cell. In some instances, a sperm cell from a mouse as described herein expresses one or more ADAM6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof. In some instances, any cell or tissue from a mouse as described herein may be isolated. In some instances, disclosed is an isolated cell and/or an isolated tissue from a mouse as described herein. In some instances, a hybridoma is disclosed, wherein the hybridoma is made with a B cell of a mouse as described herein. In some instances, a hybridoma is made with a B cell of a mouse that has been immunized with an antigen of interest. In some instances, a hybridoma is made with a B cell of a mouse that expresses an antibody that binds (e.g., specifically binds) to an epitope on an antigen of interest.

[0227] Any of the mice as described herein may be immunized with one or more antigens of interest under conditions and for a time sufficient that the mouse develops an immune response to said one or more antigens of interest. Those skilled in the art are aware of methods for immunizing mice. An exemplary, non-limiting method for immunizing mice can be found in US 2007/0280945A1.

[0228] The present disclosure provides, among other things, immunized mice as described herein, and cells and tissues isolated from the same. In some embodiments, a mouse described herein produces a population of B cells in response to immunization with an antigen that includes one or more epitopes. In some embodiments, a mouse produces a population of B cells that express antibodies that bind (e.g., specifically bind) to one or more epitopes of antigen of interest. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence and/or a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein. In some embodiments, antibodies expressed by a population of B cells produced in response to an antigen include (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein, or (iv) any combination thereof.

[0229] In some embodiments, a mouse produces a population of B cells that express antibodies that bind to one or more epitopes of antigen of interest, where antibodies expressed by the population of B cells produced in response to an antigen include: (i) a heavy chain having a human heavy chain variable domain encoded by a human heavy chain variable region sequence, (ii) a lambda light chain having a human lambda light chain variable domain encoded by a human lambda light chain variable region sequence as described herein, (iii) a kappa light chain having a human kappa light chain variable domain encoded by a human kappa light chain variable region sequence as described herein, or (iv) any combination thereof. In some embodiments, a human heavy chain variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence as described herein is somatically hypermutated. In some embodiments, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% of the B cells in a population of B cells produced in response to an antigen include a human heavy chain variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence that is somatically hypermutated.

[0230] In some embodiments, mice employed herein, in their germline genome, (1) include an engineered endogenous immunoglobulin κ light chain locus comprising (a) one or more human Vλ gene segments, (b) one or more human Jλ gene segments, and (c) a mouse Cλ gene, where the one or more human Vλ gene segments and one or more human Jλ gene segments are operably linked to the mouse Cλ gene, (2) lack a mouse Cκ gene at the engineered endogenous immunoglobulin κ locus, and (3) include an engineered endogenous immunoglobulin κ light chain locus comprising (a) one or more human Vκ gene segments, (b) one or more human Jκ gene segments, and (c) a Cκ gene, where the one or more human Vκ gene segments and one or more human Jκ gene segments are operably linked to the Cκ gene. In some embodiments, the percentage of light chains in splenocytes (e.g., as detected or observed, e.g., by flow cytometry (see, e.g., Example 3)) of such mice that are λ light chains is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, or at least 75%. In some embodiments, the percentage of light chains in splenocytes (e.g., as detected or observed, e.g., by flow cytometry (see, e.g., Example 3)) of such mice that are λ light chains is between 35-80%, between 35-75%, between 40-80%, between 40-75%, between 50-80%, between 50-75%, between 55-80%, between 55-75%, between 60-80%, or between 60-75%. In some embodiments, the percentage of light chains in splenocytes (e.g., as detected or observed, e.g., by flow cytometry (see, e.g., Example 3)) of such mice that are κ light chains is at most 65%, at most 60%, at most 55%, at most 50%, at most 45%, at most 40%, or at most 35%. In some embodiments, the percentage of light chains in splenocytes (e.g., as detected or observed, e.g., by flow cytometry (see, e.g., Example 3)) of such mice that are κ light chains is between 20-65%, between 25-65%, between 20-60%, between 25-60%, between 20-55%, between 25-55%, between 20-50%, between 25-50%, between 20-45%, between 25-45%, between 20-40%, or between 25-40%. In some embodiments, the ratio of κ:λ light chains in splenocytes (e.g., as detected or observed, e.g., by flow cytometry (see, e.g., Example 3)) of such mice is between 0.5:1 and 3:1, 0.65:1 and 3:1, between 0.8:1 and 3:1, between 1:1 and 3:1, between 1.2:1 and 3:1, between 1:1 and 2.3:1, between 1.1:1 and 1.8:1, between 1.2:1 and 2.3:1, or between 1.2:1 and 1.8:1.

Methods of making mice

[0231] Compositions and methods for making mice whose germline genome comprises an engineered Igκ light chain locus that includes human Vλ and Jλ gene segments in the place of non-human Igκ light chain sequences, including human Igλ light chain encoding sequences that include specific polymorphic forms of human Vλ and Jλ segments (e.g., specific V and/or J alleles or variants) are disclosed including compositions and methods for making mice that express antibodies comprising Igλ light chains that contain human variable regions and non-human or human constant regions, assembled from an Igκ light chain locus that contains human Vλ and Jλ gene segments operably linked to a mouse Igλ light chain constant region gene, which mouse Igλ light chain constant region gene is located in the place of a mouse Igκ light chain constant region gene that normally appears in a wild-type mouse Igκ light chain locus. In some embodiments, compositions and methods for making mice that express such antibodies under the control of an endogenous Igκ enhancer(s) and/or an endogenous Igκ regulatory sequence(s) are also disclosed. In some embodiments, compositions and methods for making that express such antibodies under the control of a heterologous Igκ enhancer(s) and/or a heterologous Igκ regulatory sequence(s) are also disclosed.

[0232] Methods described herein include inserting human Vλ and Jλ sequences encoding human Vλ domains upstream of a mouse Igλ light chain constant region gene, which mouse light chain constant region gene is located in the place of a mouse Igκ light chain constant region gene that normally appears in a wild-type mouse Igκ light chain locus, so that an antibody is expressed, which antibody is characterized by the presence of a light chain that contains a human Vλ domain and a mouse Cλ domain or by the presence of a light chain that contains human Vλ and one or more mouse Cλ domains, and is expressed both on the surface of B cells and in the blood serum of a mouse.

[0233] In some embodiments, methods include insertion of genetic material that contains human Vλ and Jλ gene segments into an Igκ light chain locus (e.g., a wild-type, modified or engineered Igκ light chain locus). In some certain embodiments, methods include insertion of genetic material that contains human Jλ gene segments into an Igκ light chain locus of a modified or engineered strain. In some embodiments, genetic material that contains human Igλ light chain sequences can be engineered or genomic (e.g., cloned from a bacterial artificial chromosome). In some embodiments, genetic material that contains human Igλ light chain sequences can be designed from published sources and/or bacterial artificial chromosomes so that said genetic material contains human Vλ and Jλ segments in an orientation that is different from that which appears in a human Igλ light chain locus yet said genetic material still contains sequences to support rearrangement of said human Vλ and Jλ segments to encode a functional human Vλ domain of an Ig light chain. To give but one example, genetic material corresponding to a plurality of human Vλ and Jλ gene segments can be designed using the guidance provided herein to construct a human Igλ light chain sequence that contains human Vλ and Jλ segments in an order and/or arrangement that is different than that which appears in a human Igλ light chain locus of a human cell (e.g., an arrangement that resembles or is similar to a human or mouse Igκ light chain locus, such as, a series of V gene segments, followed 3' by intervening DNA, followed 3' by a series of J gene segments). In such an example, genetic content of human Vλ and Jλ gene segments would be equivalent to the corresponding segments in a human cell, however, the order and arrangement would be different. When constructing an engineered Igκ light chain locus for generation of a mouse as described herein, the requisite recombination signal sequences can be configured so that the human V and J gene segments can correctly rearrange and form a functional human Vλ domain. Guidance for germline configuration of human Vλ and Jλ gene segments and sequences necessary for proper recombination can be found in, e.g., Molecular Biology of B Cells, London: Elsevier Academic Press, 2004, Ed. Honjo, T., Alt, F.W., Neuberger, M. Chapters 4 (pp. 37-59) and 5 (61-82).

[0234] In some embodiments, methods include multiple insertions in a single ES cell clone. In some embodiments, methods include sequential insertions made in a successive ES cell clones. In some embodiments, methods include a single insertion made in an engineered ES cell clone.

[0235] In some embodiments, methods include DNA insertion(s) upstream of a murine Cλ1 gene (or human Cλ2 gene) so that said DNA insertion(s) is operably linked to said murine Cλ1 gene (or human Cλ2 gene), which DNA insertion(s) comprise human Vλ gene segments Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1 or any combination thereof, and human Jλ gene segments Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof, and which murine Cλ1 gene (or human Cλ2 gene) is located in the place of a murine Cκ gene of an endogenous Igκ light chain locus.

[0236] In some embodiments, methods include DNA insertion(s) downstream of a human Vλ3-1 gene segment and upstream of a non-human Igκ intronic enhancer region (or enhancer sequence) of an engineered Igκ light chain locus, so that said DNA insertion(s) is operably linked to a murine Cλ1 gene (or human Cλ2 gene), which DNA insertion(s) comprises a human Igκ genomic sequence that naturally appears between a human Vκ4-1 gene segment and a human Jκ1 gene segment of a human Igκ light chain locus, and one or more human Jλ gene segments (e.g., one, two, three, four, five, six or seven), which murine Cλ1 gene (or human Cλ2 gene) is located in the place of a murine Cκ gene of an endogenous non-human Igκ light chain locus. In some certain embodiments, methods include DNA insertion(s) between a human Vλ3-1 gene segment and a non-human Igκ intronic enhancer, which DNA insertion(s) includes human Vκ-Jκ sequence that naturally appears between human Vκ4-1 and Jκ1 gene segments of a human Igκ light chain locus and five human Jλ gene segments (e.g., Jλ1, Jλ2, Jλ3, Jλ6 and Jλ7). In various embodiments, DNA insertion(s) including human Jλ gene segments comprises human Jκ genomic DNA with coding sequences of human Jλ gene segments and human Jλ 12RSS.

[0237] Insertion of additional human Vλ and Jλ segments may be achieved using methods described herein to further supplement the diversity of an engineered Igλ light chain locus. For example, in some embodiments, methods can include insertion of about 270kb of DNA upstream of a murine Cλ1 gene (or human Cλ2 gene) of an engineered Igκ light chain locus so that said DNA is operably linked to said murine Cλ1 gene (or human Cλ2 gene), which DNA includes human Vλ gene segments Vλ10-54, Vλ6-57, Vλ4-60, Vλ8-61 and Vλ4-69. In such embodiments, said DNA is inserted upstream of a human Vλ5-52 gene segment that is operably linked to a murine Cλ1 gene (or human Cλ2 gene) of an engineered Igκ light chain locus, which DNA includes human Vλ gene segments Vλ10-54, Vλ6-57, Vλ4-60, Vλ8-61 and Vλ4-69. In some certain embodiments, said DNA includes a human VpreB gene. Additional human Vλ gene segments described above may be cloned directly from commercially available BAC clones and arranged in smaller DNA fragment using recombinant techniques described herein or otherwise known in the art. Alternatively, additional human Vλ gene segments described above can be synthesized as an engineered DNA fragment and added to an engineered Igκ light chain locus as described above using molecular biology techniques known in the art. Likewise, additional human Jλ gene segments may be obtained from commercially available BAC clones or synthesized directly from published sequences. An exemplary illustration that shows an engineered Igκ light chain locus of mice as described herein is set forth in Figure 2B or 4B.

[0238] Where appropriate, a human Igλ light chain sequence (i.e., a sequence containing human Vλ and Jλ gene segments) encoding a human Vλ domain may separately be modified to include codons that are optimized for expression in a mouse (e.g., see U.S. Patent Nos. 5,670,356 and 5,874,304). Codon optimized sequences are engineered sequences, and preferably encode the identical polypeptide (or a biologically active fragment of a full-length polypeptide which has substantially the same activity as the full-length polypeptide) encoded by the non-codon optimized parent polynucleotide. In some embodiments, a human Igλ light chain sequence encoding a human Vλ domain may separately include an altered sequence to optimize codon usage for a particular cell type (e.g., a mouse cell). For example, the codons of each nucleotide sequence to be inserted into the genome of a mouse may be optimized for expression in a cell of the mouse. Such a sequence may be described as a codon-optimized sequence.

[0239] Insertion of nucleotide sequences encoding human Vλ domains employs a minimal modification of the germline genome of a mouse as described herein and results in expression of antibodies comprising light chains having human Vλ domains, which human Vλ domains are expressed from endogenous engineered Igκ light chain loci. Methods for generating engineered mice, including knockouts and knock-ins, are known in the art (see, e.g., Gene Targeting: A Practical Approach, Joyner, ed., Oxford University Press, Inc., 2000). For example, generation of genetically engineered mice may optionally involve disruption of the genetic loci of one or more endogenous mouse genes (or gene segments) and introduction of one or more heterologous genes (or gene segments or nucleotide sequences) into the mouse genome, in some embodiments, at the same location as an endogenous mouse gene (or gene segments). In some embodiments, nucleotide sequences encoding human Vλ domains are introduced upstream of a murine or human Igλ light chain constant region gene of a randomly inserted engineered light chain transgene in the germline genome of a mouse. In some embodiments, nucleotide sequences encoding human Vλ domains are introduced upstream of a mouse Igλ light chain constant region gene of an endogenous Igκ light chain locus in the germline genome of a mouse; in some certain embodiments, an endogenous Igκ light chain locus is altered, modified, or engineered to contain human Igλ gene segments (e.g., human V and J) operably linked to a mouse Cλ1 gene or operably linked to a human Cλ2 gene.

[0240] Schematic illustrations (not to scale) of exemplary methods for constructing an engineered Igκ light chain locus as described herein are provided in Figures 1A, 1B, 2A, 2B, 3, 4A and 4B. In particular, Figures 1A and 1B sets forth an exemplary strategy for construction of an engineered Igκ light chain locus characterized by insertion of nucleotide sequences containing a plurality of human Vλ and Jλ gene segments. As illustrated in Figures 1A and 1B, a DNA fragment containing a human Vκ-Jκ intergenic region (see U.S. Patent Nos. 9,006,511, 9,012,717, 9,029,628, 9,035,128, 9,066,502, 9,150,662 and 9,163,092) and engineered fragment containing a set of human Jλ gene segments (e.g., human Jλ1, Jλ2, Jλ3, Jλ6 and Jλ7) is operably linked to a mouse Igκ intronic enhancer region (or enhancer sequence) via a series of steps using various molecular biology

techniques described in Example 1. This engineered fragment is also engineered to contain a mouse Igλ light chain constant region that is operably linked to the human Jλ gene segments. Selection cassettes (e.g., Neomycin and Hygromycin) are included in the targeting vector to allow for selection of positive clones in bacteria and mammalian cells (e.g., embryonic stem cells). As illustrated a Neomycin resistance gene is flanked by *lox2372* site-specific recombination sites (lox) and positioned between the human Vκ-Jκ region and the set of human Jλ gene segments, while the Hygromycin selection cassette is flanked by *loxP* site-specific recombination sites and positioned 3' of the mouse Igλ light chain constant region (mCλ1) gene. The DNA fragment is then combined with a DNA fragment containing a mouse Igκ light chain 3' enhancer to create the final targeting vector (Figure 1B). The resulting targeting vector (construct G) is linearized and electroporated into mouse embryonic stem (ES) cells to create a mouse whose germline genome comprises the engineered Igκ light chain locus. As described in the examples section below, the mouse ES cells employed in electroporation of the targeting vector contained an engineered Igκ light chain locus as previously described in U.S. Patent Nos. 9,006,511, 9,012,717, 9,029,628, 9,035,128, 9,066,502, 9,150,662 and 9,163,092. Homologous recombination with the targeting vector as depicted in Figure 3 results in an engineered Igκ light chain locus characterized by a plurality of human Vλ and Jλ gene segments operably linked to a murine Cλ1 gene, which murine Cλ1 gene is located in place of a murine Cκ gene that naturally appears in a wild-type Igκ light chain locus. The human Jλ gene segments are uniquely engineered into a sequence that naturally appears in a genomic human Jκ region yet has human Jλ coding sequences and associated 12RSS in the place of human Jκ coding sequences and associated 23RSS. Positive mouse ES cell clones are confirmed using screening methods described herein and/or known in the art. Any remaining selection cassette may be deleted as desired via recombinase-mediated deletion (see Example 2).

[0241] Also referred to is a targeting vector where a human Cλ gene is employed in the targeting vector instead of a mouse Cλ gene. To give but one example, Figure 3 illustrates a targeting vector that was constructed in a similar manner as described above except that a sequence encoding a human Cλ2 gene was engineered into the targeting vector and in operable linkage with five human Jλ gene segments. Using such an approach provides an added benefit in developing human antibody therapeutics as DNA encoding the variable and constant regions of light chains may be isolated together, thereby eliminating any subsequent cloning step linking to a human light chain constant region for the preparation of fully-human antibodies.

[0242] Targeting vectors for constructing an engineered Igκ light chain locus as described herein may be incorporated into the germline genome of a non-human cell (e.g., a mouse embryonic stem cell). In some embodiments, targeting vectors as described herein are incorporated into a wild-type Igκ light chain locus in the germline genome of a non-human cell that further contains human V_H, D_H and J_H genomic DNA (e.g., containing a plurality of human V_H, D_H and J_H gene segments) operably linked with one or more immunoglobulin heavy chain constant region genes (e.g., see U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and 8,791,323). In some embodiments, targeting vectors as described herein are incorporated into a modified or engineered immunoglobulin κ light chain locus in the germline genome of a non-human cell that further contains human V_H, D_H and J_H genomic DNA (e.g., containing a plurality of human V_H, D_H and J_H gene segments) operably linked with one or more immunoglobulin heavy chain constant region genes (e.g., see U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940, 8,791,323, 9,006,511, 9,012,717, 9,029,628, 9,035,128, 9,066,502, 9,150,662 and 9,163,092).

[0243] A targeting vector is introduced into mouse (e.g., mouse) embryonic stem cells by electroporation so that the sequence contained in the targeting vector results in the capacity of a mouse cell or mouse that expresses antibodies having light chains that include human Vλ domains and mouse Cλ domains, and which light chains are expressed from an endogenous engineered immunoglobulin κ light chain locus. As described herein, a genetically engineered mouse is generated where an engineered immunoglobulin κ light chain locus has been created in the germline genome of the mouse (e.g., an endogenous immunoglobulin κ light chain locus containing a human Igλ light chain sequence (i.e., a plurality of human Vλ and Jλ gene segments) operably linked to a mouse Cλ gene in the place of an endogenous mouse Cκ gene). Antibodies are expressed on the surface of mouse B cells and in the serum of said mouse, which antibodies are characterized by light chains having human Vλ domains and mouse Cλ domains. When an endogenous immunoglobulin κ light chain locus in the germline genome of the mouse is not targeted by the targeting vector, an engineered immunoglobulin κ light chain transgene is preferably inserted at a location other than that of an endogenous mouse immunoglobulin κ light chain locus (e.g., randomly inserted transgene).

[0244] Creation of an engineered immunoglobulin κ light chain locus in a mouse as described above provides an engineered mouse strain that produces antibodies that include immunoglobulin λ light chains expressed from such an engineered immunoglobulin κ light chain locus having a human Vλ domain and a mouse Cλ domain. Leveraged with the presence of an engineered immunoglobulin heavy chain locus that includes a plurality of human V_H, D_H and J_H gene segments operably linked to immunoglobulin heavy chain constant region genes, an engineered mouse strain that produces antibodies and antibody components for the development of human antibody-based therapeutics is created. Thus, a single engineered mouse strain is realized that has the capacity to provide an alternative *in vivo* system for exploiting human Vλ domains for the development of new antibody-based medicines to treat human disease.

[0245] In some instances, a method of making a mouse whose germline genome comprises an engineered endogenous immunoglobulin κ light chain locus is disclosed, the method comprising (a) introducing a DNA fragment into a mouse embryonic stem cell, said DNA fragment comprising a nucleotide sequence that includes (i) one or more human Vλ gene segments, (ii) one or more human Jλ gene segments and (iii) a mouse Cλ gene, wherein (i)-(iii) are operably linked, and wherein the nucleotide sequence further comprises an immunoglobulin k light chain sequence between (i) and (ii), (b) obtaining the mouse embryonic stem cell generated in (a); and (c) creating a mouse using the mouse embryonic stem cell of (b).

[0246] In some instances, a method of making a mouse whose germline genome comprises an engineered endogenous immunoglobulin κ light chain locus is disclosed, the method comprising (a) introducing a DNA fragment into a non-human embryonic stem cell, said DNA fragment comprising a nucleotide sequence that includes one or more human Jλ gene segments, one or more non-human immunoglobulin κ light chain enhancers, and a mouse Cλ gene, which human Jλ gene segments are operably linked to said one or more mouse immunoglobulin κ light chain enhancers and said mouse Cλ gene, (b) obtaining the mouse embryonic stem cell generated in (a); and (c) creating a mouse using the mouse embryonic stem cell of (b).

[0247] In some instances, a method of making a mouse whose germline genome comprises an engineered endogenous immunoglobulin κ light chain locus, which engineered endogenous immunoglobulin κ light chain locus comprises insertion of one or more human Vλ gene segments, one or more human Jλ gene segments and a mouse Cλ gene, which human Vλ and Jλ gene segments are operably linked to said mouse Cλ gene, and which mouse Cλ gene is inserted in the place of a mouse Cκ gene at the endogenous immunoglobulin κ locus, is disclosed, the method comprising modifying the germline genome of a mouse so that it comprises an engineered endogenous immunoglobulin κ light chain locus that includes insertion of one or more human Vλ gene segments, one or more human Jλ gene segments and a mouse Cλ gene, which human Vλ and Jλ gene segments are operably linked to said mouse Cλ gene, and which mouse Cλ gene is inserted in the place of a mouse Cκ gene at the endogenous immunoglobulin κ locus.

[0248] In some instances of a method of making a mouse, one or more human Vλ gene segments includes at least 24, at least 34, at least 52, at least 61, or at least 70 human Vλ gene segments. In some embodiments of a method of making a mouse, one or more human Vλ gene segments include 39 human Vλ gene segments. In some certain instances of a method of making a mouse, one or more human Vλ gene segments include human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1 or any combination thereof. In some certain embodiments, one or more human Vλ gene segments include human non-coding DNA that naturally appears adjacent to the relevant human Vλ gene segments in an endogenous human λ light chain locus.

[0249] In some instances of a method of making a mouse, one or more human Jλ gene segments includes at least 1, at least 2, at least 3, at least 4 or at least 5 human Jλ gene segments. In some instances of a method of making a mouse, one or more human Jλ gene segments includes 5 human Jλ gene segments. In some instances of a method of making a mouse, one or more human Jλ gene segments comprise human Jλ1, Jλ2, Jλ3, Jλ6, Jλ7, or any combination thereof. In some certain instances, one or more human Jλ gene segments include human non-coding DNA, in whole or in part, that naturally appears adjacent to the relevant human Jλ gene segments in an endogenous human λ light chain locus. In some instances, one or more human Jλ gene segments include human non-coding DNA that naturally appears adjacent to a human Jκ1-Jκ5 in an endogenous human κ light chain locus.

[0250] In some instances of a method of making a mouse, a DNA fragment includes intergenic DNA that contains non-coding immunoglobulin DNA (e.g., DNA that naturally appears between the coding sequence of two V gene segments, a V and J gene segment or between two J gene segments). In many instances, said non-coding immunoglobulin DNA is non-coding immunoglobulin light chain DNA (e.g., human or murine). In some instances, non-coding immunoglobulin light chain DNA is immunoglobulin κ light chain DNA, immunoglobulin λ light chain DNA or combinations thereof.

[0251] In some instances of a method of making a mouse, a DNA fragment further comprises one or more selection markers. In some instances of a method of making a mouse, a

DNA fragment further comprises one or more site-specific recombination sites. In some certain instances of a method of making a mouse, a DNA fragment further comprises one or more sets of site-specific recombination sites that recombine with the same recombinase. In some certain instances of a method of making a mouse, a DNA fragment further comprises one or more sets of site-specific recombination sites that recombine with different recombinases.

[0252] In some instances s of a method of making a mouse, a DNA fragment comprises an engineered sequence that includes immunoglobulin κ light chain sequence and immunoglobulin λ light chain sequence together in a continuous sequence. In some instances of a method of making a mouse, a DNA fragment comprises an engineered sequence that includes immunoglobulin κ light chain sequence and immunoglobulin λ light chain sequence together in a single sequence yet interrupted by a non-immunoglobulin sequence (e.g., a recombination signal sequence, a resistance gene, and combinations thereof). In some certain instances of a method of making a mouse, an engineered sequence includes portions of a Jκ region and portions of a Jλ region. In some instances, an engineered sequence includes portions of a human Jκ region and portions of a human Jλ region. In some certain embodiments, portions of a human Jκ region include non-coding sequences of a human Jκ region that naturally appear in a human immunoglobulin κ light chain locus of a human cell. In some certain embodiments, portions of a human Jλ region include coding sequences and recombination signal sequences (RSS) of one or more human Jλ gene segments. In some certain embodiments of a method of making a mouse, a DNA fragment comprises an engineered sequence that is characterized, in some embodiments, by the presence of coding sequences and recombination signal sequences (RSS) of one or more human Jλ gene segments that positionally replace or substitute (i.e., positioned in the place of) the corresponding coding sequences and recombination signal sequences (RSS) of human Jκ gene segments so that said coding sequences and recombination signal sequences (RSS) of said one or more human Jλ gene segments are within, adjacent to, contiguous with or juxtaposed by said non-coding sequences of said one or more human Jκ gene segments.

[0253] In some instances of a method of making a mouse, a DNA fragment is introduced into a non-human embryonic stem cell whose germline genome comprises one or more engineered immunoglobulin loci (e.g., immunoglobulin heavy chain, immunoglobulin κ light chain, immunoglobulin λ light chain, and combinations thereof). In some certain instances, engineered immunoglobulin loci are endogenous engineered immunoglobulin loci.

[0254] In some instances of a method of making a mouse, a DNA fragment is introduced into a mouse embryonic stem cell whose germline genome comprises an endogenous immunoglobulin heavy chain locus comprising insertion of 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, which human V_H, D_H and J_H gene segments are operably linked to a non-human immunoglobulin heavy chain constant region.

[0255] In some instances of a method of making a mouse, a DNA fragment is introduced into a mouse embryonic stem cell whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising insertion of one or more human Vλ and one or more human Jλ gene segments, which human Vλ and Jλ gene segments are operably linked to a mouse immunoglobulin κ light chain constant region gene. In some certain instances of a method of making a mouse, a DNA fragment is introduced into a mouse embryonic stem cell whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising insertion of one or more human Vλ and one or more human Jλ gene segments, and a human immunoglobulin κ light chain sequence positioned, placed or located between said one or more human Vλ gene segments and said one or more human Jλ gene segments, which human Vλ and Jλ gene segments are operably linked to a mouse immunoglobulin κ light chain constant region gene.

[0256] In some instances of a method of making a mouse, modifying the germline genome of a mouse so that it comprises an engineered immunoglobulin κ light chain locus is carried out in a mouse embryonic stem cell whose germline genome comprises an endogenous immunoglobulin heavy chain locus comprising insertion of 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, which human V_H, D_H and J_H gene segments are operably linked to a non-human immunoglobulin heavy chain constant region.

[0257] In some instances of a method of making a mouse, modifying the germline genome of a mouse so that it comprises an engineered immunoglobulin κ light chain locus is carried out in a mouse embryonic stem cell whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising insertion of one or more human Vλ and one or more human Jλ gene segments, which human Vλ and Jλ gene segments are operably linked to a mouse immunoglobulin κ light chain constant region gene. In some embodiments of a method of making a mouse, modifying the germline genome of a mouse so that it comprises an engineered immunoglobulin κ light chain locus is carried out in mouse embryonic stem cell whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising insertion of one or more human Vλ and one or more human Jλ gene segments, and a human immunoglobulin κ light chain sequence positioned, placed or located between said one or more human Vλ gene segments and said one or more human Jλ gene segments, which human Vλ and Jλ gene segments are operably linked to a mouse immunoglobulin κ light chain constant region gene.

[0258] In some instances of a method of making a mouse, insertion of 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 includes human non-coding DNA that naturally appears adjacent to the human V_H gene segments, human non-coding DNA that naturally appears adj acent to the human D_H gene segments and human non-coding DNA that naturally appears adjacent to the human J_H gene segments in an endogenous human immunoglobulin locus.

[0259] A mouse made, generated, produced, obtained or obtainable from a method as described herein is also disclosed.

[0260] In some embodiments, the genome of a mouse as described herein further comprises one or more human immunoglobulin heavy variable regions as described in U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and 8,791,323. Alternatively, an engineered immunoglobulin κ light chain locus as described herein can be engineered into an embryonic stem cell of a different modified strain such as, e.g., a VELOCIMMUNE® strain (see, e.g., U.S. Patent Nos. 8,502,018 and/or 8,642,835). Homozygosity of the engineered Igκ light chain locus as described herein can subsequently be achieved by breeding. Alternatively, in the case of a randomly inserted engineered immunoglobulin κ light chain transgene (described above), mouse strains can be selected based on, among other things, expression of human Vλ domains from the transgene. In some embodiments, a VELOCIMMUNE® mouse can be a VELOCIMMUNE® 1 (VI-1) mouse, which includes eighteen human V_H gene segments, all of the human D_H gene segments, and all of the J_H gene segments. A VI-1 mouse can also include sixteen human Vκ gene segments and all of the human Jκ gene segments. In some embodiments, a VELOCIMMUNE® mouse can be a VELOCIMMUNE® 2 (VI-2) mouse, which includes thirty-nine human V_H gene segments, all of the human D_H gene segments, and all of the J_H gene segments. A VI-2 mouse can also include human thirty Vκ gene segments and all of the human Jκ gene segments. In some embodiments, a VELOCIMMUNE® mouse can be a VELOCIMMUNE® 3 (VI-3) mouse, which includes eighty human V_H gene segments, all of the human D_H gene segments, and all of the J_H gene segments. A VI-3 mouse can also include human forty Vκ gene segments and all of the human Jκ gene segments.

[0261] Alternatively, and/or additionally, in some embodiments, the germline genome of a mouse as described herein further comprises a deleted, inactivated, functionally silenced or otherwise non-functional endogenous immunoglobulin λ light chain locus. Genetic modifications to delete or render non-functional a gene or genetic locus may be achieved using methods described herein and/or methods known in the art.

[0262] A genetically engineered founder mouse can be identified based upon the presence of an engineered Igκ light chain locus in its germline genome and/or expression of antibodies having a human Vλ domain and a mouse Cλ domain in tissues or cells of the mouse. A genetically engineered founder mouse can then be used to breed additional mice carrying the engineered immunoglobulin κ light chain locus thereby creating a cohort of mice each carrying one or more copies of an engineered immunoglobulin κ light chain locus. Moreover, genetically engineered mice carrying an engineered immunoglobulin κ light chain locus as described herein can further be bred to other genetically engineered micemice carrying other transgenes (e.g., human immunoglobulin genes) or engineered immunoglobulin loci as desired.

[0263] Genetically engineered mice may also be produced to contain selected systems that allow for regulated, directed, inducible and/or cell-type specific expression of the transgene or integrated sequence(s). For example, mice as described herein may be engineered to contain one or more sequences encoding a human Vλ domain of an antibody that is/are conditionally expressed (e.g., reviewed in Rajewski, K. et al., 1996, J. Clin. Invest. 98(3):600-3). Exemplary systems include the Cre/loxP recombinase system of bacteriophage P1 (see, e.g., Lakso, M. et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:6232-6) and the FLP/Frt recombinase system of S. cerevisiae (O'Gorman, S. et al, 1991, Science 251:1351-5). Such animals can be provided through the construction of *"double"* genetically engineered animals, e.g., by mating two genetically engineered animals, one containing a transgene comprising a selected modification (e.g., an engineered Igκ light chain locus as described herein) and the other containing a transgene encoding a recombinase (e.g., a Cre recombinase).

[0264] Mice as described herein may be prepared as described above, or using methods known in the art, to comprise additional human, humanized or otherwise engineered genes, oftentimes depending on the intended use of the mouse. Genetic material of such human, humanized or otherwise engineered genes may be introduced through the further alteration of the genome of cells (e.g., embryonic stem cells) having the genetic modifications or alterations as described above or through breeding techniques known in the art with other genetically modified or engineered strains as desired. In some embodiments, mice as described herein are prepared to further comprise human IgH and/or Igκ light chain genes or gene segments (see e.g., Murphy, A.J. et al., (2014) Proc. Natl. Acad. Sci. U.S.A. 111(14):5153-5158; U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and 8,791,323; U.S. Patent No: 8,791,323; and U.S. Patent Application Publication No. 2013/0096287 A1).

[0265] In some embodiments, mice as described herein may be prepared by introducing a targeting vector described herein into a cell from a modified or engineered strain. For example, a targeting vector as described herein may be introduced into a VELOCIMMUNE® mouse. VELOCIMMUNE® mice express antibodies that have fully human variable regions and mouse constant regions. In another example, a targeting vector as described herein may be introduced into an engineered mouse as described in any one of U.S. Patent Nos. 9,006,511, 9,012,717, 9,029,628, 9,035,128, 9,066,502, 9,150,662 and 9,163,092. In some embodiments, miceas described herein are prepared to further comprise human immunoglobulin genes (variable and/or constant region genes). In some embodiments, mice as described herein comprise an engineered Igκ light chain locus as described herein and genetic material from a heterologous species (e.g., humans), wherein the genetic material encodes, in whole or in part, one or more human heavy and/or Igκ light chain variable regions.

[0266] For example, as described herein, mice comprising an engineered Igκ light chain locus as described herein may further comprise (e.g., via cross-breeding or multiple gene targeting strategies) one or more modifications as described in Murphy, A.J. et al., (2014) Proc. Natl. Acad. Sci. U.S.A. 111(14):5153-8; Macdonald, L.E. et al., 2014, Proc. Natl. Acad. Sci. U.S.A. 111(14):5147-52; U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and 8,791,323. In some embodiments, a mouse comprising an engineered immunoglobulin κ light chain locus as described herein is crossed to a mouse comprising a humanized immunoglobulin heavy chain and/or immunoglobulin κ light chain variable region locus (see, e.g., U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and/or 8,791,323). In some embodiments, a mouse comprising an engineered immunoglobulin κ light chain locus as described herein is crossed to a mouse comprising a humanized immunoglobulin heavy chain variable region locus (see, e.g., U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and/or 8,791,323) and an inactivated endogenous immunoglobulin λ light chain locus (see, e.g., U.S. Patent Nos. 9,006,511, 9,012,717, 9,029,628, 9,035,128, 9,066,502, 9,150,662 and 9,163,092).

[0267] Other methods for making a mouse comprising the genetic modification include, e.g., modifying a non-ES cell genome (e.g., a fibroblast or an induced pluripotent cell) and employing somatic cell nuclear transfer (SCNT) to transfer the genetically modified genome to a suitable cell, e.g., an enucleated oocyte, and gestating the modified cell (e.g., the modified oocyte) in a mouse under suitable conditions to form an embryo.

[0268] Methods for modifying the germline genome of a mouse include, e.g., employing a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), or a Cas protein (i.e., a CRISPR/Cas system) to include an engineered immunoglobulin κ light chain locus as described herein. Guidance for methods for modifying the germline genome of a mouse can be found in, e.g., U.S. Patent Application Nos. 14/747,461 (filed June 23, 2015), 14/948,221 (filed November 20, 2015) and 14/974,623 (filed December 18, 2015).

[0269] The non-human animal employed is a mouse.

[0270] In some embodiments, a mouse as described herein is a mouse that is a mouse of a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In some certain embodiments, a mouse as described herein is a 129-strain selected from the group consisting of a strain that is 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129/SvJae, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, e.g., Festing et al., 1999, Mammalian Genome 10:836; Auerbach, W. et al., 2000, Biotechniques 29(5):1024-1028, 1030, 1032). In some certain embodiments, a genetically modified mouse as described herein is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In some certain embodiments, a mouse as described herein is a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. In some certain embodiments, a 129 strain of the mix as described herein is a 129S6 (129/SvEvTac) strain. In some embodiments, a mouse as described herein is a BALB strain, e.g., BALB/c strain. In some embodiments, a mouse as described herein is a mix of a BALB strain and another aforementioned strain.

Specific Exemplary Embodiments - Immunoglobulin Heavy Chain Loci

[0271] In some embodiments, employed mice comprise an engineered immunoglobulin κ light chain locus as described herein and further comprise engineered IgH loci (or alleles) characterized by the presence of a plurality of human V_H, D_H and J_H gene segments arranged in germline configuration and operably linked to mouse immunoglobulin heavy chain constant region genes, enhancers and regulatory regions. In some embodiments, an engineered immunoglobulin heavy chain locus (or allele) as described herein 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 operably linked to a mouse immunoglobulin heavy chain constant region. In some certain embodiments, an engineered immunoglobulin heavy chain locus (or allele) comprises at least human V_H gene segments V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, V_H6-1, or any combination thereof. In some certain embodiments, an engineered IgH locus (or allele) comprises at least human D_H gene segments D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26, D_H7-27, or any combination thereof. In some certain embodiments, an engineered immunoglobulin heavy chain locus (or allele) comprises at least human J_H gene segments J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, or any combination thereof.

[0272] The present disclosure recognizes that a mouse as described herein will utilize human heavy chain variable region gene segments comprised in its genome in its antibody selection and generation mechanisms (e.g., recombination and somatic hypermutation). As such, in various embodiments, human immunoglobulin heavy chain variable domains generated by mice described herein are encoded by the human heavy chain variable region gene segments included in their genome or somatically hypermutated variants thereof.

[0273] In some embodiments, a mouse is employed whose genome comprises an engineered immunoglobulin κ light chain locus, where the mouse includes a B cell that includes a human heavy variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence that is somatically hypermutated. In some embodiments, a human heavy variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence present in a B cell of a mouse of the present disclosure has 1, 2, 3, 4, 5, or more somatic hypermutations. Those skilled in the art are aware of methods for identifying source gene segments in a mature antibody sequence. For example, various tools are available to aid in this analysis, such as, for example, DNAPLOT, IMGT/V-QUEST, JOINSOLVER, SoDA, and Ab-origin.

[0274] In some embodiments, a mouse immunoglobulin heavy chain constant region includes one or more mouse immunoglobulin heavy chain constant region genes such as, for example, immunoglobulin M (IgM), immunoglobulin D (IgD), immunoglobulin G (IgG), immunoglobulin E (IgE) and immunoglobulin A (IgA). In some certain embodiments, a mouse immunoglobulin heavy chain constant region includes a mouse IgM, mouse IgD, mouse IgG3, mouse IgG1, mouse IgG2b, mouse IgG2a, mouse IgE and mouse IgA constant region genes. In some embodiments, said human V_H, D_H and J_H gene segments are operably linked to one or more mouse immunoglobulin heavy chain enhancers (i.e., enhancer sequences or enhancer regions). In some embodiments, said human V_H, D_H and J_H gene segments are operably linked to one or more mousen immunoglobulin heavy chain regulatory regions (or regulatory sequences). In some embodiments, said human V_H, D_H and J_H gene segments are operably linked to one or more mouse immunoglobulin heavy chain enhancers (or enhancer sequence) and one or more mouse immunoglobulin heavy chain regulatory regions (or regulatory sequence).

[0275] In some embodiments, an engineered immunoglobulin heavy chain locus as described herein does not contain an endogenous Adam6 gene. In some embodiments, an engineered immunoglobulin heavy chain locus as described herein does not contain an endogenous Adam6 gene (or Adam6-encoding sequence) in the same germline genomic position as found in a germline genome of a wild-type mouse of the same species. In some embodiments, an engineered immunoglobulin heavy chain locus as described herein does not contain a human Adam6 pseudogene. In some embodiments, an engineered immunoglobulin heavy chain locus as described herein comprises insertion of at least one

nucleotide sequence that encodes one or more non-human (e.g., mouse) Adam6 polypeptides, functional orthologs, functional homologs, or functional fragments thereof. In some embodiments, said insertion may be outside of an engineered immunoglobulin heavy chain locus as described herein (e.g., but not limited to, upstream of a 5' most V_H gene segment), within an engineered immunoglobulin heavy chain locus or elsewhere in the germline genome of a mouse (e.g., but not limited to, a randomly introduced non-human Adam6-encoding sequence), cell or tissue.

[0276] In various embodiments, an employed mouse, mouse cell or mouse tissue as described herein does not detectably express, in whole or in part, an endogenous mouse V_H region in an antibody molecule. In various embodiments, an employed mouse, mouse cell or mouse tissue as described herein does not contain (or lacks, or contains a deletion of) one or more nucleotide sequences that encode, in whole or in part, an endogenous mouse V_H region (e.g., V_H, D_H and/or J_H) in an antibody molecule. In various embodiments, an employed mouse, mouse cell or mouse tissue as described herein has a germline genome that includes a deletion of endogenous mouse V_H, D_H and J_H gene segments, in whole or in part. In various embodiments, an employed mouse is fertile.

[0277] Guidance for the creation of targeting vectors, non-human cells and animals harboring such engineered immunoglobulin heavy chain loci (or alleles) can be found in U.S. Patent Nos. 8,502,018, 8,642,835, 8,697,940 and 8,791,323. Persons skilled in the art are aware of a variety of technologies, known in the art, for accomplishing such genetic engineering and/or manipulation of non-human (e.g., mammalian) genomes or for otherwise preparing, providing, or manufacturing such sequences for introducing into the germline genome of mice.

Specific Exemplary Embodiments - Immunoglobulin κ Light Chain Loci

[0278] In some embodiments, employed mice comprise an engineered immunoglobulin κ light chain locus characterized by the presence of a plurality of human Vλ and Jλ gene segments arranged in germline configuration (i.e., not rearranged and associated with recombination signal sequences) and inserted upstream of, and operably linked to, a mouse Cλ gene, which mouse Cλ gene is inserted in the place of a mouse Cκ gene. As described herein, such engineered immunoglobulin κ light chain locus further includes mouse immunoglobulin κ light chain enhancer regions (or enhancer sequences). In some embodiments, an engineered immunoglobulin κ light chain locus comprises one or more human Vλ gene segments and one or more human Jλ gene segments operably linked to a mouse Cλ gene. In some certain embodiments, an engineered immunoglobulin κ light chain locus (or allele) comprises human Vλ gene segments that appear in at least cluster A of a human immunoglobulin λ light chain locus; in some embodiments, cluster A and cluster B of a human immunoglobulin λ light chain locus; in some certain embodiments, cluster A, cluster B and cluster C of a human immunoglobulin λ light chain locus. In some certain embodiments, an engineered immunoglobulin κ light chain locus (or allele) comprises at least human Vλ gene segments Vκ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1 or any combination thereof. In some certain embodiments, an engineered Igκ light chain locus (or allele) comprises at least human Jλ gene segments Jλ1, Jλ2, Jλ3, Jλ6 Jλ7, or any combination thereof.

[0279] The present disclosure recognizes that a mouse as described herein will utilize human λ light chain variable region gene segments included in its genome in its antibody selection and generation mechanisms (e.g., recombination and somatic hypermutation). As such, in various embodiments, human immunoglobulin λ light chain variable domains generated by mice described herein are encoded by the human λ light chain variable region gene segments included in their genome or somatically hypermutated variants thereof.

[0280] In some embodiments, a mouse is employed whose genome comprises an engineered immunoglobulin κ light chain locus, where the mouse includes a B cell that includes a human heavy variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence that is somatically hypermutated. In some embodiments, a human heavy variable region sequence, a human λ light chain variable region sequence, and/or a human κ light chain variable region sequence present in a B cell of a mouse of the present disclosure has 1, 2, 3, 4, 5, or more somatic hypermutations. Those skilled in the art are aware of methods for identifying source gene segments in a mature antibody sequence. For example, various tools are available to aid in this analysis, such as, for example, DNAPLOT, IMGT/V-QUEST, JOINSOLVER, SoDA, and Ab-origin.

[0281] In many embodiments, an engineered immunoglobulin κ light chain locus (or allele) contains the same non-human immunoglobulin κ light chain enhancer regions (or enhancer sequences) that appear in a wild-type immunoglobulin κ light chain locus (or allele). In some embodiments, an engineered immunoglobulin κ light chain locus (or allele) contains non-human immunoglobulin κ light chain enhancer regions (or enhancer sequences) that appear in a wild-type immunoglobulin κ light chain locus (or allele) of a different species (e.g., a different mouse species).

[0282] In some embodiments, said human Vλ and Jλ gene segments are operably linked to one or more non-human immunoglobulin κ light chain enhancers (i.e., enhancer sequences or enhancer regions). In some certain embodiments, said human Vλ and Jλ gene segments are operably linked to a murine immunoglobulin κ light chain intronic enhancer region (Igκ Ei or Eiκ). In some certain embodiments, said human Vλ and Jλ gene segments are operably linked to a murine immunoglobulin κ light chain 3' enhancer region (Igκ 3'E or 3'Eκ). In some certain embodiments, said human Vλ and Jλ gene segments are operably linked to a murine Eiκ and operably linked to a murine 3'Eκ.

[0283] In some embodiments, an engineered immunoglobulin κ light chain locus (or allele) as described herein does not contain (i.e., lacks) a human VpreB gene (or human VpreB gene- encoding sequence).

[0284] In some certain embodiments, a mouse Cλ gene of an engineered Igκ light chain locus (or allele) is or comprises a mouse Cλ gene from a genetic background that includes a 129 strain, a BALB/c strain, a C57BL/6 strain, a mixed 129xC57BL/6 strain or combinations thereof.

[0285] In some embodiments, a mouse Cλ gene of an engineered Igκ light chain locus (or allele) as described herein comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO:1 (mouse Cλ1), SEQ ID NO:3 (mouse Cλ2) or SEQ ID NO:5 (mouse Cλ3). In some embodiments, a mouse Cλ gene of an engineered Igκ light chain locus (or allele) as described herein comprises a sequence that is substantially identical or identical to SEQ ID NO:1 (mouse Cλ1), SEQ ID NO:3 (mouse Cλ2) or SEQ ID NO:5 (mouse Cλ3). In some embodiments, a mouse Cλ gene of an engineered Igκ light chain locus (or allele) as described herein is or comprises the sequence of a mouse Cλ1 gene.

[0286] In some embodiments, a mouse Cλ domain encoded by a sequence positioned at an engineered Igκ light chain locus (or allele) as described herein comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to SEQ ID NO:2 (mouse Cλ1), SEQ ID NO:4 (mouse Cλ2) or SEQ ID NO:6 (mouse Cλ3). In some embodiments, a mouse Cλ domain encoded by a sequence positioned at an engineered Igκ light chain locus (or allele) as described herein comprises a sequence that is substantially identical or identical to SEQ ID NO:2 (mouse Cλ1), SEQ ID NO:4 (mouse Cλ2) or SEQ ID NO:6 (mouse Cλ3). In some embodiments, a mouse Cλ gene encoded by a sequence positioned at an engineered Igκ light chain locus (or allele) as described herein is or comprises a mouse Cλ1 domain polypeptide.

[0287] Among other things, the present disclosure demonstrates that the presence of human Vλ and Jλ gene segments at Igκ light chain loci (or alleles) increases the diversity of the light chain repertoire of an employed mouse as compared to the diversity of the light chains in the expressed antibody repertoire of a mouse that does not comprise such engineered Igκ light chain loci.

Specific Exemplary Embodiments - Immunoglobulin λ Light Chain Loci

[0288] In some embodiments, employed mice comprise an engineered immunoglobulin κ light chain locus as described herein and further comprise wild-type or inactivated immunoglobulin λ light chain loci (or alleles).

[0289] In some embodiments, employed mice, mouse cells and/or mouse tissues as described herein comprise a deletion, in whole or in part, of an endogenous immunoglobulin λ light chain locus. In some embodiments, employedmice, mouse cells and/or mouse tissues as described herein comprise an insertion within an endogenous immunoglobulin λ light

chain locus, wherein said insertion renders the endogenous immunoglobulin λ light chain locus non-functional. In some embodiments, employed mice, mouse cells and/mouse tissues as described herein comprise a deletion of one or more gene segments of an endogenous immunoglobulin λ light chain locus such that the endogenous immunoglobulin λ light chain locus is unable to recombine and/or express a functional light chain of an antibody.

[0290] In some embodiments, an employed mouse, mouse cell or mouse tissue as described herein does not detectably express, in whole or in part, an endogenous non-human Vλ region in an antibody molecule. In some embodiments, an employed mouse, mouse cell or mouse tissue as described herein does not contain (or lacks, or contains a deletion of) one or more nucleotide sequences that encode, in whole or in part, an endogenous non-human Vλ region in an antibody molecule. In some embodiments, an employed mouse, mouse cell or mouse tissue as described herein has a germline genome that includes a deletion of endogenous mouse Vλ and Jλ gene segments, in whole or in part. In some embodiments, an employed mouse, mouse cell or mouse tissue as described herein as a germline genome that includes a deletion of endogenous mouse Vλ, Jλ and Cλ gene segments, in whole or in part.

[0291] Guidance for the creation of targeting vectors, non-human cells and animals harboring inactivated Igλ light chain loci (or alleles) can be found in, e.g., U.S. Patent Nos. 9,006,511, 9,012,717, 9,029,628, 9,035,128, 9,066,502, 9,150,662 and 9,163,092. Those skilled in the art are aware of a variety of technologies, known in the art, for accomplishing genetic inactivation of specific loci and/or manipulation of non-human (e.g., mammalian) genomes or for otherwise preparing, providing, or manufacturing such genetic inactivation (e.g., gene deletions) for introducing into the germline genome of mice

Specific Exemplary Embodiments - Combinations of Immunoglobulin loci

[0292] In some embodiments, employed mice comprise an engineered immunoglobulin κ light chain locus as described herein and further comprise one or more additional human or humanized genes (e.g., via cross-breeding or multiple gene targeting strategies). Such mice may be prepared as described above, or using methods known in the art, to achieve a desired engineered genotype depending on the intended use of the mouse. Genetic material of such additional human or humanized genes may be introduced through the further alteration of the genome of cells (e.g., embryonic stem cells) having the genetic modifications as described above or through breeding techniques known in the art with other genetically modified strains as desired.

[0293] In some embodiments, employed mice are prepared to further comprise a human or humanized immunoglobulin heavy chain locus (e.g., including but not limited to, a plurality of human V_H, D_H and J_H gene segments operably linked to one or more mouse immunoglobulin heavy chain constant region genes at an endogenous immunoglobulin heavy chain locus). In some embodiments, employed mice are heterozygous for an engineered immunoglobulin κ light chain locus as described herein and heterozygous for a human or humanized immunoglobulin heavy chain locus. In some embodiments, employed mice are homozygous for an engineered immunoglobulin κ light chain locus as described herein and homozygous for a human or humanized immunoglobulin heavy chain locus.

[0294] In some embodiments, employed mice are prepared to further comprise a human or humanized immunoglobulin heavy chain locus (e.g., including but not limited to, a plurality of human V_H, D_H and J_H gene segments operably linked to one or more mouse immunoglobulin heavy chain constant region genes at an endogenous immunoglobulin heavy chain locus) and a humanized immunoglobulin κ light chain locus (e.g., including but not limited to, a plurality of human Vκ and Jκ gene segments operably linked to a mouse immunoglobulin κ light chain constant region gene at an endogenous immunoglobulin κ light chain locus. In some embodiments, employed mice are heterozygous for a human or humanized immunoglobulin heavy chain locus and further comprise one endogenous immunoglobulin κ light chain locus that contains a plurality of human Vλ and Jλ gene segments operably linked to a mouse immunoglobulin λ light chain constant region gene (i.e., an engineered Igκ light chain locus as described herein), and another endogenous immunoglobulin κ light chain locus having a plurality of human Vκ and Jκ gene segments operably linked to a mouse immunoglobulin κ light chain constant region gene. In some embodiments, employed mice are homozygous for a human or humanized immunoglobulin heavy chain locus and further comprise an endogenous immunoglobulin κ light chain locus that contains a plurality of human Vλ and Jλ gene segments operably linked to a mouse immunoglobulin λ light chain constant region gene (i.e., an engineered Igκ light chain locus as described herein), and another endogenous immunoglobulin κ light chain locus having a plurality of human Vκ and Jκ gene segments operably linked to a mouse immunoglobulin κ light chain constant region gene.

[0295] In some embodiments, employed mice have a genome comprising (a) a homozygous or heterozygous human or humanized immunoglobulin heavy chain locus comprising human V_H, D_H and J_H gene segments operably linked to one or more endogenous non-human immunoglobulin heavy chain constant regions such that the mouse expresses an immunoglobulin heavy chain that comprises a human V_H domain sequence fused with a non-human C_H domain sequence; (b) a first immunoglobulin κ light chain locus comprising human Vλ and Jλ gene segments operably linked to a mouseimmunoglobulin Cλ gene such that the mouse expresses an immunoglobulin light chain that comprises a human Vλ domain sequence fused with a mouse Cλ domain sequence; and (c) a second immunoglobulin κ light chain locus comprising human Vκ and Jκ gene segments operably linked to an endogenous mouse Cκ gene such that the mouse expresses an immunoglobulin light chain that comprises a human Vκ domain sequence fused with a mouse Cκ domain sequence.

[0296] In some embodiments, employed mice have a genome comprising (a) a homozygous or heterozygous human or humanized immunoglobulin heavy chain locus comprising human V_H, D_H and J_H gene segments operably linked to one or more endogenous mouse immunoglobulin heavy chain constant regions such that the mouse expresses an immunoglobulin heavy chain that comprises a human V_H domain sequence fused with a mouse C_H domain sequence; (b) a first immunoglobulin κ light chain locus comprising human Vλ and Jλ gene segments operably linked to a mouse immunoglobulin Cλ gene such that the mouse expresses an immunoglobulin light chain that comprises a human Vλ domain sequence fused with a mouse Cλ domain sequence; (c) a second immunoglobulin κ light chain locus comprising human Vκ and Jκ gene segments operably linked to an endogenous mouse Cκ gene such that the mouse expresses an immunoglobulin light chain that comprises a human Vκ domain sequence fused with a mouse Cκ domain sequence; and (d) a homozygous or heterozygous functionally inactivated or deleted, in whole or in part, endogenous immunoglobulin λ light chain locus.

[0297] For example, as described herein, mice comprising an engineered immunoglobulin κ light chain locus as described herein may further comprise (e.g., via cross-breeding or multiple gene targeting strategies) one or more modifications as described in U.S. Patent Nos. 8,642,835, 8,697,940, 9,006,511, 9,035,128, 9,066,502, 9,150,662 and 9,163,092 In some embodiments, employed mice further comprise a humanized immunoglobulin heavy chain locus (e.g., an immunoglobulin heavy chain locus comprising human V_H, D_H and J_H gene segments operably linked to one or more non-human immunoglobulin heavy chain constant region genes). In some embodiments, employed mice further comprise a humanized immunoglobulin heavy chain locus and a non-functional endogenous immunoglobulin λ light chain locus (e.g., deleted in whole or in part, or otherwise rendered non-functional).
In some embodiments, employed mice comprise an immunoglobulin κ light chain locus having human Vλ and Jλ gene segments operably linked to a human or mouse Cλ gene positioned in the place of a mouse Cκ gene and a second immunoglobulin κ light chain locus comprising human Vκ and Jκ gene segments operably linked to an endogenous mouse Cκ gene. In such embodiments, employed mice are referred to as hemizygous for an engineered immunoglobulin κ light chain locus. In some embodiment, said hemizygous mice employed herein further comprise a humanized immunoglobulin heavy chain locus. In some embodiments, said hemizygous mice employed herein further comprise a humanized immunoglobulin heavy chain locus and a non-functional endogenous immunoglobulin λ light chain locus.

Methods of using the described mice, cells or tissues

[0298] Mice as described herein can be used as a platform for the development of antibodies. In particular, the mice described herein represent a particularly advantageous platform for the generation and identification of human lambda light chain variable domains and antibodies that include such human lambda light chain variable domains. In particular, a method of producing an antibody in a genetically modified mouse is provided, the method comprising the steps of:

1. (a) immunizing the genetically modified mouse with an antigen of interest,
   wherein the genetically modified mouse has a germline genome comprising:

   a first engineered endogenous immunoglobulin κ light chain locus comprising:

1. (i) one or more human Vλ gene segments,
2. (ii) one or more human Jλ gene segments, and
3. (iii) one mouse Cλ gene,

wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are operably linked to the one mouse Cλ gene of (iii);

wherein the one mouse Cλ gene (iii) is in the place of a mouse Cκ gene at the first engineered endogenous immunoglobulin κ light chain locus;

wherein the one or more human Vλ gene segments of (i) and the one or more human Jλ gene segments of (ii) are in place of one or more endogenous mouse Vκ gene segments and one or more endogenous mouse Jκ gene segments;

wherein the genetically modified mouse lacks a mouse Cκ gene at the first engineered endogenous immunoglobulin k light chain locus;

2. (b) maintaining the genetically modified mouse under conditions sufficient for the genetically modified mouse to produce an immune response to the antigen of interest; and
3. (c) recovering from the genetically modified mouse:
    1. (i) an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain,
    2. (ii) a nucleotide that encodes a human light chain variable domain or human heavy chain variable domain, a light chain, or a heavy chain of an antibody that binds the antigen of interest, wherein the antibody that binds the antigen of interest comprises a light chain comprising a human λ variable domain and a mouse λ constant domain, or
    3. (iii) a cell that expresses an antibody that binds the antigen of interest, wherein the antibody comprises a light chain comprising a human λ variable domain and a mouse λ constant domain.

[0299] Accordingly, the present disclosure provides that mice described herein can be used in methods of making antibodies. Antibodies made in accordance with the present disclosure can include, for example, human antibodies, chimeric antibodies, reverse chimeric antibodies, fragments of any of these antibodies, or combinations thereof.

[0300] In some embodiments, mice as described herein may be employed for making a human antibody (e.g., a fully human antibody), which human antibody comprises variable regions derived from nucleic acid sequences encoded by genetic material of a cell of a mouse as described herein. In some embodiments, a mouse as described herein is immunized with an antigen of interest under conditions and for a time sufficient that the mouse develops an immune response to said antigen of interest. Antibodies and/or antibody sequences (i.e., sequences that encode for part of an antibody, e.g., a variable region sequence) are isolated and/or identified from the mouse (or one or more cells, for example, one or more B cells) so immunized and characterized using various assays measuring, for example, affinity, specificity, epitope mapping, ability for blocking ligand-receptor interaction, inhibition receptor activation, etc. In various embodiments, antibodies produced by mice as described herein comprise one or more human variable regions that are derived from one or more human variable region nucleotide sequences isolated from the mouse. In some embodiments, anti-drug antibodies (e.g., anti-idiotype antibody) may be raised in mice as described herein. In various embodiments, antibodies produced by mice as described herein are reverse chimeric antibodies that include a human light chain variable domain and a mouse light chain constant domain and/or a human heavy chain variable domain and a mouse heavy chain constant domain.

[0301] In various embodiments, antibodies produced by mice include heavy and light chains having a human variable domain and a mouse constant domain. In some embodiments, antibodies produced by mice as described herein are reverse chimeric antibodies that include a human light chain variable domain and a mouse light chain constant domain. In some embodiments, antibodies produced by mice as described herein are reverse chimeric antibodies that include a human heavy chain variable domain and a mouse heavy chain constant domain.

[0302] In some embodiments, provided methods include immunizing a mouse as described herein with an antigen of interest. In some embodiments, provided methods include identifying a lymphocyte (e.g., a clonally selected lymphocyte) from said mouse, where the lymphocyte expresses an antibody that binds (e.g., specifically binds) the antigen of interest. In some embodiments, a lymphocyte is a B cell. In some embodiments, a human heavy chain variable region sequence, a human lambda light chain variable region sequence, and/or a human kappa light chain variable region sequence is obtained from the lymphocyte (e.g., B cell) and/or identified (e.g., genotyped, e.g., sequenced). In some embodiments, an amino acid sequence of a human heavy chain variable domain, a human lambda light chain variable domain, and/or a human kappa light chain variable domain is obtained from the lymphocyte (e.g., B cell) and/or identified (e.g., sequenced). In some embodiments, a human heavy chain variable region sequence, a human lambda light chain variable region sequence, and/or a human kappa light chain variable region sequence from a B cell of a mouse is expressed in a host cell. In some embodiments, a variant of a human heavy chain variable region sequence, a human lambda light chain variable region sequence, and/or a human kappa light chain variable region sequence from a B cell of a mouse is expressed in a host cell. In some embodiments, a variant includes one or more mutations. In some embodiments, one or more mutations can improve a pharmacokinetic and/or a pharmacodynamic property of an antibody including a variant. In some embodiments, one or more mutations can improve the specificity, the affinity, and/or the immunogenicity of an antibody including a variant.

[0303] In some embodiments, methods of making a human antibody include identifying a nucleotide sequence encoding a human immunoglobulin heavy chain variable domain and/or a human immunoglobulin light chain variable domain from a mouse described herein; and (i) joining or ligating the nucleotide sequence encoding the human immunoglobulin heavy chain variable domain to a nucleotide sequence encoding a human immunoglobulin heavy chain constant domain, thereby forming a human immunoglobulin heavy chain sequence encoding a fully human immunoglobulin heavy chain, (ii) joining or ligating the nucleotide sequence encoding the human immunoglobulin λ light chain variable domain to a nucleotide sequence encoding a human immunoglobulin λ light chain constant domain, thereby forming a human immunoglobulin λ light chain sequence encoding a fully human immunoglobulin λ light chain, and/or (iii) joining or ligating the nucleotide sequence encoding the human immunoglobulin κ light chain variable domain to a nucleotide sequence encoding a human immunoglobulin κ light chain constant domain, thereby forming a human immunoglobulin κ light chain sequence encoding a fully human immunoglobulin κ light chain. In certain embodiments, a human immunoglobulin heavy chain sequence, and (i) a human immunoglobulin λ light chain sequence, or (ii) a human immunoglobulin κ light chain sequence are expressed in a cell (e.g., a host cell, a mammalian cell) so that fully human immunoglobulin heavy chains and (i) fully human immunoglobulin λ light chains or (ii) fully human immunoglobulin κ light chains are expressed and form human antibodies. In some embodiments, human antibodies are isolated from the cell or culture media including the cell.

[0304] Mice as described herein may be employed for identifying a nucleotide or nucleic acid sequence encoding a human variable domain generated by a mouse described herein, e.g., as part of an antibody against an epitope or antigen.

[0305] Mice as described herein may be employed for identifying an amino acid sequence of a human variable domain generated by a mouse described herein, e.g., as part of an antibody against an epitope or antigen.

[0306] Mice as described herein provide an improved *in vivo* system and source of biological materials (e.g., cells, nucleotides, polypeptides, protein complexes) for producing human antibodies that are useful for a variety of assays. In various embodiments, mice as described herein are used to develop therapeutics that target a polypeptide of interested (e.g., a transmembrane or secreted polypeptide) and/or modulate one or more activities associated with said polypeptide of interest and/or modulate interactions of said polypeptide of interest with other binding partners (e.g., a ligand or receptor polypeptide). For example, in various embodiments, mice as described herein are used to develop therapeutics that target one or more receptor polypeptides, modulate receptor polypeptide activity and/or modulate receptor polypeptide interactions with other binding partners. In various embodiments, mice as described herein are used to identify, screen and/or develop candidate therapeutics (e.g., antibodies, siRNA, etc.) that bind one or more polypeptides of interest. In various embodiments, mice as described herein are used to screen and develop candidate therapeutics (e.g., antibodies, siRNA, etc.) that block activity of one or more polypeptides of interest or that block the activity of one or more receptor polypeptides of interest. In various embodiments, mice as described herein are used to determine the binding profile of antagonists and/or agonists of one or more polypeptides of interest. In some embodiments, mice as described herein are used to determine the epitope or epitopes of one or more candidate therapeutic antibodies that bind one or more polypeptides of interest.

[0307] In various embodiments, mice as described herein are used to determine the pharmacokinetic profiles of one or more human antibody candidates. In various embodiments, one or more mice as described herein and one or more control or reference mice are each exposed to one or more human antibody candidates at various doses (e.g., 0.1 mg/kg,

0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/mg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, or 50 mg/kg or more). Candidate therapeutic antibodies may be dosed via any desired route of administration including parenteral and non-parenteral routes of administration. Parenteral routes include, e.g., intravenous, intraarterial, intraportal, intramuscular, subcutaneous, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intracranial, intrapleural or other routes of injection. Non-parenteral routes include, e.g., oral, nasal, transdermal, pulmonary, rectal, buccal, vaginal, ocular. Administration may also be by continuous infusion, local administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection. Blood is isolated from mice (humanized and control) at various time points (e.g., 0 hr, 6 hr, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, or up to 30 or more days). Various assays may be performed to determine the pharmacokinetic profiles of administered candidate therapeutic antibodies using samples obtained from mice as described herein including, but not limited to, total IgG, anti-therapeutic antibody response, agglutination, etc.

[0308] In various embodiments, mice as described herein are used to measure the therapeutic effect of blocking or modulating the activity of a polypeptide of interest and the effect on gene expression as a result of cellular changes or, in the context of a receptor polypeptide, the density of a receptor polypeptide on the surface of cells in the mice. In various embodiments, a non-human animal as described herein or cells isolated therefrom are exposed to a candidate therapeutic that binds a polypeptide of interest and, after a subsequent period of time, analyzed for effects on specific cellular processes that are associated with said polypeptide of interest, for example, ligand-receptor interactions or signal transduction.

[0309] Mice as described herein express human antibody variable regions, thus cells, cell lines, and cell cultures can be generated to serve as a source of human antibody variable regions for use in binding and functional assays, e.g., to assay for binding or function of an antagonist or agonist, particularly where the antagonist or agonist is specific for a human antigen of interest or specific for an epitope that functions in ligand-receptor interaction (binding). In various embodiments, epitopes bound by candidate therapeutic antibodies or siRNAs can be determined using cells isolated from mice as described herein.

[0310] Cells from provided mice can be isolated and used on an ad hoc basis, or can be maintained in culture for many generations. In various embodiments, cells from a provided mouse are immortalized (e.g., via use of a virus) and maintained in culture indefinitely (e.g., in serial cultures).

[0311] In some embodiments, a mouse cell is a mouse lymphocyte. In some embodiments, a mouse cell is selected from a B cell, dendritic cell, macrophage, monocyte and a T cell. In some embodiments, a mouse cell is an immature B cell, a mature naive B cell, an activated B cell, a memory B cell, and/or a plasma cell.

[0312] In some embodiments, a mouse cell is a non-human embryonic stem (ES) cell. In some embodiments, a mouse ES cell is a mouse ES cell. In some certain embodiments, a mouse ES cell is from a 129 strain, C57BL strain, BALB/c or a mixture thereof. In some certain embodiments, a a mouse embryonic stem cell is a mixture of 129 and C57BL strains. In some certain embodiments, a a mouse embryonic stem cell is a mixture of 129, C57BL and BALB/c strains.

[0313] In some embodiments, use of a mouse ES cell as described herein to make a mouse is provided. In some certain embodiments, a mouse ES cell is used to make a mouse comprising engineered immunoglobulin κ light chain locus as described herein.

[0314] In some embodiments, a mouse tissue is selected from adipose, bladder, brain, breast, bone marrow, eye, heart, intestine, kidney, liver, lung, lymph node, muscle, pancreas, plasma, serum, skin, spleen, stomach, thymus, testis, ovum, and a combination thereof.

[0315] In some embodiments, an immortalized cell made, generated, produced or obtained from an isolated mouse cell or tissue as described herein is described.

[0316] In some embodiments, a mouse embryo made, generated, produced, or obtained from a mouse ES cell as described herein is described.

[0317] Mice as described herein provide an *in vivo* system for the generation of variants of human antibody variable regions that binds a polypeptide of interest (e.g., human Vλ domain variants). Such variants include human antibody variable regions having a desired functionality, specificity, low cross-reactivity to a common epitope shared by two or more variants of a polypeptide of interest. In some embodiments, mice as described herein are employed to generate panels of human antibody variable regions that contain a series of variant variable regions that are screened for a desired or improved functionality.

[0318] Mice as described herein provide an *in vivo* system for generating human antibody variable region libraries (e.g., a human Vλ domain library). Such libraries provide a source for heavy and/or light chain variable region sequences that may be grafted onto different Fc regions based on a desired effector function, used as a source for affinity maturation of the variable region sequence using techniques known in the art (e.g., site-directed mutagenesis, error-prone PCR, etc.) and/or used as a source of antibody components for the generation of antibody-based therapeutic molecules such as, for example, chimeric antigen receptors (i.e., a molecule engineered using antibody components, e.g., an scFv), multi-specific binding agents (e.g., bi-specific binding agents) and fusion proteins (e.g., single domain antibodies, scFvs, etc.).

[0319] In some embodiments, a method of producing an antibody in a mouse is provided, the method comprising the steps of (a) immunizing a mouse as described herein with an antigen of interest; (b) maintaining the mouse under conditions sufficient that the mouse produces an immune response to the antigen of interest; and (c) recovering an antibody from the mouse, or a mouse cell, that binds the antigen of interest.

[0320] In some embodiments of a method of producing an antibody in a mouse, a mouse cell is a B cell. In some embodiments of a method of producing an antibody in a mouse, a mouse cell is a hybridoma.

[0321] In some embodiments, a mouse is employed whose germline genome comprises a homozygous endogenous immunoglobulin κ light chain locus comprising (i) humanVλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3, Vλ3-1 or any combination thereof, (ii) human J Jλ1, Jλ2, Jλ3, Jλ6, Jλ7 or any combination thereof, and (iii) a mouse Cλ gene, wherein (i)-(iii) are operably linked to each other, the mouse Cλ gene is inserted in the place of a mouse Cκ gene of the endogenous immunoglobulin κ light chain locus, the human Vλ gene segment(s) include human non-coding DNA that naturally appears adjacent to the corresponding human Vλ gene segments in an endogenous human λ light chain locus, and the human Jλ gene segment(s) include human non-coding DNA that naturally appears adjacent to the corresponding human Vλ gene segments in an endogenous human λ light chain locus.In some certain embodiments of an employed mouse, a mouse Cλ gene is or comprises a mouse Cλ1 gene. In some certain embodiments of an employed mouse, a human Cλ gene is or comprises a human Cλ2 gene. In some certain embodiments of an employed mouse, the endogenous immunoglobulin κ light chain locus further comprises non-human immunoglobulin κ light chain enhancers Eκi and 3' Eκ. In some certain embodiments of an employed mouse, the endogenous immunoglobulin κ light chain locus includes a deletion of mouse Vκ and Jκ gene segments.

[0322] Also disclosed is an antibody prepared by a method, the method comprising the steps of: (a) providing a mouse as described herein; (b) immunizing the mouse with an antigen of interest; (c) maintaining the mouse under conditions sufficient that the mouse produces an immune response to the antigen of interest; and (d) recovering an antibody from the mouse, or a mouse cell, that binds the antigen of interest, wherein the antibody of (d) includes human V_H and Vλ domains.

[0323] In some instances of an antibody prepared by a method, a human V_H domain encoded by a rearranged human heavy chain variable region comprising a human V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2 V_H6-1, or somatically hypermutated variant thereof.

[0324] In some instances of an antibody prepared by a method, a human Vλ domain encoded by a rearranged human λ light chain variable region comprising a human Vλ4-69, Vλ8-61, Vλ4-60, Vλ6-57, Vλ10-54, Vλ5-52, Vλ1-51, Vλ9-49, Vλ1-47, Vλ7-46, Vλ5-45, Vλ1-44, Vλ7-43, Vλ1-40, Vλ5-39, Vλ5-37, Vλ1-36, Vλ3-27, Vλ3-25, Vλ2-23, Vλ3-22, Vλ3-21, Vλ3-19, Vλ2-18, Vλ3-16, Vλ2-14, Vλ3-12, Vλ2-11, Vλ3-10, Vλ3-9, Vλ2-8, Vλ4-3 Vλ3-1, or somatically hypermutated variant thereof.

[0325] Also disclosed is a mouse, mouse cell or mouse tissue as described herein for use in the manufacture and/or development of a drug (e.g., an antibody or fragment thereof)

for therapy or diagnosis.

[0326] I Also disclosed is a mouse, mouse cell or mouse tissue as described herein for use in the manufacture of a medicament for the treatment, prevention or amelioration of a disease, disorder or condition.

[0327] Also disclosed is use of a mouse, mouse cell or mouse tissue as described herein in the manufacture and/or development of a drug or vaccine for use in medicine, such as use as a medicament.

[0328] Also disclosed is use of a mouse or cell as described herein in the manufacture and/or development of an antibody or fragment thereof.

[0329] Mice as described herein provide an *in vivo* system for the analysis and testing of a drug or vaccine. In various embodiments, a candidate drug or vaccine may be delivered to one or more mice as described herein, followed by monitoring of the mice to determine one or more of the immune response to the drug or vaccine, the safety profile of the drug or vaccine, or the effect on a disease or condition and/or one or more symptoms of a disease or condition. Exemplary methods used to determine the safety profile include measurements of toxicity, optimal dose concentration, antibody (i.e., anti-drug) response, efficacy of the drug or vaccine and possible risk factors. Such drugs or vaccines may be improved and/or developed in such mice.

[0330] Vaccine efficacy may be determined in a number of ways. Briefly, mice as described herein are vaccinated using methods known in the art and then challenged with a vaccine or a vaccine is administered to already-infected mice. The response of a mouse or mice to a vaccine may be measured by monitoring of, and/or performing one or more assays on, the mice (or cells isolated therefrom) to determine the efficacy of the vaccine. The response of a mouse or mice to the vaccine is then compared with control animals, using one or more measures known in the art and/or described herein.

[0331] Vaccine efficacy may further be determined by viral neutralization assays. Briefly, mice as described herein are immunized and serum is collected on various days post-immunization. Serial dilutions of serum are pre-incubated with a virus during which time antibodies in the serum that are specific for the virus will bind to it. The virus/serum mixture is then added to permissive cells to determine infectivity by a plaque assay or microneutralization assay. If antibodies in the serum neutralize the virus, there are fewer plaques or lower relative luciferase units compared to a control group.

[0332] Mice as described herein produce human antibody variable regions and, therefore, provide an *in vivo* system for the production of human antibodies for use in diagnostic applications (e.g., immunology, serology, microbiology, cellular pathology, etc.). In various embodiments, mice as described herein may be used to produce human antibody variable regions that bind relevant antigenic sites for identification of cellular changes such as, for example, expression of specific cell surface markers indicative of pathological changes. Such antibodies can be conjugated to various chemical entities (e.g., a radioactive tracer) and be employed in various *in vivo* and/or *in vitro* assays as desired.

[0333] Mice as described herein provide an improved *in vivo* system for development and selection of human antibodies for use in oncology and/or infectious diseases. In various embodiments, mice as described herein and control mice (e.g., having a genetic modification that is different than as described herein or no genetic modification, i.e., wild-type) may be implanted with a tumor (or tumor cells) or infected with a virus (e.g., influenza, HIV, HCV, HPV, etc.). Following implantation of infection, mice may be administered a candidate therapeutic. The tumor or virus may be allowed sufficient time to be established in one or more locations within the mice prior to administration of a candidate therapeutic. Alternatively, and/or additionally, the immune response may be monitored in such mice so as to characterize and select potential human antibodies that may be developed as a therapeutic.

Pharmaceutical Compositions

[0334] An antibody, a nucleic acid, or a therapeutically relevant portion thereof produced by a mouse disclosed herein or derived from an antibody, a nucleic acid, or a therapeutically relevant portion thereof produced by a mouse disclosed herein can be administered to a subject (e.g., a human subject). A pharmaceutical composition may include an antibody produced by a mouse disclosed herein. A pharmaceutical composition can include a buffer, a diluent, an excipient, or any combination thereof. A composition, if desired, can also contain one or more additional therapeutically active substances.

[0335] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions that are suitable for ethical administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation.

[0336] For example, a pharmaceutical composition described herein may be in a sterile injectable form (e.g., a form that is suitable for subcutaneous injection or intravenous infusion). For example, a pharmaceutical compositionmay be in a liquid dosage form that is suitable for injection. In some instances, a pharmaceutical composition is provided as powders (e.g., lyophilized and/or sterilized), optionally under vacuum, which can be reconstituted with an aqueous diluent (e.g., water, buffer, salt solution, etc.) prior to injection. In some instances, a pharmaceutical compositions is diluted and/or reconstituted in water, sodium chloride solution, sodium acetate solution, benzyl alcohol solution, phosphate buffered saline, etc. In some instance, a powder should be mixed gently with the aqueous diluent (e.g., not shaken).

[0337] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a diluent or another excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0338] In some instances, a pharmaceutical composition including an antibody produced by a non-human animal disclosed herein can be included in a container for storage or administration, for example, an vial, a syringe (e.g., an IV syringe), or a bag (e.g., an IV bag). A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a *"unit dose"* is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0339] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0340] A pharmaceutical composition may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, it may be used.

[0341] In some instances, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some instances, an excipient is approved for use in humans and for veterinary use. In some instances, an excipient is approved by the United States Food and Drug Administration. In some instances, an excipient is pharmaceutical grade. In some instances, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[0342] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical formulations. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

[0343] In some instances, a described pharmaceutical composition comprises one or more pharmaceutically acceptable excipients (e.g., preservative, inert diluent, dispersing agent, surface active agent and/or emulsifier, buffering agent, etc.). In some instances, a pharmaceutical composition comprises one or more preservatives. In some instances, pharmaceutical compositions comprise no preservative.

[0344] In some instances, a pharmaceutical composition is in a form that can be refrigerated and/or frozen. In some instances, a pharmaceutical composition is in a form that cannot be refrigerated and/or frozen. In some instances, reconstituted solutions and/or liquid dosage forms may be stored for a certain period of time after reconstitution (e.g., 2 hours, 12 hours, 24 hours, 2 days, 5 days, 7 days, 10 days, 2 weeks, a month, two months, or longer). In some instances, storage of antibody compositions for longer than the specified time results in antibody degradation.

[0345] Liquid dosage forms and/or reconstituted solutions may comprise particulate matter and/or discoloration prior to administration. In some instances, a solution should not be used if discolored or cloudy and/or if particulate matter remains after filtration.

[0346] General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005.

Kits

[0347] Also described is a pack or kit comprising one or more containers filled with at least one mouse, mouse cell, DNA fragment, targeting vector, or any combination thereof, as described herein. Kits may be used in any applicable method (e.g., a research method). Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, and/or (c) a contract that governs the transfer of materials and/or biological products (e.g., a mouse or mouse cell as described herein) between two or more entities and combinations thereof.

[0348] In some instances, a kit comprising a mouse, mouse cell, mouse tissue, immortalized cell, mouse ES cell, or mouse embryo as described herein is described. Also disclosed is a kit comprising an amino acid (e.g., an antibody or fragment thereof) from a mouse, mouse cell, mouse tissue, immortalized cell, mouse ES cell, mouse embryo as described herein is disclosed. In some embodiments, a kit comprising a nucleic acid (e.g., a nucleic acid encoding an antibody or fragment thereof) from a mouse, mouse cell, mouse tissue, immortalized cell, mouse ES cell, or mouse embryo as described hereinAlso disclosed is a kit comprising a sequence (amino acid and/or nucleic acid sequence) identified from a mouse, mouse cell, mouse tissue, immortalized cell, mouse ES cell, or mouse embryo as described herein.

[0349] Also disclosed is a kit as described herein for use in the manufacture and/or development of a drug (e.g., an antibody or fragment thereof) for therapy or diagnosis.

[0350] Also disclosed is a kit as described herein for use in the manufacture and/or development of a drug (e.g., an antibody or fragment thereof) for the treatment, prevention or amelioration of a disease, disorder or condition.

[0351] Other features of certain embodiments will become apparent in the course of the following descriptions of exemplary embodiments, which are given for illustration and are not intended to be limiting thereof.

EXAMPLES

[0352] The following examples are provided so as to describe to the skilled artisan how to make and use methods and compositions described herein, and are not intended to limit the scope of what the inventors of the present disclosure regard as their invention. Unless indicated otherwise, temperature is indicated in Celsius and pressure is at or near atmospheric.

Example 1. Construction of a targeting vectors for generating a mouse expressing at least one lambda light chain from a kappa light chain locus

Example 1.1. Engineering a targeting vector comprising a mouse lambda constant region

[0353] This example illustrates exemplary methods of constructing a targeting vector for insertion into the genome of a mouse. Furthermore, this example demonstrates production of a mouse whose germline genome comprises an engineered immunoglobulin κ light chain locus. In particular, this example demonstrates construction of a targeting vector for engineering an endogenous immunoglobulin κ light chain locus in a mouse so that the mouse expresses and/or produces antibodies that include immunoglobulin λ light chains having human variable regions and mouse immunoglobulin λ constant (Cλ) regions from said immunoglobulin κ light chain locus in the germline genome of the mouse. As described below in Example 2, DNA fragments containing multiple human Jλ (e.g., Jλ1, Jλ2, Jλ3, Jλ6 and Jλ7) coding sequences and a mouse Cλ (e.g., a mouse Cλ1) coding sequence are inserted into an endogenous mouse immunoglobulin κ light chain locus. In particular exemplified embodiments, a mouse Cλ1 gene is inserted in the place of a mouse Cκ gene and in operable linkage with mouse Igκ enhancers (e.g., Eκi and 3'Eκ). An exemplary strategy for creation of a targeting vector for generating an engineered immunoglobulin κ light chain locus in a mouse characterized by the presence of a plurality of human Vλ and Jλ gene segments operably linked to a mouse Cλ gene and operably linked to endogenous Igκ enhancers is set forth in Figure 1 (Figure 1A: initial steps of construction of targeting vector; Figure 1B: additional subsequent steps of construction of a targeting vector; a human immunoglobulin κ light chain sequence between human Vλ and Jλ gene segments is indicated by an open bar filed with wide downward diagonal lines (e.g., see U.S. Patent Nos. 9,006,511, 9,035,128, 9,066,502, 9,150,662 and 9,163,092), lox: *lox*2372; NEO: Neomycin resistance gene (neo^R) under transcriptional control of a ubiquitin promoter, HYG: Hygromycin resistance gene (hyg^R) under transcriptional control of a ubiquitin promoter, Spec: Spectinomycin resistance gene ($Spec^R$), R6K: R6K origin of replication).

[0354] A targeting vector containing human Jλ and mouse Cλ coding sequences for insertion into a mouse immunoglobulin κ light chain locus was created using VELOCIGENE® technology (see, e.g., U.S. Patent No. 6,586,251 and Valenzuela et al., 2003, Nature Biotech. 21(6):652-9) and molecular biology techniques known in the art. Those of ordinary skill, reading the present example, will appreciate that the described technologies and approach can be employed to utilize any human Jλ and any Cλ coding sequences, or combination of coding sequences (or sequence fragments) as desired.

[0355] Briefly, a 2.7kb DNA fragment containing human Jλ gene segments Jλ1, Jλ2, Jλ3, Jλ6 and Jλ7 and unique 5' and 3' overlap regions corresponding to a human Vκ-Jκ genomic (non-coding) sequence and a genomic sequence 5' of a mouse Cκ gene, respectively, was made by *de novo* DNA synthesis (pA; Figure 1A, top left, Blue Heron Biotech, Bothell, WA). Various restriction enzyme recognition sites were included in the DNA fragment to facilitate subsequent cloning of selection markers and other DNA fragments (described below). The DNA fragment was uniquely designed to contain non-coding human Jκ sequences juxtaposed with human Jλ coding sequences and human Jλ recombination signal sequences (RSS). As in known in the art, an RSS consists of a conserved block of seven nucleotides (heptamer) followed by a spacer either 12 or 23 base pairs in length and followed by a second conserved block of nine nucleotides (nonamer). Thus, an RSS has a configuration of 7-12-9 (12RSS) or 7-23-9 (23RSS) depending on the

associated gene segment (see, e.g., Figure 5.4 in Murphy, Kenneth, et al. "Chapter 5." Janeway's Immunobiology, 8th ed., Garland Science/Taylor & Francis Group, LLC, 2012). In particular, human Jλ gene segments (i.e., human Jλ coding sequences) and their associated 12RSS were substituted in the place of human Jκ gene segments (i.e., human Jκ coding sequences) and their associated 23RSS. Thus, this fragment contained human Jλ and Jκ DNA sequences. Inclusion of such sequences in targeting vectors described herein can provide for (or promote) efficient joining of human Vλ and Jλ gene segments within an engineered mouse Igκ light chain locus.

[0356] Plasmid A (pA) was digested with AgeI and EcoRI and ligated to a Neomycin selection cassette (i.e., a Neo^R gene under control of a ubiquitin promoter flanked by *lox2372* sites) containing compatible ends to create plasmid B (pB) (Figure 1A). Separately, unique DNA fragments containing a mouse Igκ intronic enhancer (Ei), a mouse Cλ1 gene (from BAC clone RP23-60e14), a DNA fragment containing 316bp of sequence immediately downstream of a mouse Cκ coding sequence and 80bp of overlap sequence to facilitate isothermal assembly, and restriction enzyme recognition sites (NotI, MluI) to facilitate subsequent cloning steps, and an R6K-Spec (a Spectinomycin Adenylytransferase gene and a R6K origin of replication) were amplified by polymerase chain reaction (PCR) and combined together by isothermal assembly (see, e.g., Gibson, D.G. et.al., 2009, Nat. Meth. 6(5):343-5; Gibson, D.G. et al., 2010, Nat. Meth. 7:901-903) to create plasmid C (pC), which was subsequently digested with NotI and MluI and ligated to a Hygromycin selection cassette (i.e., a Hyg^R gene under control of a ubiquitin promoter flanked by *loxP* sites) containing compatible ends to create plasmid D (pD; Figure 1A, top and middle right). This resulting plasmid (pD; Figure 1A, middle) was then digested with PI-SceI and AscI and ligated with plasmid B (pB) containing compatible ends (Figure 1A, bottom) to generate plasmid E (pE).

[0357] In a next step, a targeting vector containing human Vλ and Jλ gene segments operably linked to a mouse Cκ gene and a human immunoglobulin κ light chain sequence positioned between the human Vλ and Jλ gene segments (see, e.g., U.S. Patent No. 9,006,511) was separately digested with NotI and religated to remove the human Vλ region including a human immunoglobulin κ sequence (Figure 1B, top), which resulted in a deletion of ~137kb. The resulting construct (construct F) was combined with plasmid E (pE) using a CRISPR/Cas9 isothermal assembly method (see, e.g., U.S. Patent No. 9,738,897, and U.S. Publication No. 2016/0145646) so that the human Jκ region with human Jλ-12RSS coding sequence (CDS) was operably linked with a human Vκ-Jκ intergenic (non-coding) region (see, e.g., U.S. Patent No. 9,006,511) and a mouse Igκ 3' enhancer (Figure 1B). Positive bacterial clones were selected on media containing Kanamycin, Hygromycin and Spectinomycin. The resulting targeting vector (construct G) contained, from 5' to 3', a *loxP* recognition site, a NotI site, a human Vκ-Jκ intergenic sequence (see, e.g., U.S. Patent No. 9,006,511), a Neomycin selection cassette flanked by *lox2372* recognition sites, a human Jκ region with five human Jλ gene segments and their respective 12RSS, a mouse immunoglobulin κ intronic enhancer (Eiκ), a mouse Cλ1 gene, a Hygromycin selection cassette flanked by *loxP* recognition sites, a mouse immunoglobulin κ 3' enhancer (3' Eκ) and a Spectinomycin selection cassette (Figure 1B).

Example 1.2. Engineering a targeting vector comprising a human lambda constant region

[0358] This example illustrates exemplary methods of constructing a targeting vector for insertion into the genome of a mouse. Futhermore, this example demonstrates production of a mouse whose germline genome comprises an engineered immunoglobulin κ light chain locus. In particular, this example demonstrates construction of a targeting vector for engineering an endogenous immunoglobulin κ light chain locus in a mouse so that the mouse expresses and/or produces antibodies that include immunoglobulin λ light chains having human variable regions and human immunoglobulin λ constant (Cλ) regions from said immunoglobulin κ light chain locus in the germline genome of the mouse. As described below in Example 2, DNA fragments containing multiple human Jλ (e.g., Jλ1, Jλ2, Jλ3, Jλ6 and Jλ7) coding sequences and a human Cλ (e.g., a human Cλ2) coding sequence are inserted into an endogenous mouse immunoglobulin κ light chain locus. In particular, a human Cλ2 gene is inserted in the place of a mouse Cκ gene and in operable linkage with mouse immunoglobulin κ enhancers (e.g., Eiκ and 3'Eκ). An exemplary strategy for creation of a targeting vector is set forth in Figure 3.

[0359] A targeting vector containing human Jλ and human Cλ coding sequences for insertion into a mouse Igκ light chain locus was created using VELOCIGENE® technology (see, e.g., U.S. Patent No. 6,586,251 and Valenzuela et al., 2003, Nature Biotech. 21(6):652-9) and molecular biology techniques known in the art. Those of ordinary skill, reading the present example, will appreciate that the described approach and technologies can be employed to utilize any human Jλ and Cλ coding sequences, or combination of coding sequences (or sequence fragments) as desired.

[0360] Briefly, an 871bp DNA fragment containing a human Cλ coding sequence and unique 5' and 3' overlap regions corresponding to genomic sequences 5' and 3' of a mouse Cκ gene, respectively, was made by *de novo* DNA synthesis (pH; Figure 3, top left, Blue Heron Biotech, Bothell, WA). Various restriction enzyme recognition sites were included in the DNA fragment to allow for subsequent cloning of selection markers and other DNA fragments (described below). Plasmid H (pH) was digested with AgeI and XhoI and ligated to a Hygromycin selection cassette (i.e., a Hyg^R gene under control of a ubiquitin promoter flanked by *loxP* sites) containing compatible ends to create plasmid J (pJ; Figure 3). An intermediate construct (construct K, generated from construct F and plasmid B using Cas9 and isothermal assembly) containing an engineered human Jκ region with human Jλ coding sequences (described above) operably linked to a mouse Cκ gene and mouse Igκ enhancers was combined with plasmid J using a CRISPR/Cas9 isothermal assembly method (see, e.g., U.S. Patent No. 9,738,897, and U.S. Publication No. 2016/0145646) so that the human Jκ region with human Jλ-12RSS coding sequence (CDS) was operably linked with the human Cλ2 coding sequence of plasmid J (Figure 3). Positive bacterial clones were selected on media containing Kanamycin, Hygromycin and Spectinomycin. The resulting targeting vector (construct L) contained, from 5' to 3', a *loxP* recognition site, a NotI site, a human Vκ-Jκ intergenic sequence (see, e.g., U.S. Patent No. 9,006,511), a Neomycin selection cassette flanked by *lox*2372 recognition sites, a human Jκ region with five human Jλ gene segments and their respective 12RSS, a mouse Igκ intronic enhancer (Eiκ), a human Cλ2 gene, a Hygromycin selection cassette flanked by *loxP* recognition sites, a mouse immunoglobulin κ 3' enhancer (3' Eκ) and a Spectinomycin selection cassette (Figure 3).

Example 2. Generation of mice having an engineered light chain locus

[0361] This example demonstrates production of mice whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising insertion of a plurality of human Vλ and Jλ gene segments and a mouse Cλ gene, which human Vλ and Jλ gene segments are operably linked to said mouse Cλ gene, and which mouse Cλ gene is inserted in the place of a mouse Cκ gene of an endogenous immunoglobulin κ light chain locus. Such mice are characterized, in some embodiments, by expression of immunoglobulin λ light chains (variable and constant domains) from an endogenous immunoglobulin κ light chain locus.

[0362] Targeted insertion of targeting vectors described in Examples 1.1 and 1.2 were confirmed by polymerase chain reaction. Targeted BAC DNA, confirmed by polymerase chain reaction, was introduced into F1 hybrid (C57BL6NTac/129S6SvEvTac) mouse embryonic stem (ES) cells via electroporation followed by culturing in selection medium.

[0363] ES cells used for electroporation of construct G (mouse Cλ1) had a germline genome that included a heterozygous immunoglobulin κ light chain locus containing a plurality of human Vλ and Jλ gene segments operably linked to a mouse immunoglobulin κ light chain constant region gene including mouse immunoglobulin κ light chain enhancers, and a human immunoglobulin κ light chain sequence positioned between the human Vλ and Jλ gene segments and one wild-type mouse immunoglobulin κ light chain locus. ES cells before and after electroporation are depicted in Figure 2A (1741HET: a mouse ES cell clone having a genome heterozygous for an engineered immunoglobulin κ light chain locus containing human Vλ and Jλ gene segments operably linked to a mouse immunoglobulin κ light chain constant region gene including mouse immunoglobulin κ light chain enhancers, and a human immunoglobulin κ light chain sequence positioned between the human Vλ and Jλ gene segments indicated by an open bar filed with wide downward diagonal lines, and wild-type immunoglobulin heavy and λ light chain loci, e.g., see U.S. Patent Nos. 9,006,511, 9,035,128, 9,066,502, 9,150,662 and 9,163,092; 6557HET: a mouse ES cell clone after insertion of construct G resulting in a genome heterozygous for an engineered immunoglobulin κ light chain locus including mouse immunoglobulin κ light chain enhancers, which engineered immunoglobulin κ light chain locus is characterized by the presence of a plurality of human Vλ and Jλ gene segments, which human Jλ gene segments are contained within a human Jκ region sequence with human Jλ gene segment coding sequences and human Jλ 12RSS in place of the corresponding human Jκ gene segment coding sequences and human Jκ 23RSS, and which human Vλ and Jλ gene segments are operably linked to a mouse immunoglobulin λ light chain constant region gene (e.g., mCλ1); lox: *lox*2372; NEO: Neomycin resistance gene (neo^R) under transcriptional control of a ubiquitin promoter; HYG: Hygromycin resistance gene (hyg^R) under transcriptional control of a ubiquitin promoter; locations of selected primer/probe sets for screening ES cells clones are indicated near the locations of regions within the engineered Igκ light chain locus detected in an assay described below).

[0364] ES cells used for electroporation of construct L (human Cλ2) had a germline genome that included a heterozygous immunoglobulin κ light chain locus containing a plurality of human Vλ and Jλ gene segments operably linked to a mouse immunoglobulin κ light chain constant region gene including mouse immunoglobulin κ light chain enhancers, and a

human immunoglobulin κ light chain sequence positioned between the human Vλ and Jλ gene segments and one wild-type mouse Igκ locus. ES cells before and after electroporation are depicted in Figure 4A (1741HET: *supra;* 20029HET: a mouse ES cell clone after insertion of a targeting vector having a genome heterozygous for an engineered Igκ light chain locus including mouse immunoglobulin κ light chain enhancers, which engineered Igκ light chain locus is characterized by the presence of a plurality of human Vλ and Jλ gene segments, which human Jλ gene segments are contained within a human Jκ region sequence with human Jλ gene segment coding sequences and human Jλ 12RSS in place of the corresponding human Jκ gene segment coding sequences and human Jκ 23RSS, and which human Vλ and Jλ gene segments are operably linked to a human Igλ light chain constant region gene (e.g., hCλ2); lox: *lox*2372; NEO: Neomycin resistance gene (neo^R) under transcriptional control of a ubiquitin promoter; HYG: Hygromycin resistance gene (hyg^R) under transcriptional control of a ubiquitin promoter; locations of selected primer/probe sets for screening ES cells clones are indicated near the locations of regions within the engineered Igκ light chain locus detected in an assay described below).

[0365] Drug-resistant colonies were picked 10 days after electroporation and screened by TAQMAN™ and karyotyping for correct targeting as previously described (Valenzuela et al., *supra;* Frendewey, D. et al., 2010, Methods Enzymol. 476:295-307;). Table 1 sets forth exemplary primers/probes sets used for screening positive ES cell clones (F: forward; R: reverse; P: probe; GOA: gain of allele; LOA: loss of allele; WT: wild-type).

[0366] The VELOCIMOUSE® method (DeChiara, T.M. et al., 2010, Methods Enzymol. 476:285-294; DeChiara, T.M., 2009, Methods Mol. Biol. 530:311-324; Poueymirou et al., 2007, Nat. Biotechnol. 25:91-99;), in which targeted ES cells were injected into uncompacted 8-cell stage Swiss Webster embryos, was used to produce healthy fully ES cell-derived F0 generation mice heterozygous for the engineered Igκ light chain locus (Figure 2A and 4A). F0 generation heterozygous mice were crossed with C57Bl6/NTac mice to generate F1 heterozygotes that were intercrossed to produce homozygous F2 generation animals for phenotypic analyses.

[0367] Alternatively, murine ES cells bearing an engineered immunoglobulin κ locus as described above can be modified to remove one or more selection cassettes introduced with a targeting vector as desired (Figure 2B: 6557HET: *supra;* 6558HET: a mouse ES cell clone after recombinase-mediated excision of Neomycin and Hygromycin selection cassettes inserted after homologous recombination with a targeting vector; Figure 4B: 20029HET: *supra;* 20030HET: a mouse ES cell clone after recombinase-mediated excision of Neomycin and Hygromycin selection cassettes inserted after homologous recombination with a targeting vector. Cre: Cre recombinase). For example, the Neomycin and Hygromycin cassette introduced by the targeting vectors may be removed in engineered ES cells (or embryos) by transient recombinase expression or by breeding to a recombinase-expressing genetically engineered strain (see e.g., Lakso, M. et al., 1992, Proc. Natl. Acad. Sci. USA 89: 6232-6; Orban, P.C. et al., 1992, Proc. Natl. Acad. Sci. U.S.A. 89:6861-5; Gu, H. et al., 1993, Cell 73(6):1155-64; Araki, K. et al., 1995, Proc. Natl. Acad. Sci. U.S.A. 92:160-4; Dymecki, S.M., 1996, Proc. Natl. Acad. Sci. U.S.A. 93(12):6191-6).

[0368] Taken together, this example illustrates the generation of a mouse) whose germline genome comprises an engineered Igκ light chain locus characterized by the presence of a plurality of human Vλ and Jλ gene segments operably linked to a mouse Cλ gene, which mouse Cλ gene is inserted in the place of a mouse Cκ gene of an endogenous Igκ light chain locus. An engineered Igκ light chain locus as described includes plurality of human Vλ and Jλ gene segments in a non-endogenous arrangement. The strategy described herein for inserting human Vλ and Jλ gene segments, and a mouse Cλ gene into the place of a mouse Cκ gene, enables the construction of a mouse that expresses antibodies that exclusively contain human Vλ domains. It was unclear if such an engineered Igκ light chain locus that includes exclusively λ gene segments (outside of the endogenous λ locus, in a non-endogenous orientation) would be able to produce functional light chains. As described herein, such human Vλ domains are expressed from endogenous Igκ light chain loci in the germline genome of provided mice.

Table 1. Representative primer/probe sets for screening positive ES cell clones

Name		Sequence (5'-3')	Assay
mIgKC-1	F	GTGGAAGATTGATGGCAGTGAAC (SEQ ID NO:25)	LOA
	R	GTGCTGCTCATGCTGTAGGT (SEQ ID NO:26)	
	P	AAATGGCGTCCTGAACAGTTGGACTGA (SEQ ID NO:27)	
mIgKC-2	F	CCATCCAGTGAGCAGTTAACATC (SEQ ID NO:28)	LOA
	R	TGTCGTTCACTGCCATCAATC (SEQ ID NO:29)	
	P	AGGTGCCTCAGTCGTGTGCTTC (SEQ ID NO:30)	
mIgKLC1-1	F	GGAGCCCTTCCTTGTTACTTCA (SEQ ID NO:31)	GOA-6557
	R	AGGTGGAAACAGGGTGACTGATG (SEQ ID NO:32)	
	P	TCCTCTGTGCTTCCTTCCTCAGGC (SEQ ID NO:33)	
mIgKLC1-2	F	TCCTTGTTACTTCATACCATCCTCT (SEQ ID NO:34)	GOA-6557
	R	AGGGTGACTGATGGCGAAGACT (SEQ ID NO:35)	
	P	TTCCTTCCTCAGGCCAGCCC (SEQ ID NO:36)	
hIgKLJ-1	F	GAGGCTTGCTGAGCTTTCAG (SEQ ID NO:37)	GOA
	R	AGGACGGTCAGCTTGGTC (SEQ ID NO:38)	
	P	TATGAGCCTGTGTCACAGTGTTGGG (SEQ ID NO:39)	
hIgKLJ-2	F	GCTGACCCAGGACTCTGTTC (SEQ ID NO:40)	GOA
	R	TCCCAGTTCCGAAGACATAACAC (SEQ ID NO:41)	
	P	CCCTTTGGTGAGAAGGGTTTTGGTC (SEQ ID NO:42)	
hIgLC2-1	F	TACGCGGCCAGCAGCTAT (SEQ ID NO:43)	GOA-20029
	R	TGGCAGCTGTAGCTTCTGT (SEQ ID NO:44)	
	P	CTGAGCCTGACGCCTGAGCAG (SEQ ID NO:45)	
1561hJ1	F	TCAACCTTTCCCAGCCTGTCT (SEQ ID NO:46)	LOA
	R	CCCCAGAGAGAGAAAACAGATTTT (SEQ ID NO:47)	
	P	ACCCTCTGCTGTCCCT (SEQ ID NO:49)	
Neo	F	GGTGGAGAGGCTATTCGGC (SEQ ID NO:50)	GOA
	R	GAACACGGCGGCATCAG (SEQ ID NO:51)	
	P	TGGGCACAACAGACAATCGGCTG (SEQ ID NO:52)	
Hyg	F	TGCGGCCGATCTTAGCC (SEQ ID NO:53)	GOA
	R	TTGACCGATTCCTTGCGG (SEQ ID NO:54)	
	P	ACGAGCGGGTTCGGCCCATTC (SEQ ID NO:55)	
1468h2	F	GGGCTACTTGAGGACCTTGCT (SEQ ID NO:56)	Parental
	R	GACAGCCCTTACAGAGTTTGGAA (SEQ ID NO:57)	
	P	CAGGGCCTCCATCCCAGGCA (SEQ ID NO:58)	
1525hk-VJ1	F	ATCTCCCTACTTCCTGGCTAATG (SEQ ID NO:59)	Parental
	R	GCTTGGAACCTGATTGGTTGTC (SEQ ID NO:60)	
	P	AGCCTTGATCCTTGGGAATCCAGGACA (SEQ ID NO:61)	
mIgKd2	F	GCAAACAAAAACCACTGGCC (SEQ ID NO:62)	WT
	R	GGCCACATTCCATGGGTTC (SEQ ID NO:63)	

Name		Sequence (5'-3')	Assay
	P	CTGTTCCTCTAAAACTGGACTCCACAGTAAATGGAAA (SEQ ID NO:64)	

Example 3. Characterization of mice having an engineered immunoglobulin light chain locus Example 3.1. Phenotypic assessment of immune cells in mice having an engineered immunoglobulin light chain locus

[0369] This example demonstrates the characterization of various immune cell populations in mice (e.g., mice) engineered to contain a plurality of human Vλ and Jλ gene segments operably linked to a mouse Cλ gene, and mouse immunoglobulin κ light chain enhancer and regulatory regions, within an endogenous immunoglobulin κ light chain locus. In particular, this example specifically demonstrates that mice having engineered immunoglobulin κ light chain loci described herein display a unique light chain expression profile as compared to wild-type mice. This example also demonstrates that provided mice express a broad repertoire of human Vλ regions from the engineered immunoglobulin κ light chain locus.

[0370] Briefly, spleens and femurs were harvested from wild-type (WT, 75% C57BL/6NTac 25% 129SvEvTac) and 6558HO (homozygous LiK, 75% C57BL/6NTac 25% 129SvEvTac) mice. Bone marrow was collected from femurs by flushing with 1x phosphate buffered saline (PBS, Gibco) with 2.5% fetal bovine serum (FBS). Red blood cells from spleen and bone marrow preparations were lysed with ACK lysis buffer (Gibco) followed by washing with 1xPBS with 2.5% FBS.

[0371] Isolated cells ($1x10^6$) were incubated with selected antibody cocktails for 30 min at +4°C: anti-mIgκ-FITC (187.1, BD Biosciences), anti-mIgλ-PE (RML-42, BioLegend; 1060-09, Southern Biotech), anti-mIgλ-FITC (106002, Bio-Rad; ABIN303989, Antibodies-online), anti-mouse IgM-PeCy7 (II/41, eBioscience), anti-mouse IgD-PerCP/Cy5.5 (11-26c.2a, BioLegend), anti-mouse CD3-Pacific Blue (17A2, BioLegend), anti-mouse B220-APC (RA3-6B2, eBioscience), anti-mouse CD19-APC-H7 (ID3, BD Biosciences). Following staining, cells were washed and fixed in 2% formaldehyde. Data acquisition was performed on a BD LSRFORTESSA™ flow cytometer and analyzed with FLOWJO™ software. Representative results are set forth in Figures 5-7.

[0372] As shown in Figures 5 and 6, LiK mice demonstrate similar distributions of $CD19^+$ and immature/mature B cells as compared to wild-type mice in the spleen and bone marrow compartments, respectively. However, LiK mice demonstrate a unique light chain expression as compared to wild-type mice in that only $Ig\lambda^+$ expression was observed in these mice (Figure 7). In particular, >90% of $CD19^+$ B cells in LiK mice express immunoglobulin λ light chain thereby confirming proper recombination and expression at the engineered immunoglobulin κ locus. As expected given these mice lack a mouse Cκ gene, LiK mice demonstrate no detectable immunoglobulin κ expression by flow cytometry (i.e., the anti-mIgκ antibody detects the constant region). Similar levels of immunoglobulin λ light chain expression were observed from additional LiK mice littermates (data not shown). Expression of human Vλ regions in the context of a mouse Cλ region from the LiK locus was confirmed by, among other things, immunoglobulin repertoire analysis using Next Generation Sequencing techniques (described in Example 3.2 below).

Example 3.2. Immunoglobulin repertoire in mice having an engineered immunoglobulin light chain locus

[0373] Usage of human antibody genes (i.e., VDJ gene segments) in the engineered mouse strain described above was determined by Next Generation Sequencing antibody repertoire analysis. In particular, RT-PCR sequencing was conducted on RNA isolated from splenocytes of mice homozygous for the LiK locus (6558 HO) to confirm correct transcription and recombination of the LiK locus. A representative illustration of a rearranged LiK locus is set forth in Figure 12 (LiK locus: engineered immunoglobulin κ light chain locus as described herein; rearranged LiK locus: representative rearrangement of engineered immunoglobulin κ light chain locus (referred to herein as "LiK locus") resulting in human Vλ-Jλ recombination; rearranged LiK mRNA: representative transcription and mRNA processing of rearranged LiK locus).

[0374] Briefly, splenic B cells were positively enriched from total splenocytes by magnetic cell sorting using mouse anti-CD19 magnetic beads and MACS® columns (Miltenyi Biotech). Total RNA was isolated from purified splenic B cells using an RNeasy Plus RNA isolation kit (Qiagen) according to manufacturer's specifications. Reverse transcription was performed to generate cDNA containing immunoglobulin λ constant region gene sequence, using a SMARTer™ RACE cDNA Amplification Kit (Clontech) and immunoglobulin λ specific primers (see below). During this process, a DNA sequence, reverse compliment to 3' of a template switching (TS) primer, was attached to the 3' end of newly synthesized cDNAs. Purified Igλ-specific cDNAs were then amplified by a 1^{st} round PCR reaction using the TS specific primer and reverse primers specific to sequences of mouse Cλ1. PCR products ranging from ~450-700bp were isolated using Pippin Prep (SAGE Science) and then these fragments were further amplified by a 2^{nd} round PCR reaction. Table 2 sets forth the sequences of selected primers used for repertoire library construction (for: forward primer; rev: reverse primer). PCR products ranging from ~400bp-700bp were isolated, purified, and quantified by qPCR using a KAPA Library Quantification Kit (KAPA Biosystems) before loading onto a Miseq sequencer (Illumina) for sequencing using Miseq Reagent Kits v3 (2x300 cycles).

[0375] For bioinformatic analysis, Raw Illumina sequences were de-muliplexed and filtered based on quality, length and match to corresponding constant region gene primer. Overlapping paired-end reads were merged and analyzed using custom in-house pipeline. The pipeline used local installation of IgBLAST (NCBI, v2.2.25+) to align rearranged light chain sequences to human germline Vλ and Jλ gene segment database, and denoted productive and non-productive joins along with the presence of stop codons. CDR3 sequences and expected non-template nucleotides were extracted using boundaries as defined in International Immunogenetics Information System (IMGT).

Table 2. Representative primers for repertoire library construction

Primer Name	Sequence (5'-3')
TS primer	CACCATCGAT GTCGACACGC CTAGGG (SEQ ID NO:65)
IgλC (RT primer)	CACCAGTGTG GCCTTGTTAG TCTC (SEQ ID NO:66)
IgλC (1^{st} PCR)	ACACTCTTTC CCTACACGAC GCTCTTCCGA TCTCAGGGTG ACTGATGGCG AAGAC (SEQ ID NO:67)
TS specific (1^{st} PCR)	GTGACTGGAG TTCAGACGTG TGCTCTTCCG ATCTCACCAT CGATGTCGAC ACGCCTA (SEQ ID NO:68)
for (2^{nd} PCR)	AATGATACGG CGACCACCGA GATCTACAC XXXXXX ACACTCTTTC CCTACACGAC GCTCTTCCGA TCT (SEQ ID NO:69)
rev (2^{nd} PCR)	CAAGCAGAAG ACGGCATACG AGAT XXXXXX GTGACTGGAG TTCAGACGTG TGCTCTTCCG ATCT (SEQ ID NO:70)

[0376] The majority of the functional human Vλ and Jλ gene segments included in the LiK locus in engineered mice exemplified herein were represented in the expressed antibody repertoire of LiK mice comprising a plurality of human Vλ and Jλ gene segments operably linked to a mouse Cλ gene at the endogenous kappa locus (data not shown). Overall, the inventors observed that the B cells of LiK mice expressed antibodies having light chains expressed from the LiK locus as expected. No altered splicing products, insertions, deletions or otherwise unexpected mutations were observed in the transcripts analyzed. These results confirm that recombination at the LiK locus generates functional light chains

as part of the antibody repertoire of these mice. Similar analysis was performed in mice comprising a plurality of human Vλ and Jλ gene segments operably linked to a human Cλ gene at the endogenous kappa locus, where the expression of a plurality of human Vλ and Jλ gene segments was detected (data not shown).

Example 3.3. Antibody expression in mice having an engineered light chain locus

[0377] This example demonstrates expression of antibodies (e.g., IgG) from mice, which antibodies contain light chains characterized by the presence of human Vλ regions and mouse an Cλ regions, and which light chains are expressed from an engineered endogenous mouse immunoglobulin κ light chain locus. Among other things, this example specifically demonstrates expression of IgG antibodies (in dimeric and monomeric forms) in the serum of mice whose germline genome comprises an endogenous immunoglobulin κ light chain locus comprising insertion of one or more human Vλ gene segments, one or more human Jλ gene segments and a mouse Cλ gene, which human Vλ and Jλ gene segments are operably linked to said mouse Cλ gene, and which mouse Cλ gene is inserted in the place of a mouse Cκ gene of an endogenous mouse Igκ light chain locus.

[0378] Blood was drawn from wild-type (WT, 75% C57BL/6NTac 25% 129SvEvTac) and 6558 homozygous ("LiK", 75% C57BL/6NTac 25% 129SvEvTac) mice. Serum was separated from blood using Eppendorf tubes centrifuged at 9000 rpm for five minutes at 4°C. Collected serum was used for immunoblotting to identify expression of IgG antibodies.

[0379] Mouse sera were diluted 1:100 or 1:500 in PBS (without $Ca2^{+}$ and $Mg2^{+}$) and run on 4-20% Novex Tris-Glycine gels under reducing and non-reducing conditions. Gels were transferred to Polyvinylidene difluoride (PVDF) membranes according to manufacturer's specifications. Blots were blocked overnight with 5% nonfat milk in Tris-Buffered Saline with 0.05% Tween-20 (TBST, Sigma). PVDF membranes were exposed to primary antibody (goat anti-mIgG1 conjugated to HRP, Southern Biotech) diluted 1: 1000 in 0.1% nonfat milk in TBST for one hour at room temperature. Blots were washed four times for ten minutes per wash and developed for five minutes with Amersham ECL Western Blotting Detection Reagent (GE Healthcare Life Sciences) according to manufacturer's specifications. Blots were then imaged using GE Healthcare ImageQuant LAS-4000 Cooled CCD Camera Gel Documentation System. Images were captured at 15 second intervals until 20 images were captured or images were fully exposed, whichever came first. Representative results are set forth in Figure 13 (lane numbers are indicated at the top of each gel image and lane assignments are the same for both images; top left: reduced samples; bottom left: non-reduced samples; LiK HO: 6558 homozygous; WT: wild-type; molecular weights are indicated on the left of each gel image).

[0380] As shown in Figure 13, the size of IgG antibodies expressed in LiK mice is similar to the size observed for IgG antibodies expressed in wild-type mice, which demonstrates that LiK mice produce functional antibodies that bind antigen and can be used as an *in vivo* system for the production of human antibodies and human antibody components for use in the treatment of human disease(s).

Example 4. Generation and characterization of mice comprising several engineered immunoglobulin loci

[0381] LiK mice as described herein are separately bred with multiple engineered mouse strains over multiple breedings using techniques known in the art to establish mice strains containing the following engineered immunoglobulin loci: (1) a mouse strain homozygous for humanized immunoglobulin heavy chain (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940), homozygous for an immunoglobulin κ light chain locus comprising human Vλ and Jλ gene segments operably linked to a Cλ gene as described herein and homozygous for an inactivated endogenous immunoglobulin λ light chain locus (see, e.g., U.S. Patent No. 9,006,511), in some embodiments referred to herein as HoH/ LiK/$\lambda^{-/-}$ mice, (2) a mouse strain homozygous for a humanized immunoglobulin heavy chain locus (*supra*), homozygous for an inactivated endogenous immunoglobulin λ light chain locus (*supra*), and hemizygous for an immunoglobulin κ light chain locus having a first immunoglobulin κ light chain locus comprising human Vλ and Jλ gene segments operably linked to a Cλ gene as described herein and a second immunoglobulin κ light chain locus comprising human Vκ and Jκ gene segments operably linked to an endogenous mouse Cκ gene (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940), in some embodiments referred to herein as HoH/KoK/LiK/$\lambda^{-/-}$ mice. Alternatively, such mice maybe generated by introducing targeting vectors comprising engineered loci into ES cells already comprising several engineered immunoglobulin loci. In some embodiments, the immunoglobulin heavy chain locus in said mice comprises a functional and expressed mouse Adam6 gene.

[0382] Specifically, LiK mice were bred with multiple engineered mouse strains over multiple breedings to establish HoH/LiK/$\lambda^{-/-}$ and HoH/KoK/LiK/$\lambda^{-/-}$ mice.

[0383] Once established, various immune cell populations were characterized in these humanized mice by flow cytometry. Briefly, spleens and femurs were harvested from HoH/LiK/$\lambda^{-/-}$ (n=3), HoH/KoK/LiK/$\lambda^{-/-}$ (n=4) and VELOCIMMUNE® ("HoH/KoK"; n=3; see U.S. Patent Nos. 8,642,835 and 8,697,940) mice and prepared for flow cytometry analysis as described above. Representative results are set forth in Figures 8-11. Average light chain expression (κ:λ) observed in splenocytes of engineered mouse strains tested was approximately as follows: HoH/LiK/$\lambda^{-/-}$: 0:100, HoH/KoK/LiK/$\lambda^{-/-}$: 40:60, HoH/KoK: 85:15.

Example 5. Production of antibodies in engineered mice

[0384] This example demonstrates production of antibodies in a mouse that comprises an engineered endogenous immunoglobulin κ light chain locus as described above using an antigen of interest (e.g., a single-pass or multi-pass membrane protein, etc.). The methods described in this example, or immunization methods well known in the art, can be used to immunize mice containing an engineered endogenous immunoglobulin κ light chain locus as described with various antigens (e.g., polypeptides, etc.). Any genetically modified mice described herein above, e.g., LiK mice - mice comprising an immunoglobulin κ light chain locus comprising human Vλ and Jλ gene segments operably linked to a Cλ gene (such as mice homozygous for the LiK locus); HoH/LiK/$\lambda^{-/-}$ mice - mice comprising an LiK locus (such as mice homozygous for the LiK locus) and also comprising humanized immunoglobulin heavy chain locus (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940) and an inactivated endogenous immunoglobulin λ light chain locus (see, e.g., U.S. Patent No. 9,006,511); and HoH/KoK/LiK/$\lambda^{-/-}$ mice - mice hemizygous for immunoglobulin κ light chain locus having a first immunoglobulin κ light chain locus comprising LiK and the second immunoglobulin κ light chain locus comprising human Vκ and Jκ gene segments operably linked to an endogenous mouse Cκ gene (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940), and also comprising humanized immunoglobulin heavy chain locus (see, e.g., U.S. Patent Nos. 8,642,835 and 8,697,940) and an inactivated endogenous immunoglobulin λ light chain locus (see, e.g., U.S. Patent No. 9,006,511), may be used for production of antibodies after immunization with an antigen of interest. Such mice are suitable for immunization and production of human antibodies and/or human antibody fragments.

[0385] LiK mice that further include the engineered immunoglobulin loci described above are challenged with an antigen of interest using immunization methods known in the art. The antibody immune response is monitored by an ELISA immunoassay (i.e., serum titer). When a desired immune response is achieved, splenocytes (and/or other lymphatic tissue) are harvested and fused with mouse myeloma cells to preserve their viability and form immortal hybridoma cell lines. Generated hybridoma cell lines are screened (e.g., by an ELISA assay) and selected to identify hybridoma cell lines that produce antigen-specific antibodies. Hybridomas may be further characterized for relative binding affinity and isotype as desired. Using this technique, and the immunogen described above, several antigen-specific chimeric antibodies (i.e., antibodies possessing human variable domains and mouse constant domains) are obtained.

[0386] DNA encoding the variable regions of heavy chain and light chains may be isolated or otherwise prepared, and may be linked to human heavy chain and light chain constant regions (e.g., of a desired isotype)for the preparation of fully-human antibodies. Such fully-human antibodies (and/or heavy or light chains thereof) may be produced in a cell, typically a mammalian cell such as a CHO cell. Fully human antibodies may then be characterized for relative binding affinity and/or neutralizing activity of the antigen of interest.

[0387] DNA encoding antigen-specific chimeric antibodies produced by B cells of the engineered mice described and/or exemplified herein, and/or the variable domains of light and/or heavy chains thereof, may be isolated directly from antigen-specific lymphocytes. For example, high affinity chimeric antibodies having a human variable region and a mouse constant region may be isolated and characterized so that particular antibodies (and/or B cells that produce them) of interest are defined. To give but a few examples, assessed characteristics of such antibodies, and/or variable and/or constant regions thereof, may be or inclue one or more of affinity, selectivity, identity of epitope, etc.

[0388] Mouse constant regions are replaced with a desired human constant region to generate fully-human antibodies. While the constant region selected may vary according to

specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region. Alternatively, when employing LiK mice containing a human Cλ2 gene in the place of a mouse Cκ gene as described herein, a step of replacing a mouse constant region in an antibody isolated from an immunized mouse is omitted. Antigen-specific antibodies are also isolated directly from antigen-positive B cells (from immunized mice) without fusion to myeloma cells, as described in, e.g., U.S. Patent No. 7,582,298. Using this method, several fully human antigen-specific antibodies (i.e., antibodies possessing human variable domains and human constant domains) are made.

Example 6. Generation of mice having an engineered light chain locus and expressing Human Terminal deoxynucleotidyl transferase (Td1) gene

Example 6.1. Generation of Mice Having an Engineered Light Chain Locus and Expressing Human TDT

[0389] This example illustrates the generation of mice whose germline genome comprises an engineered immunoglobulin κ light chain locus as described herein and further expressing human TdT. Mice expressing human TdT were made as described in Example 1.1. of WO 2017/210586,. Mice having a genome comprising both an engineered immunoglobulin κ light chain locus as described herein and further expressing human TdT were generated by multiple breedings to establish cohorts of mouse strains containing both modifications.

Example 6.2. Phenotypic Assessment of Mice Having an Engineered Kappa Locus and Expressing Human TDT

[0390] Once established, immune cell populations were characterized in these humanized mice by flow cytometry. Briefly, spleens and femurs were harvested from HoH/LiK/$\lambda^{-/-}$/TdT (n=4) and HoH/KoK/LiK/$\lambda^{-/-}$/TdT (n=6) mice and prepared for flow cytometry analysis as described above (see Example 3 above). Representative results are set forth in Figures 14-17. Average light chain expression (κ:λ) observed in splenocytes of engineered mouse strains tested was as follows: HoH/LiK/$\lambda^{-/-}$/TdT: 0:100, HoH/KoK/LiK/$\lambda^{-/-}$/TdT: 45:55.

Example 6.3. Human Immunoglobulin Kappa Junctional Diversity and Non-germline Additions in LiK Mice Comprising Human TdTS

[0391] As demonstrated in WO 2017/210586, mice comprising exogenously introduced TdT exhibited increases in both junctional diversity and non-germline nucleotide additions (also "non-template nucleotide additions" as used herein) in their light chains. The mice comprising HoH/LiK/$\lambda^{-/-}$/TdT and HoH/KoK/LiK/$\lambda^{-/-}$/TdT were assessed to determine their immunoglobulin repertoire sequence diversity and presence of non-template nucleotide additions in their CDR3 using Next Generation Sequencing technology.

[0392] Briefly, splenocytes were harvested from mice and B cells were positively enriched from total splenocytes by anti-mouse CD19 magnetic beads and MACS columns (Miltenyi Biotech). Total RNA was isolated from splenic B cells using the RNeasy Plus kit (Qiagen).

[0393] Reverse transcription with an oligo-dT primer followed by gene specific PCR was performed to generate cDNA containing mouse Cλ1 sequence, using SMARTer™ RACE cDNA Amplification Kit (Clontech). During reverse transcription, a specific DNA sequence (PIIA: 5'-CCCATGTACT CTGCGTTGAT ACCACTGCTT-3', SEQ ID NO:71) was attached to the 3' end of the newly synthesized cDNAs. The cDNAs were purified by the NucleoSpin Gel and PCR Clean-Up Kit (Clontech), then further amplified using a primer reverse compliment to PIIA (5' - AAGCAGTGGT ATCAACGCAG AGTACAT - 3', SEQ ID NO:72) paired with mouse Cλ1 specific primer (5'-CACCAGTGTG GCCTTGTTAG TCTC-3', SEQ ID NO:73).

[0394] Purified amplicons were then amplified by PCR using a PIIA specific primer (5'-GTGACTGGAG TTCAGACGTG TGCTCTTCCG ATCTAAGCAG TGGTATCAAC GCAGAGT-3', SEQ ID NO:74 and a nested mouse Cλ1 specific primer (5'-ACACTCTTTC CCTACACGAC GCTCTTCCGA TCTAAGGTGG AAACAGGGTG ACTGATG-3', SEQ ID NO:75. PCR products between 450-690bp were isolated and collected by Pippin Prep (SAGE Science). These fragments were further amplified by PCR using following primers: 5'-AATGATACGG CGACCACCGA GATCTACACX XXXXXACACT CTTTCCCTAC ACGACGCTCT TCCGATC-3', SEQ ID NO:76 and 5'-CAAGCAGAAG ACGGCATACG AGATXXXXXX GTGACTGGAG TTCAGACGTG TGCTCTTCCG ATCT-3', SEQ ID NO:77 ("XXXXXX" represents a 6bp index sequence to enable multiplexing samples for sequencing). PCR products between 490bp-710bp were isolated and collected by Pippin Prep, then quantified by qPCR using a KAPA Library Quantification Kit (KAPA Biosystems) before loading onto Miseq sequencer (Illumina) for sequencing (v3, 600-cycles).

[0395] For bioinformatic analysis, the resulting Illumina sequences were demultiplexed and trimmed for quality. Overlapping paired-end reads were then assembled and annotated using local installation of igblast (NCBI, v2.2.25+). Reads were aligned to human germline Vλ and Jλ segments database and sorted for the best hit. A sequence was marked as ambiguous and removed from analysis when multiple best hits with identical score were detected. A set of in-house perl scripts was developed to analyze results.

[0396] Lambda light chains from splenic B cells from HoH/LiK/$\lambda^{-/-}$/TdT mice, and both lambda and kappa light chains from splenic B cells from HoH/KoK/LiK/$\lambda^{-/-}$/TdT mice, were tested for an increase in non-template additions and junctional diversity at lambda and/or kappa loci. Light chains from HoH/LiK/$\lambda^{-/-}$/TdT and HoH/KoK/LiK/$\lambda^{-/-}$/TdT mice exhibited at least a 2 fold increase in junctional diversity as measured by number of unique CDR3/ 10,000 reads (data not shown). In addition, about 50% of light chains (lambda and/or kappa) from in HoH/LiK/$\lambda^{-/-}$/TdT and HoH/KoK/LiK/$\lambda^{-/-}$/TdT mice exhibited non-template additions as compared to light chains from control mice without TdT, which only exhibited about 10% non-template additions (data not shown).

Example 7. Immunization of engineered mice and analysis of immune response to immunogens

[0397] This example illustrates immunization of LiK/VI-3 and LiK/VI-3/TdT mice, and the analysis of serum antibody responses to the immunogens. Briefly, (1) VI-3/TdT (e.g., a positive control for human kappa light chain, which also has endogenous mouse lambda light chain loci) and VI-3 mice with human lambda light chains, (2) LiK/VI-3 and (3) LiK/VI-3/TdT, respectively, were immunized with protein immunogens using standard protocols and adjuvants. The mice were bled prior to the initiation of immunization and periodically bled following immunogen boosts. Antiserum titers were assayed on respective antigens.

[0398] Antibody titers in serum against immunogens were determined using ELISA. Ninety six-well microtiter plates (Pierce) were coated with antigens at 2 µg/ml in phosphate-buffered saline (PBS, Irvine Scientific) overnight at 4°C. Plates were washed with PBS containing 0.05% Tween-20 (PBS-T, Sigma-Aldrich) and blocked with 250 µl of 0.5% bovine serum albumin (BSA, Sigma-Aldrich) in PBS for 1 hour at room temperature. The plates were washed with PBS-T. Pre-immune and immune anti-sera were serially diluted three-fold in 1% BSA-PBS and added to the plates for 1 hour at room temperature. The plates were washed and goat anti-mouse IgG-Fc-Horse Radish Peroxidase (HRP) conjugated secondary antibodies (Jackson Immunoresearch), goat anti-mouse Kappa-HRP (SouthernBiotech) or goat anti-mouse Lambda-HRP (SouthernBiotech) were added at 1:5000 dilution to the plates and incubated for 1 hour at room temperature. Plates were washed and developed using TMB/H_2O_2 as substrate by incubating for 15-20 minutes. The reaction was stopped with acid and plates read on a spectrophotometer (Victor, Perkin Elmer) at 450 nm. Antibody titers were computed using Graphpad PRISM software. The titer is defined as interpolated serum dilution factor of which the binding signal is 2-fold over background.

[0399] The humoral immune responses in LiK/VI-3, LiK/VI-3/TdT, and VI-3/TdT mice were investigated following immunization with a protein immunogen. Antisera from mice immunized with protein show high titers on antigen in LiK/VI-3 and LiK/VI-3/TdT strains comparable to VI-3/TdT strain (Figure 18). High lambda titers were elicited in both LiK/VI-3 and LiK/VI-3/TdT mice. In VI-3/TdT strain mice, lambda titers were not observed in three mice, while low titers were observed in two mice, which corresponds to the low usage of mouse lambda variables in this strain. As expected, no kappa titers were elicited in LiK/VI-3 and LiK/VI-3/TdT mice as they lack the kappa light chain, while the VI-3/TdT showed high kappa titers. Figure 19 shows baseline titers (lowest serum dilution) were observed on irrelevant protein antigen for His tag in all three mice strains with anti-Fc and anti-kappa

detection, while very low titers were observed with anti-lambda detection in LiK/VI-3 and LiK/VI-3/TdT mice.

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PATENTKRAV

1. Fremgangsmåde til fremstilling af et antistof i en genetisk modificeret mus, hvor fremgangsmåden omfatter følgende trin:

(a) immunisering af den genetisk modificerede mus med et antigen af interesse,

hvor den genetisk modificerede mus har et kimbanegenom omfattende:

et første konstrueret, endogent immunglobulin-κ-letkædelocus omfattende:

(i) ét eller flere humane Vλ-gensegmenter,

(ii) ét eller flere humane Jλ-gensegmenter og

(iii) ét muse-Cλ-gen,

hvor det ene eller flere humane Vλ-gensegmenter fra (i) og det ene eller flere humane Jλ-gensegmenter fra (ii) er operativt bundet til det ene muse-Cλ-gen fra (iii);

hvor det ene muse-Cλ-gen (iii) er i stedet for et muse-Cκ-gen ved det første konstruerede, endogene immunglobulin-κ-letkædelocus;

hvor det ene eller flere humane Vλ-gensegmenter fra (i) og det ene eller flere humane Jλ-gensegmenter fra (ii) er i stedet for ét eller flere endogene muse-Vκ-gensegmenter og ét eller flere endogene muse-Jκ-gensegmenter;

hvor den genetisk modificerede mus mangler et muse-Cκ-gen ved det første konstruerede, endogene immunglobulin-k-letkædelocus;

(b) at holde den genetisk modificerede mus under forhold, der er tilstrækkelige for den genetisk modificerede mus til at frembringe et immunrespons mod antigenet af interesse; og

(c) genvinding fra den genetisk modificerede mus:

(i) et antistof, der binder antigenet af interesse, hvor antistoffet omfatter en letkæde omfattende et variabelt humant λ-domæne og et konstant muse-λ-domæne,

(ii) et nukleotid, der koder for et variabelt humant letkædedomæne eller variabelt, humant tungkædedomæne, en letkæde eller en tungkæde af et antistof, der binder antigenet af interesse, hvor antistoffet, der binder antigenet af interesse, omfatter en letkæde omfattende et variabelt humant λ-domæne og et konstant muse-λ-domæne, eller

(iii) en celle, der udtrykker et antistof, der binder antigenet af interesse, hvor antistoffet omfatter en letkæde omfattende et variabelt humant λ-domæne og et konstant muse-λ-domæne.

2. Fremgangsmåde ifølge krav 1, hvor den genetisk modificerede mus er homozygot for det første konstruerede, endogene immunglobulin-κ-letkædelocus.

3. Fremgangsmåde ifølge krav 1, hvor den genetisk modificerede mus er heterozygot for det første konstruerede, endogene immunglobulin-κ-letkædelocus.

4. Fremgangsmåde ifølge krav 3, hvor den genetisk modificerede mus' kimbanegenom omfatter et andet konstrueret, endogent immunglobulin-κ-letkædelocus omfattende:

(a) ét eller flere humane Vκ-gensegmenter og

(b) ét eller flere humane Jκ-gensegmenter,

hvor det ene eller flere humane Vκ-gensegmenter og det ene eller flere humane Jκ-gensegmenter er operativt bundet til et Cκ-gen.

5. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor musens kimbanegenom endvidere omfatter:

et konstrueret, endogent immunglobulin-tungkædelocus, omfattende:

(a) ét eller flere humane V_H-gensegmenter,

(b) ét eller flere humane D_H-gensegmenter og

(c) ét eller flere humane J_H-gensegmenter,

hvor det ene eller flere humane V_H-gensegmenter fra (a), det ene eller flere humane D_H-gensegmenter fra (b) og det ene eller flere humane J_H-gensegmenter fra (c) er operativt bundet til ét eller flere gener fra det konstante muse-immunglobulin-tungkædeområde ved det konstruerede endogene immunglobulin-tungkædelocus.

6. Fremgangsmåde ifølge krav 5, hvor det ene eller flere humane V_H-gensegmenter fra (a), ét eller flere humane D_H-gensegmenter fra (b) og ét eller flere humane J_H-gensegmenter fra (c) er i stedet for ét eller flere muse-V_H-gensegmenter, ét eller flere muse-D_H-gensegmenter, ét eller flere muse-J_H-gensegmenter eller en kombination deraf.

7. Fremgangsmåde ifølge krav 5 eller 6, hvor det konstruerede endogene immunglobulin-tungkædelocus endvidere omfatter:

(i) én eller flere humane, ikke-kodende V_H-sekvenser, der hver støder op til mindst ét af det ene eller flere humane V_H-gensegmenter, hvor hver af den ene eller flere V_H ikke-kodende sekvenser naturligt forekommer stødende op til et humant V_H-gensegment i et endogent, humant immunglobulin-tungkædelocus;

(ii) én eller flere humane, ikke-kodende D_H-sekvenser, der hver støder op til mindst ét af det ene eller flere humane D_H-gensegmenter, hvor hver af den ene eller flere ikke-kodende D_H-sekvenser naturligt forekommer stødende op til et humant D_H-gensegment i et endogent, humant immunglobulin-tungkædelocus;

(iii) én eller flere humane, ikke-kodende J_H-sekvenser, der hver støder op til mindst ét af det ene eller flere humane J_H-gensegmenter, hvor hver af den ene eller flere ikke-kodende J_H-sekvenser naturligt forekommer stødende op til et humant J_H-gensegment i et endogent, humant immunglobulin-tungkædelocus; eller

(iv) en hvilken som helst kombination deraf.

8. Fremgangsmåde ifølge et hvilket som helst af kravene 5 til 7, hvor det ene eller flere gener fra det konstante muse-immunglobulin-tungkædeområde er ét eller flere endogene gener fra det konstante immunglobulin-tungkædeområde.

9. Fremgangsmåde ifølge et hvilket som helst af kravene 5 til 8, hvor:

(i) det ene eller flere humane V_H-gensegmenter omfatter V_H3-74, V_H3-73, V_H3-72, V_H2-70, V_H1-69, V_H3-66, V_H3-64, V_H4-61, V_H4-59, V_H1-58, V_H3-53, V_H5-51, V_H3-49, V_H3-48, V_H1-46, V_H1-45, V_H3-43, V_H4-39, V_H4-34, V_H3-33, V_H4-31, V_H3-30, V_H4-28, V_H2-26, V_H1-24, V_H3-23, V_H3-21, V_H3-20, V_H1-18, V_H3-15, V_H3-13, V_H3-11, V_H3-9, V_H1-8, V_H3-7, V_H2-5, V_H7-4-1, V_H4-4, V_H1-3, V_H1-2, V_H6-1 eller en kombination deraf,

(ii) det ene eller flere humane D_H-gensegmenter omfatter D_H1-1, D_H2-2, D_H3-3, D_H4-4, D_H5-5, D_H6-6, D_H1-7, D_H2-8, D_H3-9, D_H3-10, D_H5-12, D_H6-13, D_H2-15, D_H3-16, D_H4-17, D_H6-19, D_H1-20, D_H2-21, D_H3-22, D_H6-25, D_H1-26, D_H7-27 eller en kombination deraf, og

(iii) det ene eller flere humane J_H-gensegmenter omfatter J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, eller en kombination deraf.

10. Fremgangsmåde ifølge et hvilket som helst af kravene 5 til 9, hvor den genetisk modificerede mus er homozygot for det konstruerede endogene immunglobulin-tungkædelocus.

11. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det ene eller flere humane Vλ-gensegmenter fra (i) og det ene eller flere humane Jλ-gensegmenter fra (ii) erstatter ét eller flere muse-Vκ-gensegmenter, ét eller flere muse-Jκ-gensegmenter eller en kombination deraf.

12. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det første konstruerede, endogene immunglobulin-κ-letkædelocus endvidere omfatter en ikke-kodende κ-letkædesekvens mellem det ene eller flere humane Vλ-gensegmenter og det ene eller flere humane Jλ-gensegmenter.

13. Fremgangsmåde ifølge krav 12, hvor den ikke-kodende κ-letkædesekvens har en sekvens, der naturligt forekommer mellem et humant Vκ4-1-gensegment og et human Jκ1-gensegment i et endogent, humant immunglobulin-κ-letkædelocus.

14. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor de endogene Vλ-gensegmenter, de endogene Jλ-gensegmenter og de endogene Cλ-gener deleteres helt eller delvist.

15. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor hvert variable humane letkædedomæne kodes for af en omarrangeret sekvens af det variable humane immunglobulin λ-letkædeområde omfattende ét af det ene eller flere humane Vλ-gensegmenter fra (i) eller en somatisk hypermuteret variant deraf, og ét af det ene eller flere humane Jλ-gensegmenter fra (ii) eller en somatisk hypermuteret variant deraf.

16. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor kimbanegenomet endvidere omfatter en nukleinsyresekvens, der koder for en exogen, terminal deoxynucleotidyltransferase (TdT) operativt bundet til et transskriptionsstyreelement.

DRAWINGS

Drawing

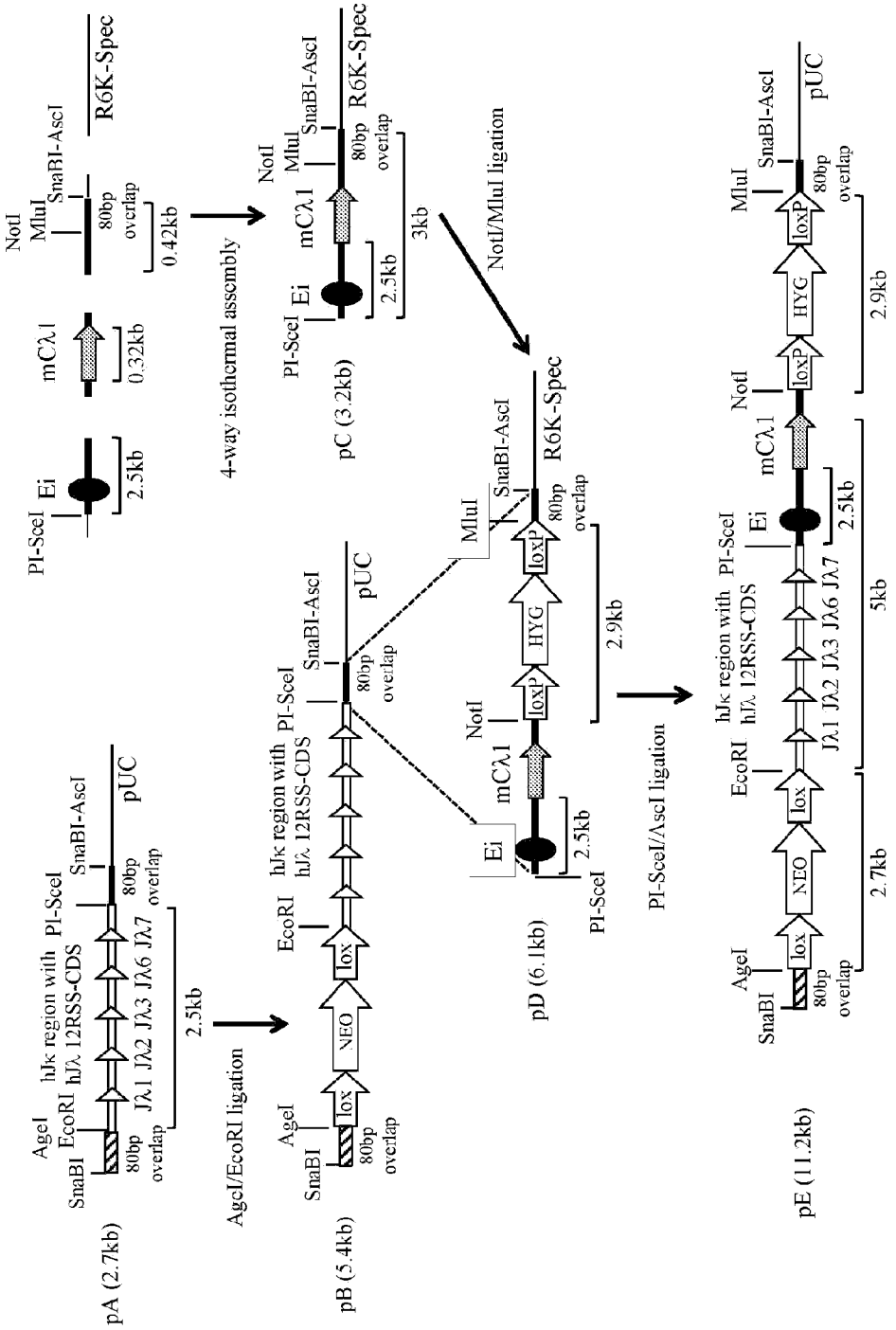


Figure 1A

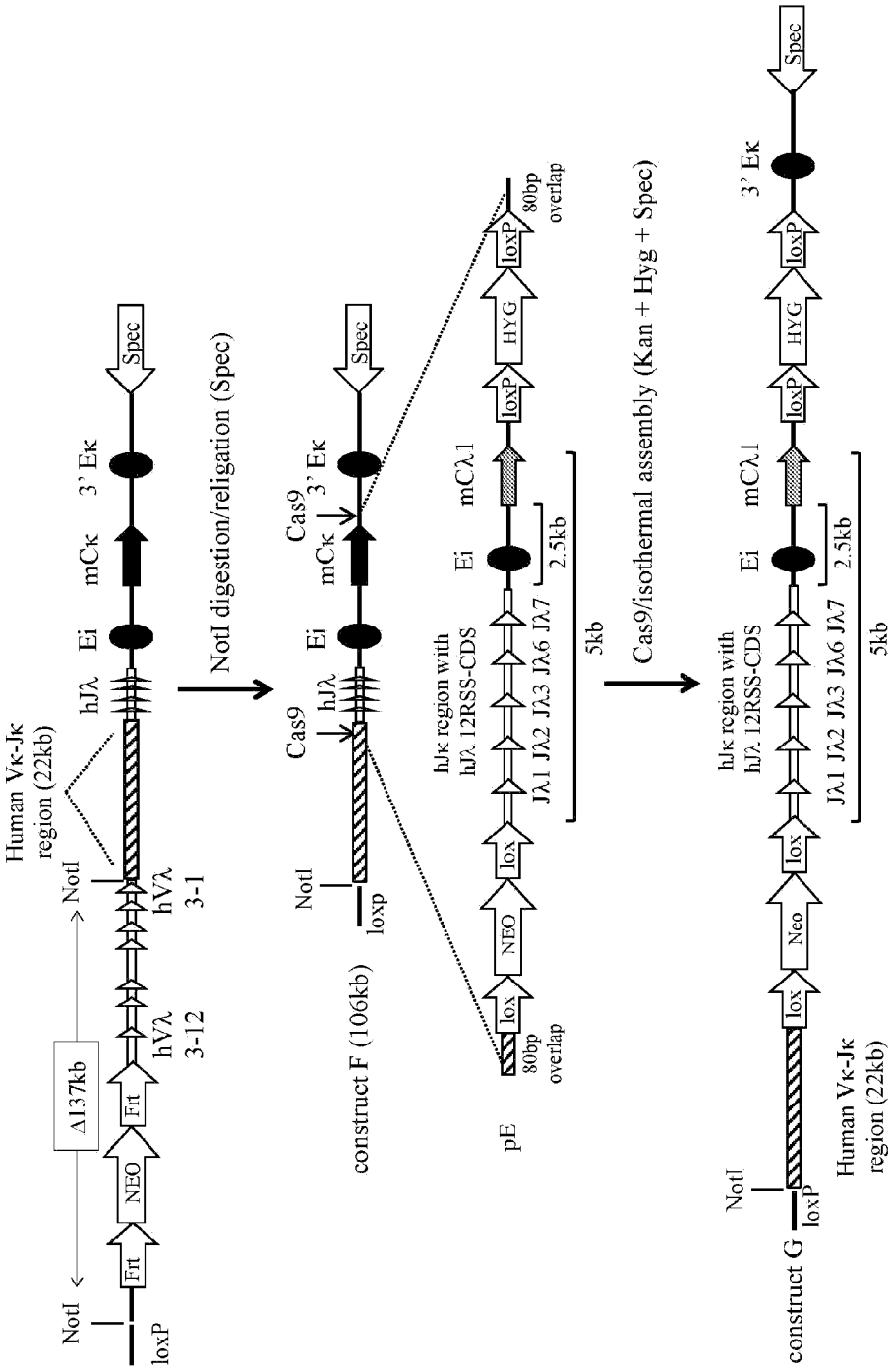
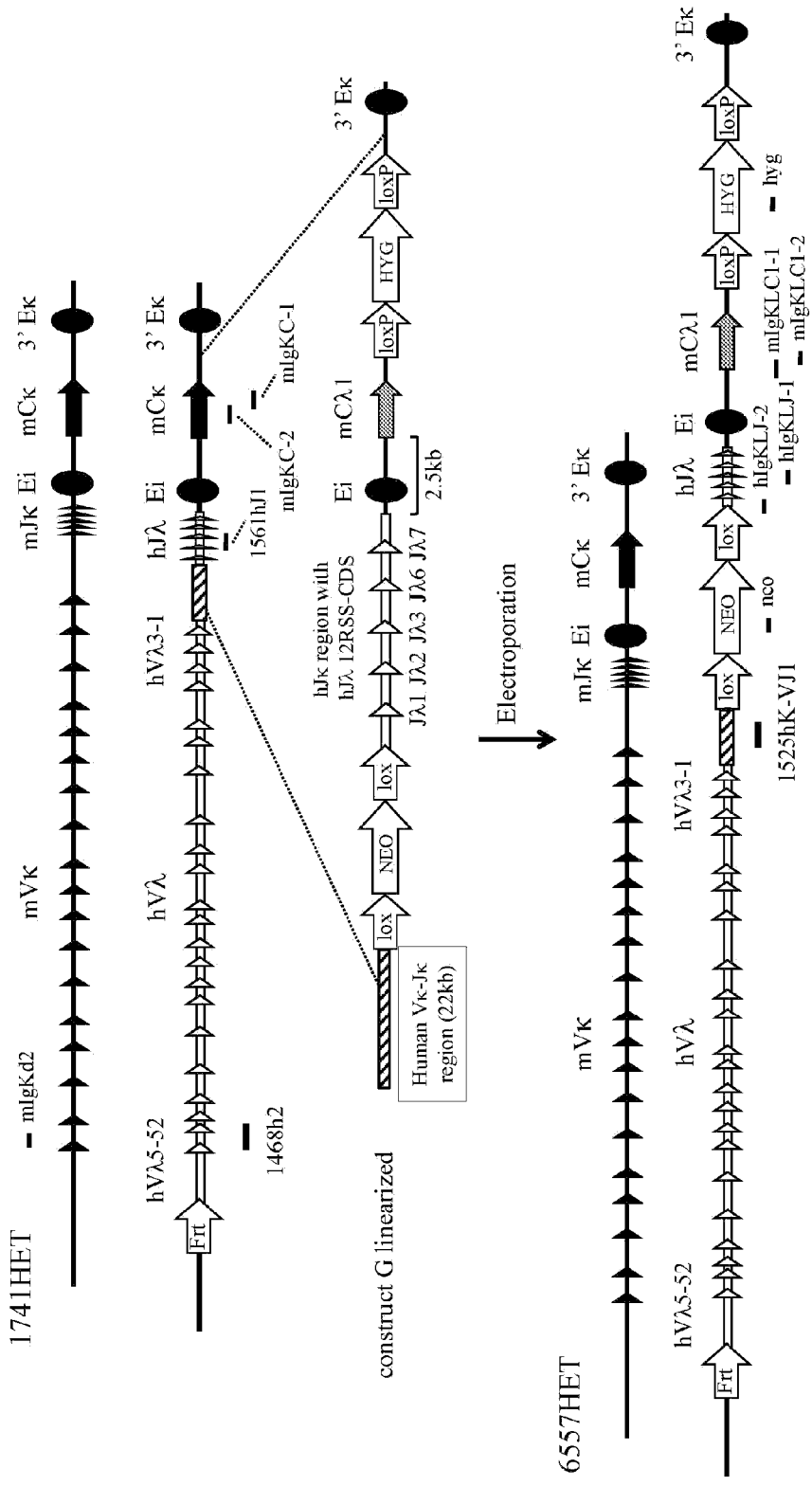

Human Vκ-Jκ region (22kb)
NotI
loxP
Frt
NEO
Frt
Δ137kb
hVλ 3-12
hVλ 3-1
hJλ
Ei
mCκ
3' Eκ
Spec
NotI digestion/religation (Spec)
construct F (106kb)
Cas9
NotI
loxp
hJλ
Ei
mCκ
Cas9
3' Eκ
Spec
pE
lox
NEO
lox
80bp overlap
hJκ region with hJλ 12RSS-CDS
Jλ1 Jλ2 Jλ3 Jλ6 Jλ7
2.5kb
5kb
Ei
mCλ1
loxP
HYG
loxP
80bp overlap
Cas9/isothermal assembly (Kan + Hyg + Spec)
construct G
NotI
loxP
Human Vκ-Jκ region (22kb)
lox
Neo
lox
hJκ region with hJλ 12RSS-CDS
Jλ1 Jλ2 Jλ3 Jλ6 Jλ7
5kb
Ei
mCλ1
2.5kb
loxP
HYG
loxP
3' Eκ
Spec

Figure 1B


1741HET
mIgKd2
mVκ
mJκ Ei mCκ 3' Eκ
hVλ5-52
hVλ
hVλ3-1
hJλ Ei mCκ 3' Eκ
Frt
1468h2
1561hJ1
mIgKC-2
mIgKC-1
construct G linearized
Human Vκ-Jκ region (22kb)
lox
NEO
lox
hJκ region with
hJλ 12RSS-CDS
Jλ1 Jλ2 Jλ3 Jλ6 Jλ7
Ei mCλ1
loxP
HYG
loxP
2.5kb
3' Eκ
Electroporation
6557HET
mVκ
mJκ Ei mCκ 3' Eκ
hVλ5-52
hVλ
hVλ3-1
hJλ Ei mCλ1
Frt
lox
NEO
lox
1525hK-VJ1
neo
hIgKLJ-2
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loxP
HYG
loxP
mIgKLC1-1
mIgKLC1-2
hyg
3' Eκ

Figure 2A

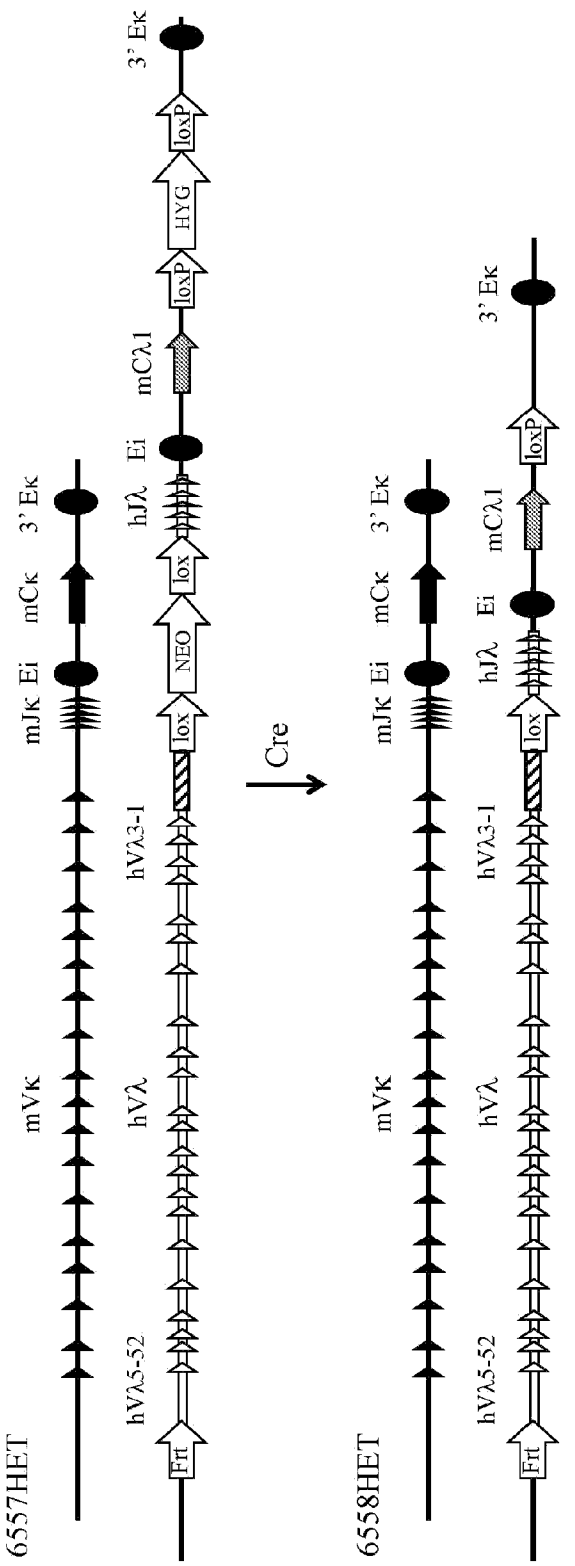

6557HET
mVκ
mJκ
Ei
mCκ
3' Eκ
hVλ
hVλ5-52
hVλ3-1
Frt
lox
NEO
lox
hJλ
Ei
mCλ1
loxP
HYG
loxP
3' Eκ
Cre
6558HET
mVκ
mJκ
Ei
mCκ
3' Eκ
hVλ
hVλ5-52
hVλ3-1
Frt
lox
hJλ
Ei
mCλ1
loxP
3' Eκ

Figure 2B

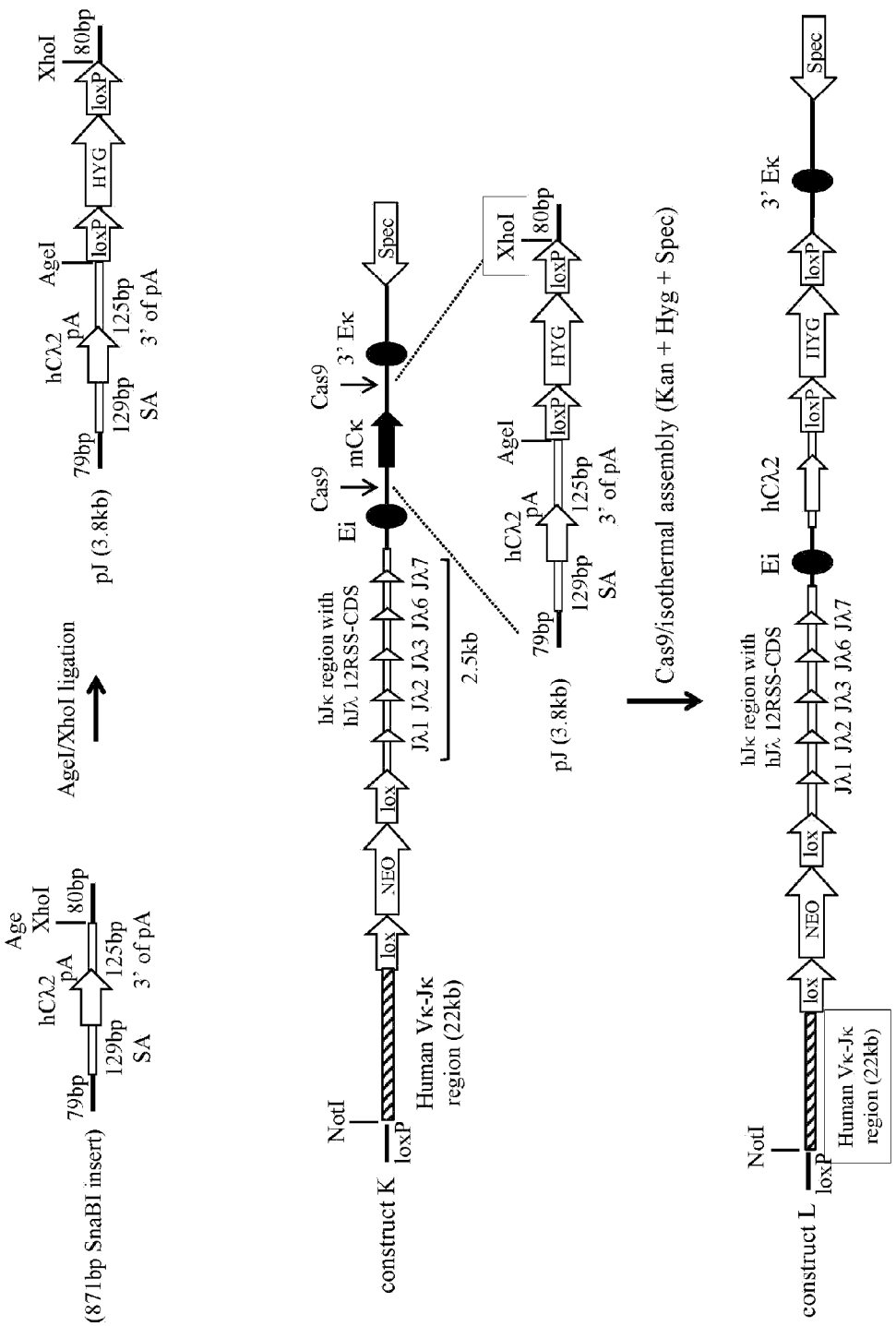

pH (871bp SnaBI insert)
Age
XhoI
80bp
hCλ2
pA
125bp
79bp
129bp
SA
3' of pA
AgeI/XhoI ligation
pJ (3.8kb)
AgeI
XhoI
80bp
loxP
HYG
loxP
hCλ2
pA
125bp
79bp
129bp
SA
3' of pA
construct K
NotI
loxP
Human Vκ-Jκ
region (22kb)
lox
NEO
lox
hJκ region with
hJλ 12RSS-CDS
Jλ1 Jλ2 Jλ3 Jλ6 Jλ7
2.5kb
Cas9
Ei
Cas9
mCκ
3' Eκ
Spec
Cas9/isothermal assembly (Kan + Hyg + Spec)
construct L
NotI
loxP
Human Vκ-Jκ
region (22kb)
lox
NEO
lox
hJκ region with
hJλ 12RSS-CDS
Jλ1 Jλ2 Jλ3 Jλ6 Jλ7
Ei
hCλ2
loxP
HYG
loxP
3' Eκ
Spec

Figure 3

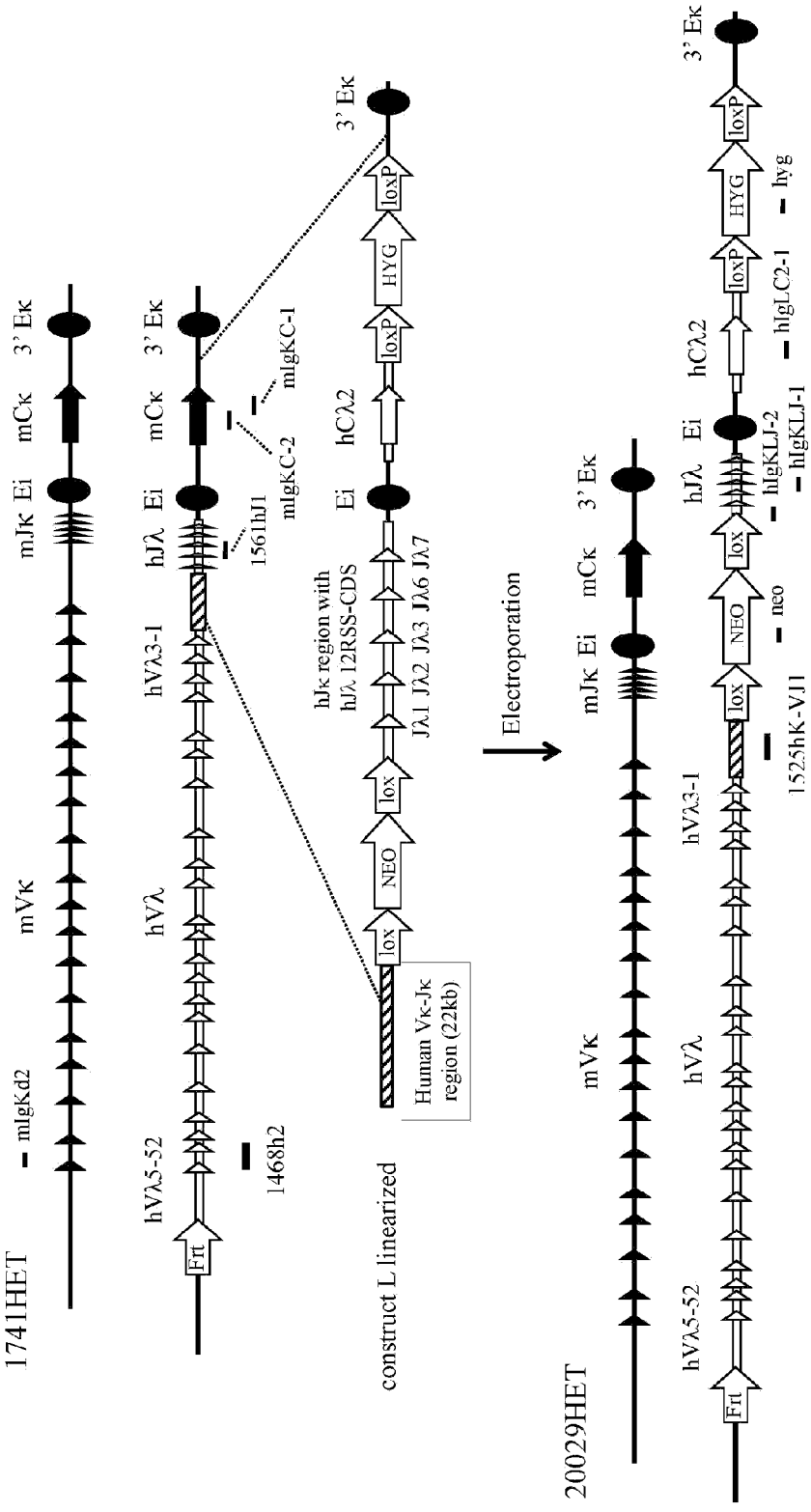

1741HET
mIgKd2
mVκ
mJκ Ei
mCκ
3' Eκ
hVλ5-52
Frt
1468h2
hVλ
hVλ3-1
hJλ Ei
mCκ
3' Eκ
1561hJ1
mIgKC-2
mIgKC-1
construct L linearized
lox
NEO
lox
Human Vκ-Jκ region (22kb)
hJκ region with hJλ 12RSS-CDS
Jλ1 Jλ2 Jλ3 Jλ6 Jλ7
Ei
hCλ2
loxP
HYG
loxP
3' Eκ
Electroporation
20029HET
mVκ
mJκ Ei
mCκ
3' Eκ
hVλ5-52
Frt
hVλ
hVλ3-1
lox
1525hK-VJ1
neo
NEO
lox
hJλ Ei
hCλ2
hIgKLJ-2
hIgKLJ-1
loxP
HYG
loxP
hIgLC2-1
hyg
3' Eκ

Figure 4A

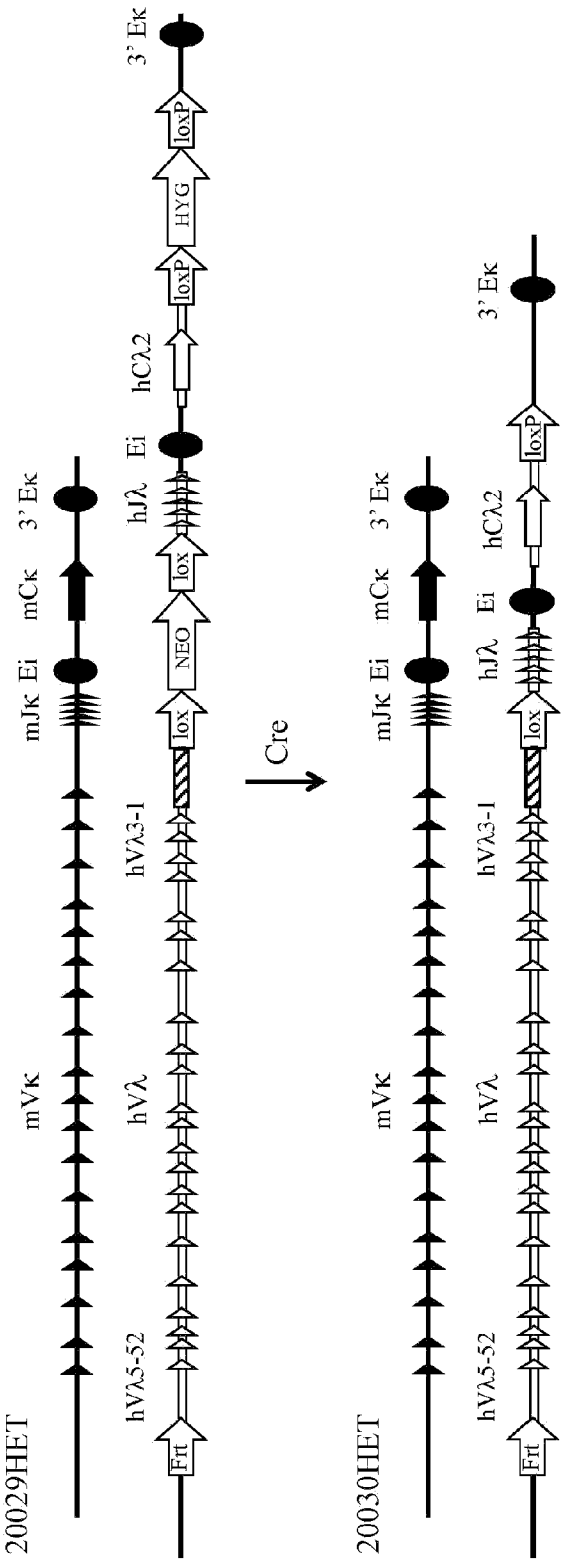

20029HET
mVκ
mJκ
Ei
mCκ
3' Eκ
hVλ
hVλ3-1
hJλ
Ei
hCλ2
hVλ5-52
Frt
lox
NEO
lox
loxP
HYG
loxP
3' Eκ
Cre
20030HET
mVκ
mJκ
Ei
mCκ
3' Eκ
hVλ
hVλ3-1
hJλ
Ei
hCλ2
hVλ5-52
Frt
lox
loxP
3' Eκ

Figure 4B

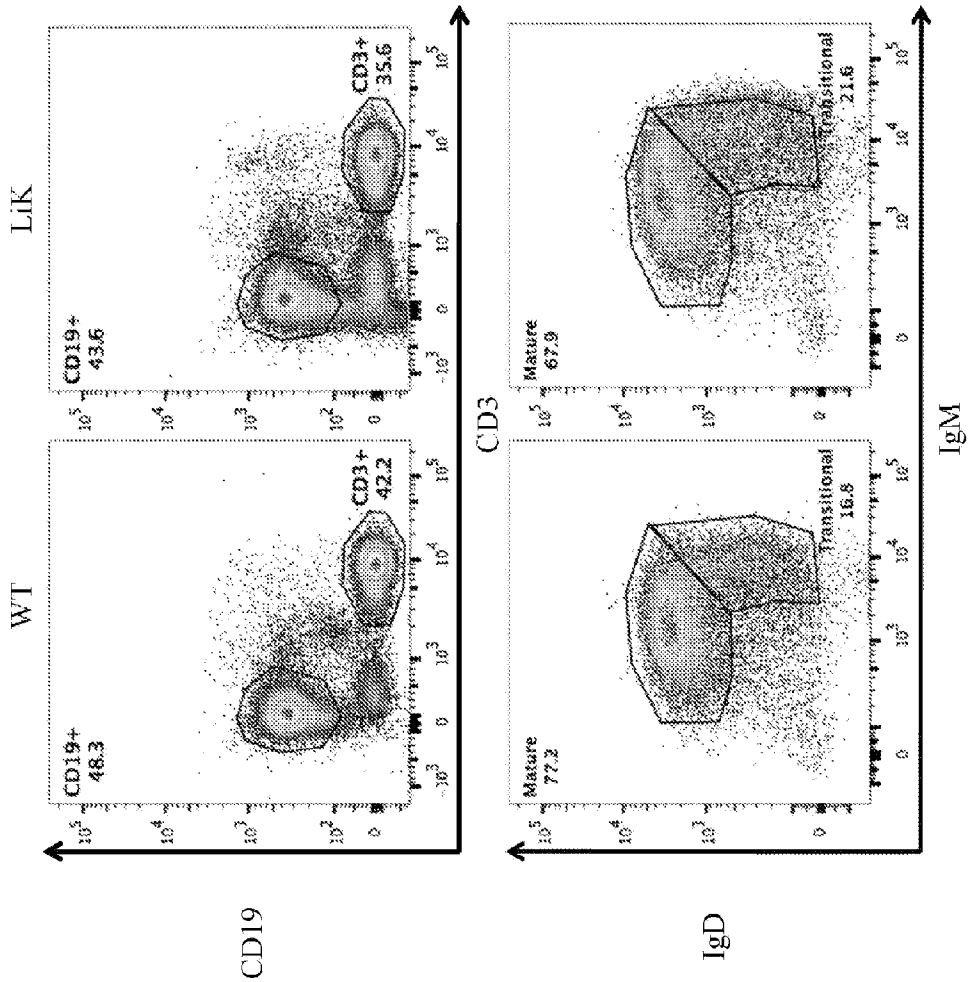

WT
LiK
CD19+
48.3
CD3+
42.2
CD19+
43.6
CD3+
35.6
CD19
CD3
Mature
77.2
Transitional
16.8
Mature
67.9
Transitional
21.6
IgD
IgM

Figure 5

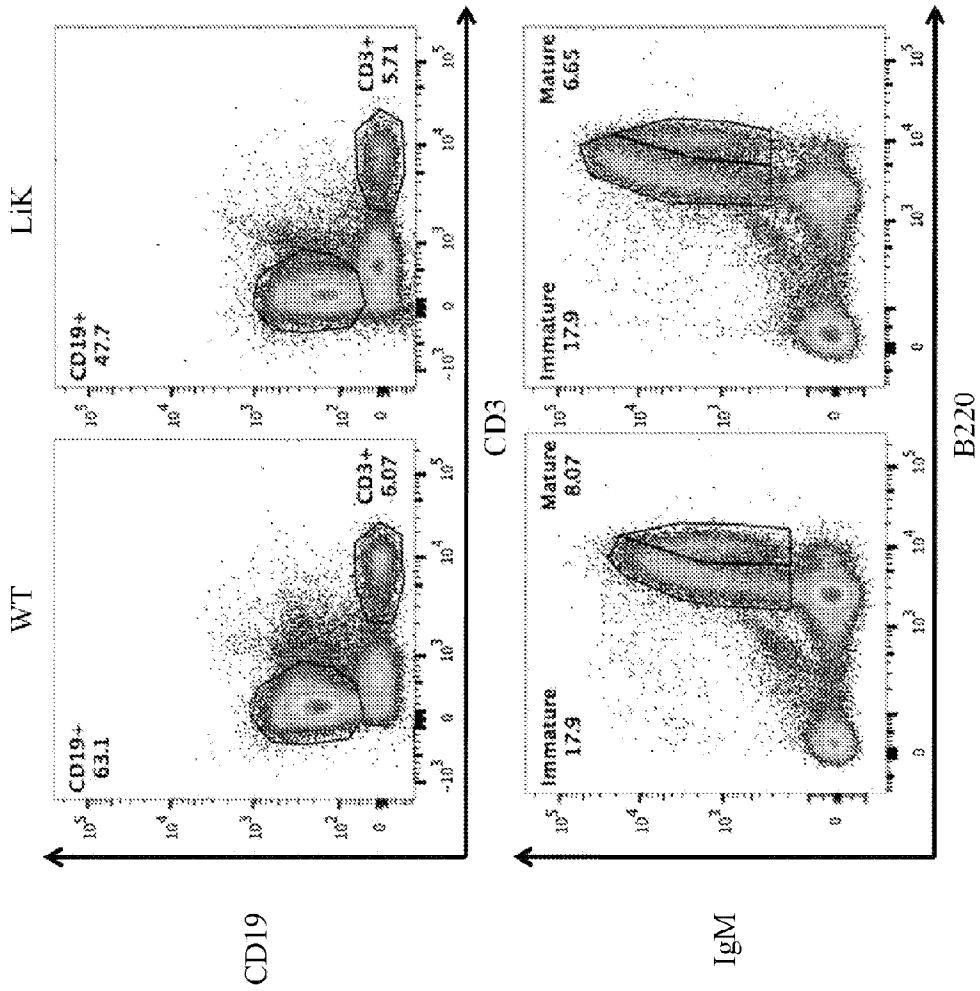

WT
LiK
CD19+
63.1
CD3+
6.07
CD19+
47.7
CD3+
5.71
CD19
CD3
Immature
17.9
Mature
8.07
Immature
17.9
Mature
6.65
IgM
B220

Figure 6

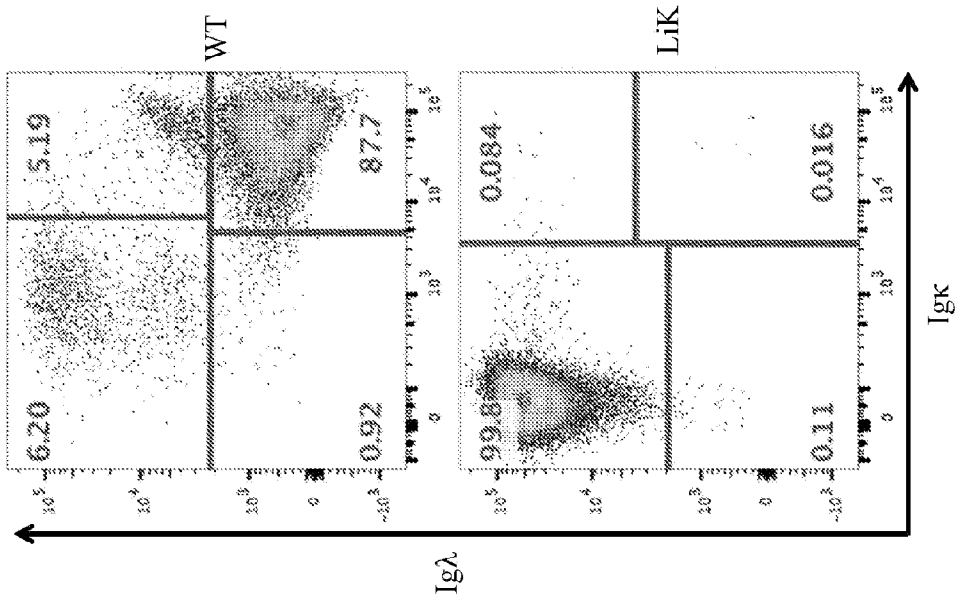


Figure 7

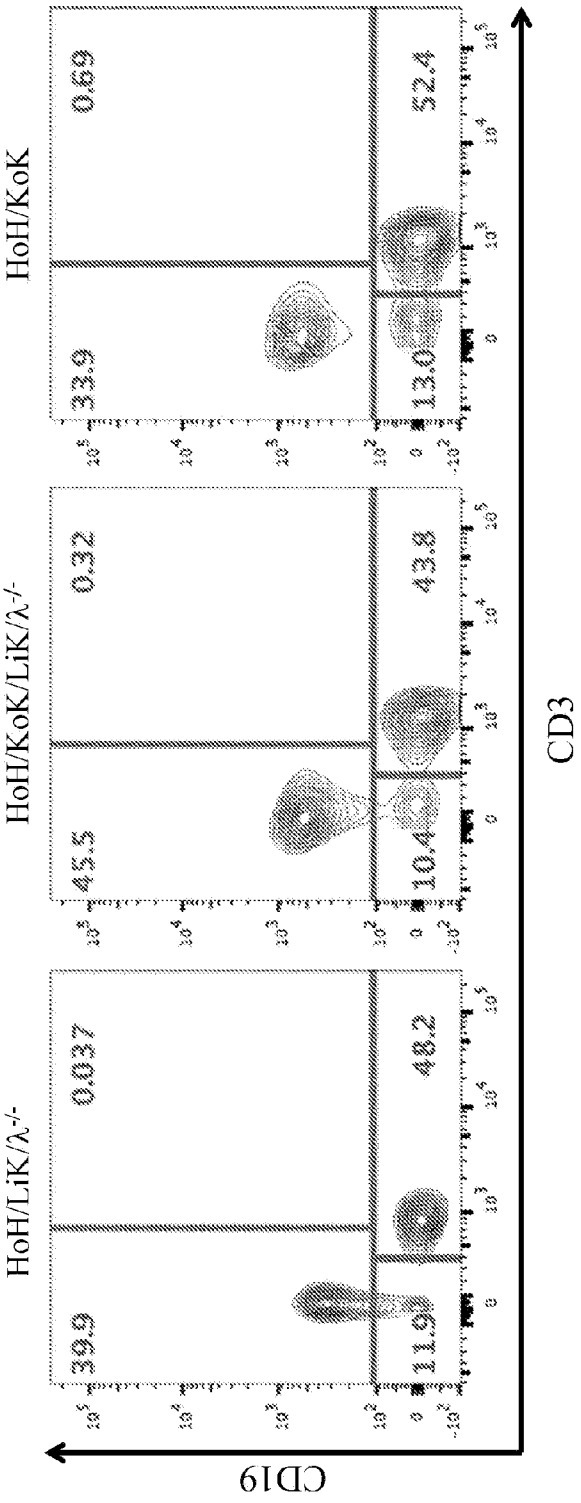

HoH/LiK/λ-/-
HoH/KoK/LiK/λ-/-
HoH/KoK
39.9
11.9
0.037
48.2
45.5
10.4
0.32
43.8
33.9
13.0
0.69
52.4
CD19
CD3

Figure 8

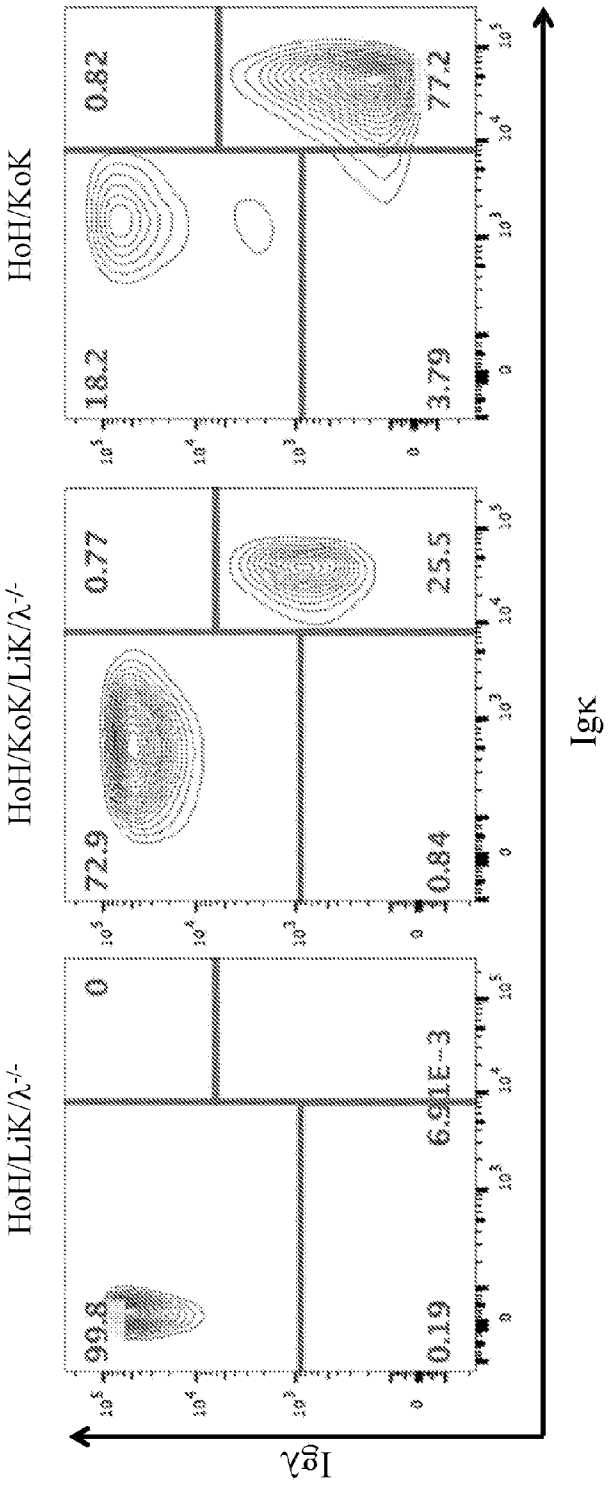

HoH/LiK/λ-/-
HoH/KoK/LiK/λ-/-
HoH/KoK
99.8
0.19
0
6.91E-3
72.9
0.84
0.77
25.5
18.2
3.79
0.82
77.2
Igλ
Igκ

Figure 9

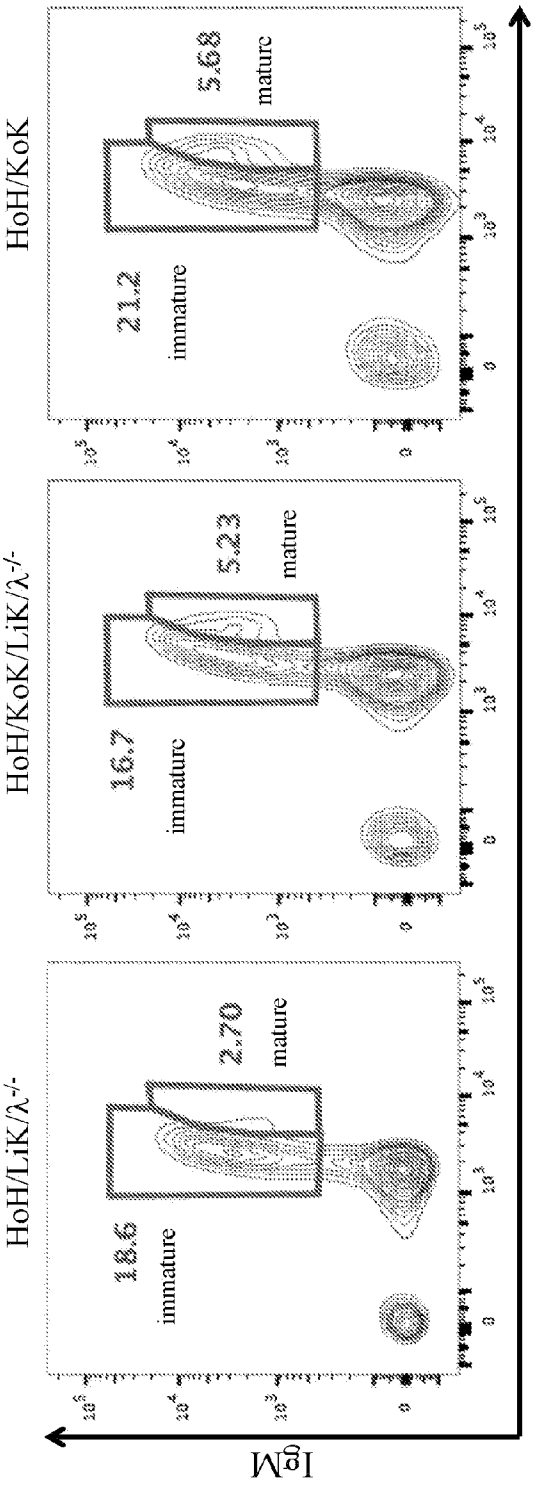

HoH/LiK/λ-/-
HoH/KoK/LiK/λ-/-
HoH/KoK
18.6
immature
2.70
mature
16.7
immature
5.23
mature
21.2
immature
5.68
mature
IgM
B220

Figure 10

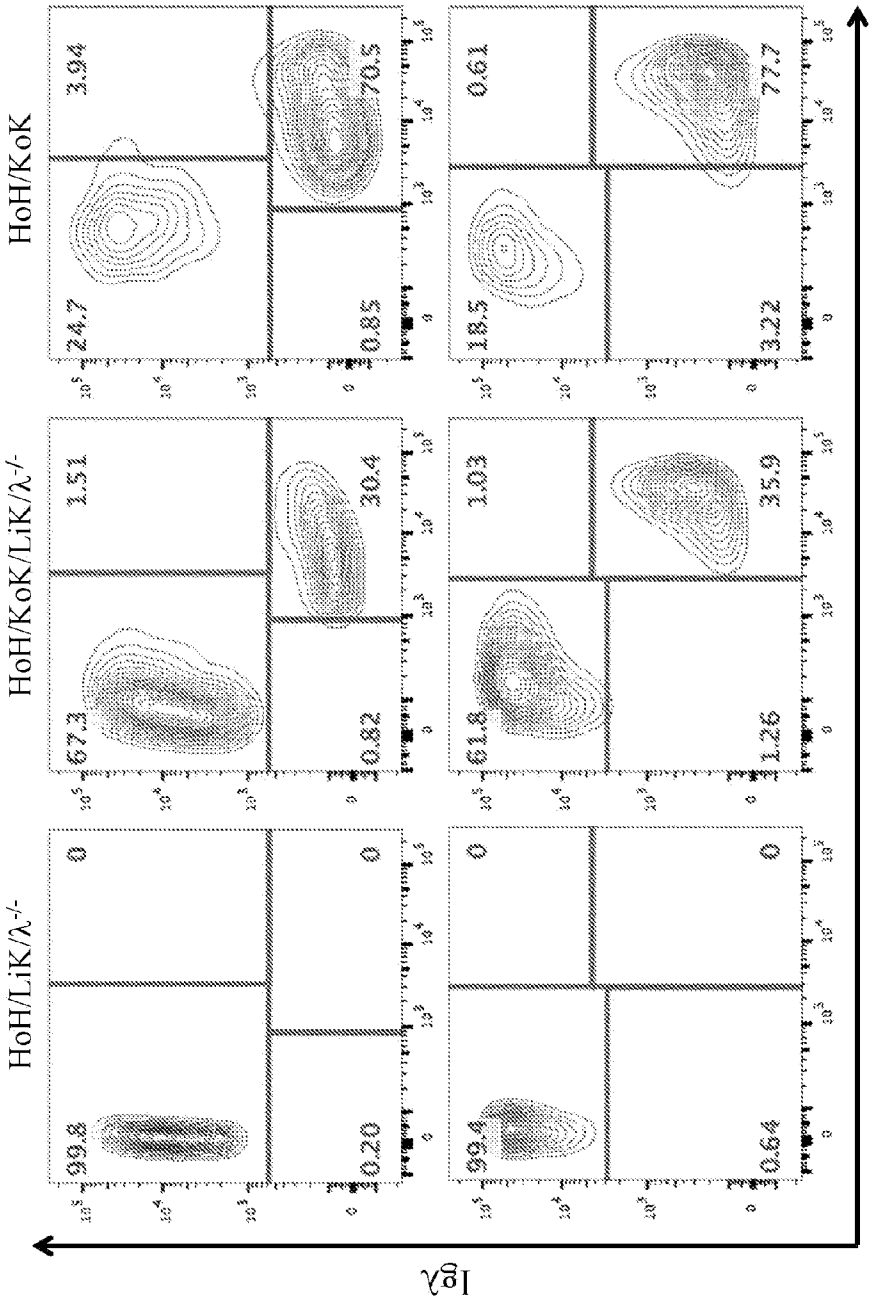

HoH/LiK/λ-/-
HoH/KoK/LiK/λ-/-
HoH/KoK
Igλ
Igκ
99.8
0.20
0
0
67.3
0.82
1.51
30.4
24.7
0.85
3.94
70.5
99.4
0.64
0
0
61.8
1.26
1.03
35.9
18.5
3.22
0.61
77.7

Figure 11

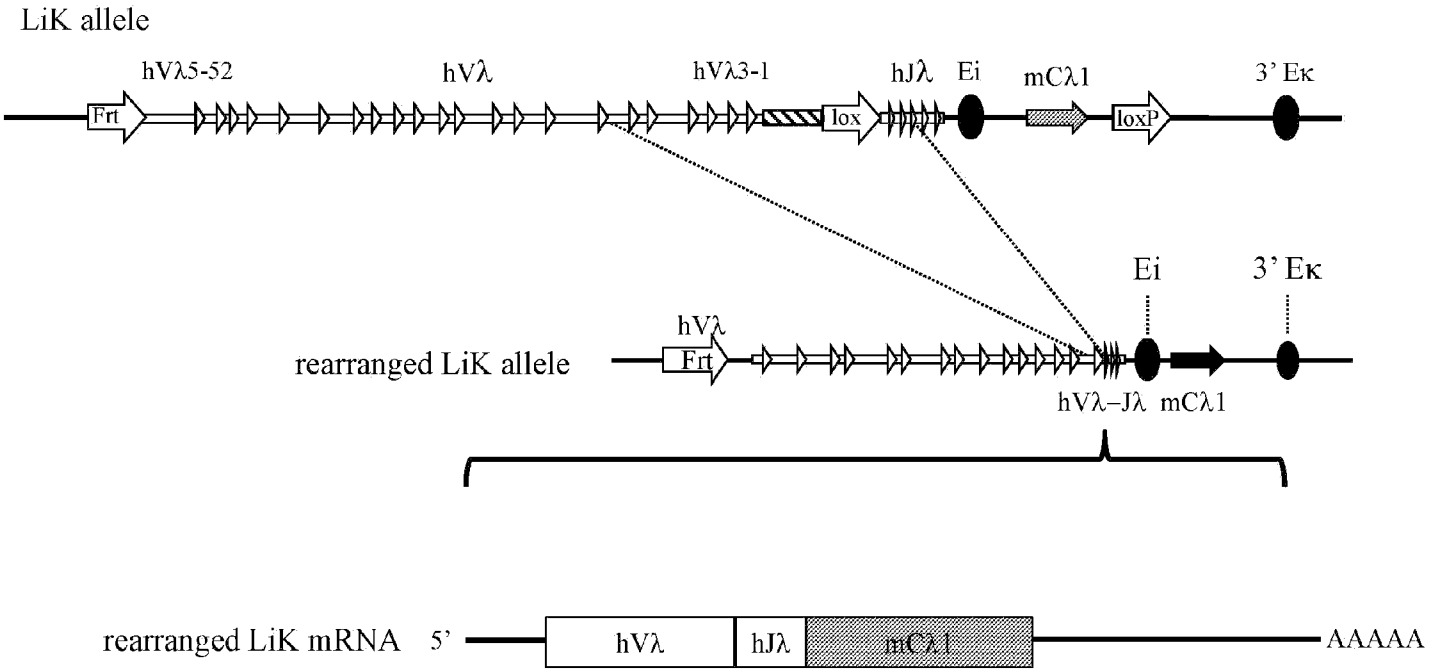
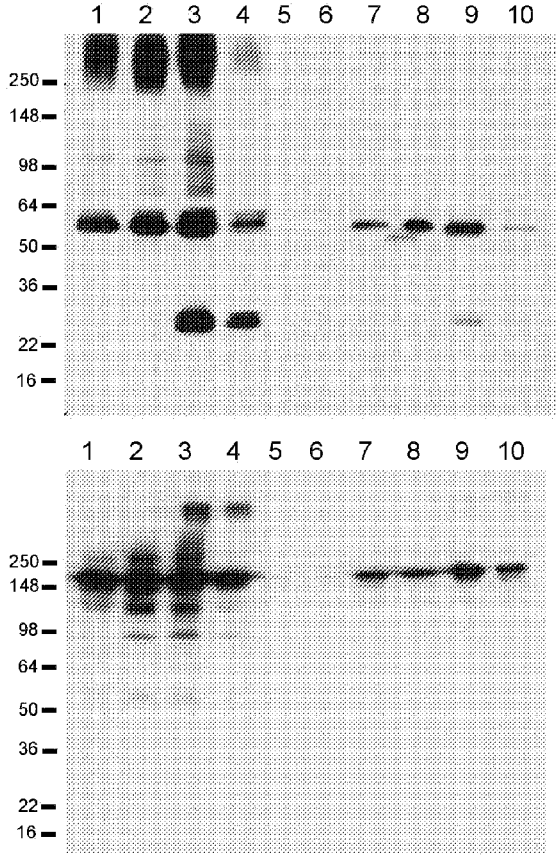


Figure 12



Lane	Genotype	Dilution
1	LiK HO	1:100
2	LiK HO	1:100
3	WT	1:100
4	WT	1:100
5	Blank	N/A
6	Blank	N/A
7	LiK HO	1:500
8	LiK HO	1:500
9	WT	1:500
10	WT	1:500

Figure 13

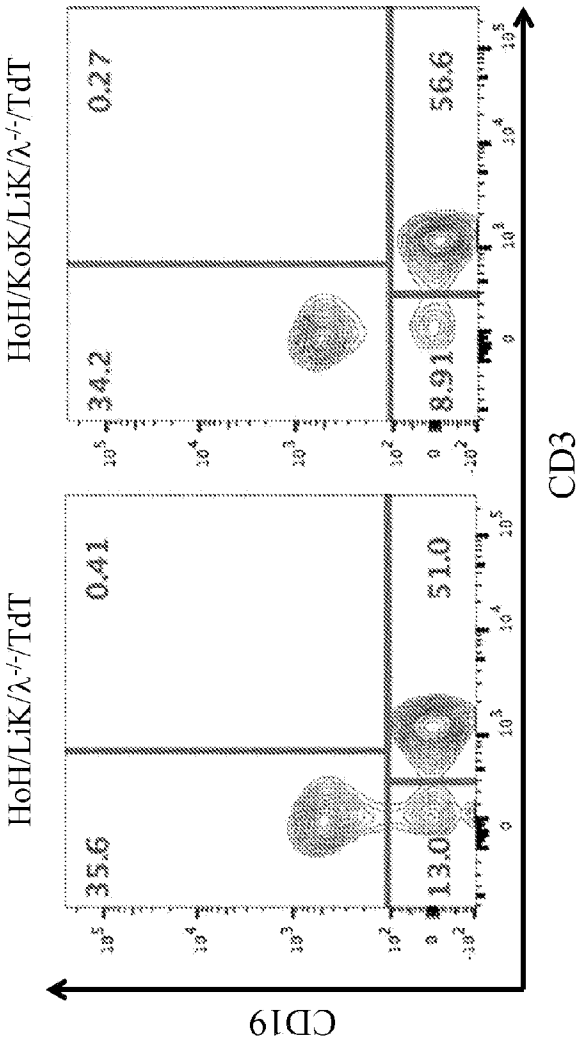

HoH/LiK/λ-/-/TdT
HoH/KoK/LiK/λ-/-/TdT
CD19
CD3
35.6
0.41
13.0
51.0
34.2
0.27
8.91
36.6

Figure 14

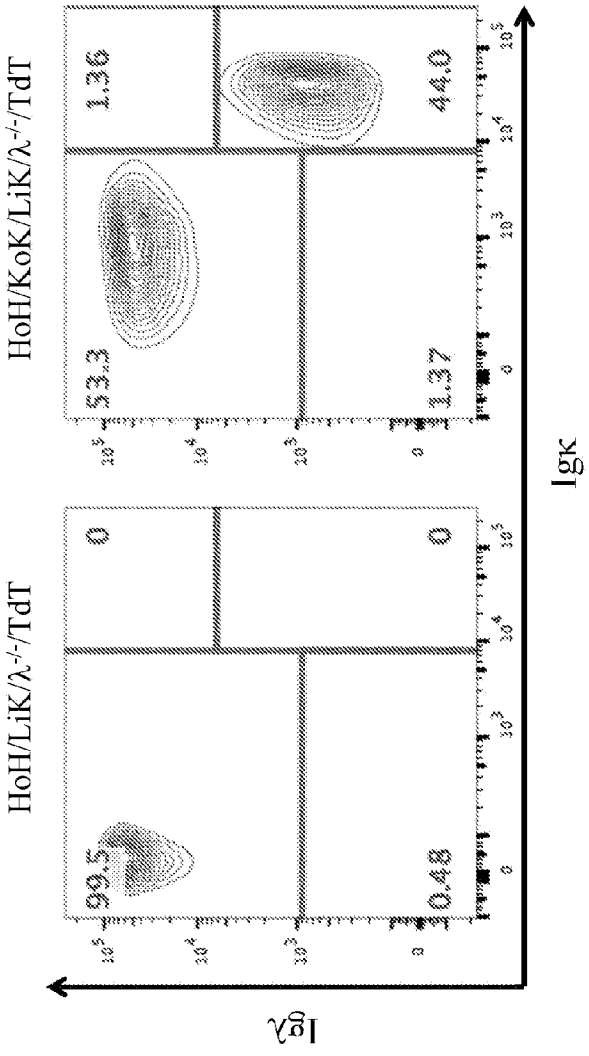


Figure 15

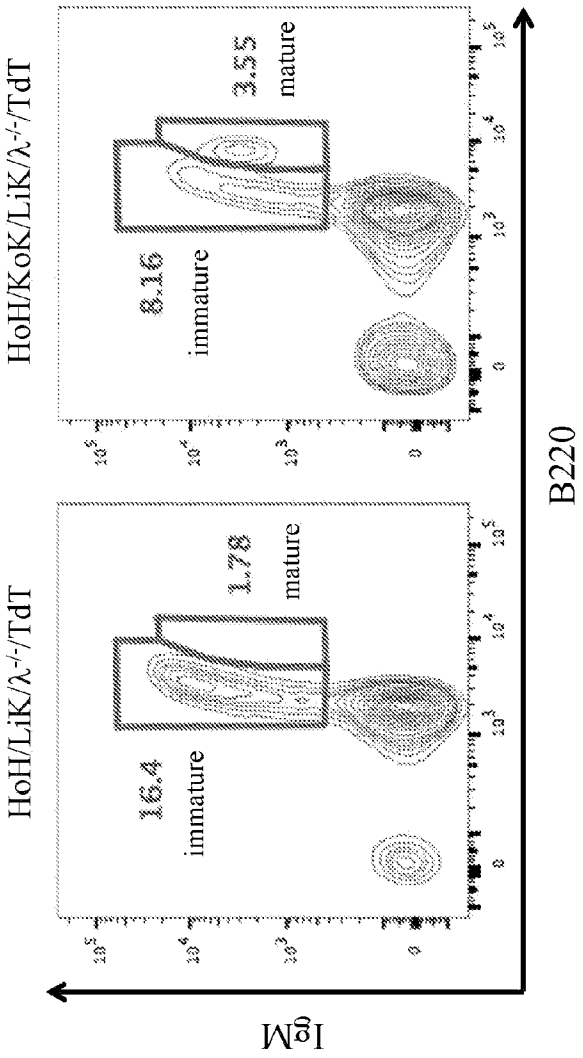

HoH/LiK/λ-/-/TdT
HoH/KoK/LiK/λ-/-/TdT
16.4
immature
1.78
mature
8.16
immature
3.35
mature
IgM
B220

Figure 16

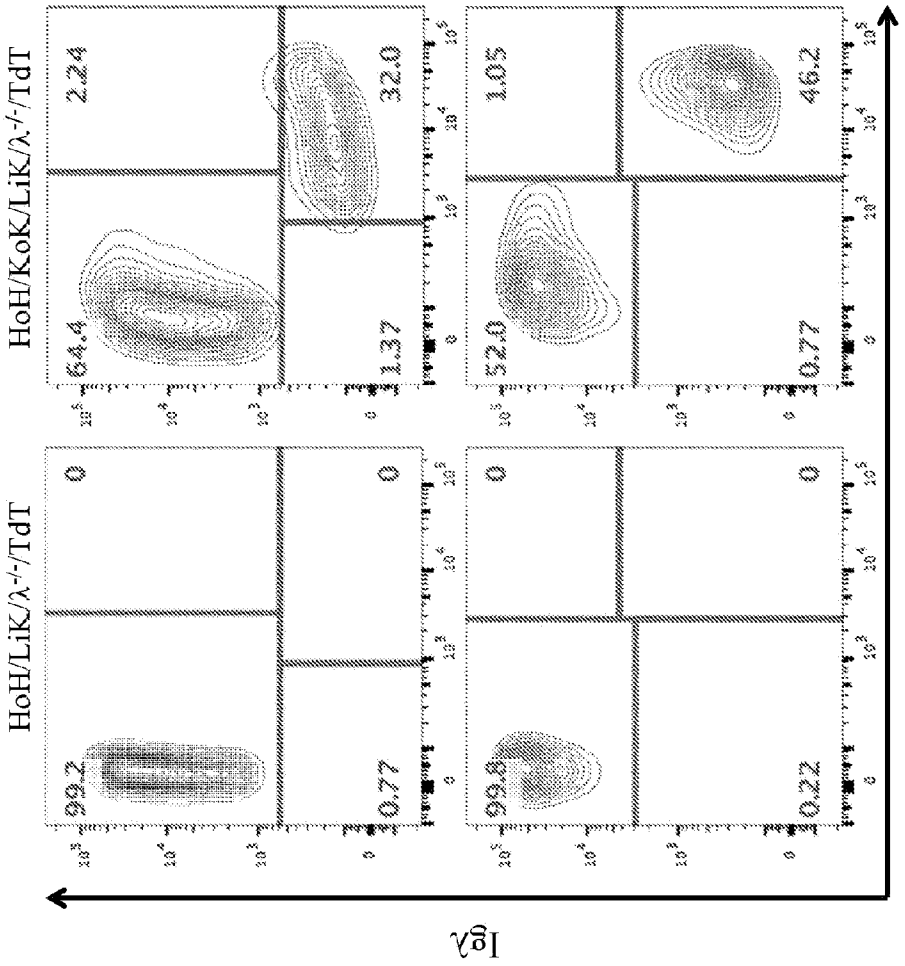

HoH/LiK/λ-/-/TdT
HoH/KoK/LiK/λ-/-/TdT
0
99.2
0.72
0
64.4
1.37
32.0
2.24
0
99.8
0.22
0
32.0
0.77
49.2
1.05
Igλ
Igκ

Figure 17

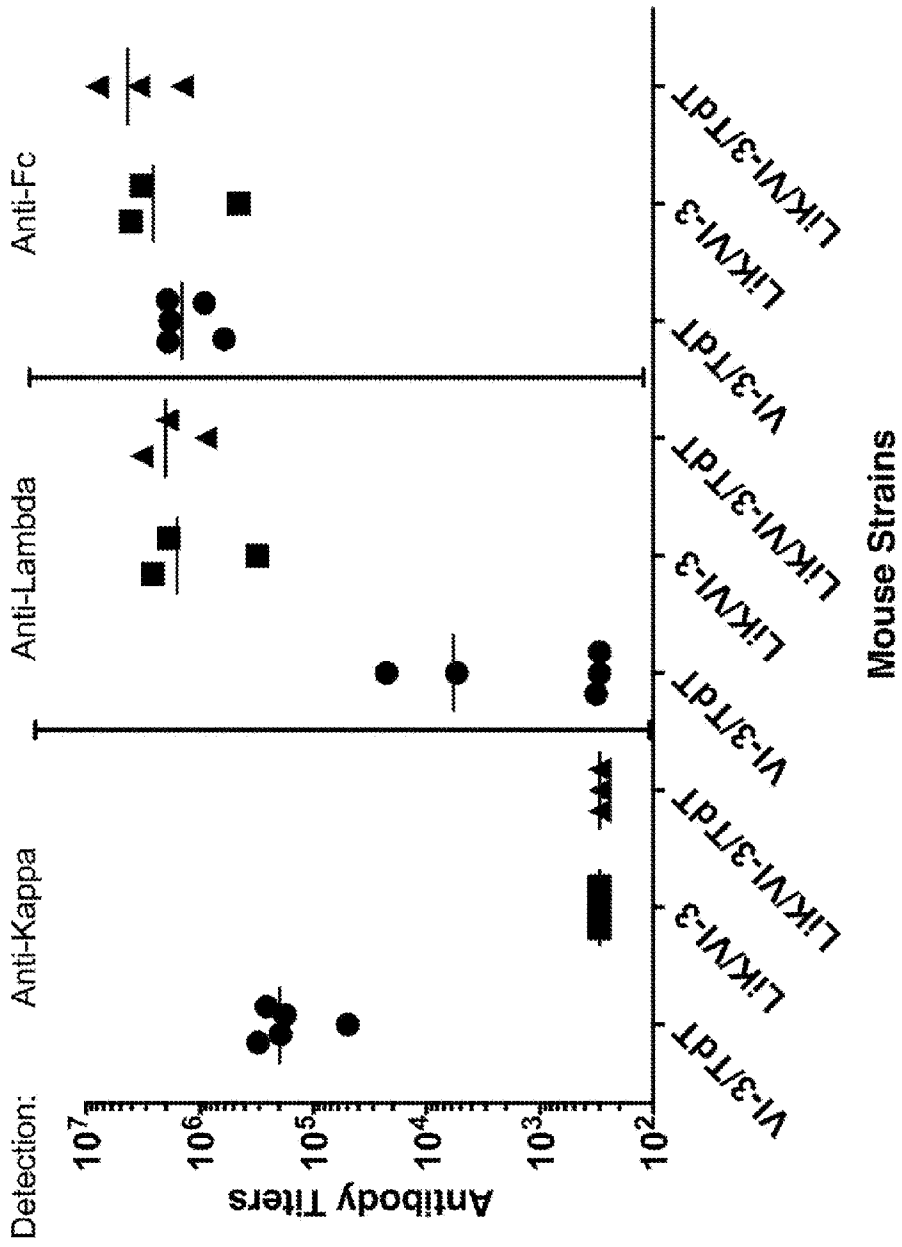

Detection:
Anti-Kappa
Anti-Lambda
Anti-Fc
Antibody Titers
10^7
10^6
10^5
10^4
10^3
10^2
Vl-3/Tdt
Li/KVl-3
Vl-3/Tdt
Li/KVl-3/Tdt
Vl-3/Tdt
Li/KVl-3
Vl-3/Tdt
Li/KVl-3/Tdt
Vl-3/Tdt
Li/KVl-3
Vl-3/Tdt
Li/KVl-3/Tdt
Mouse Strains

Figure 18

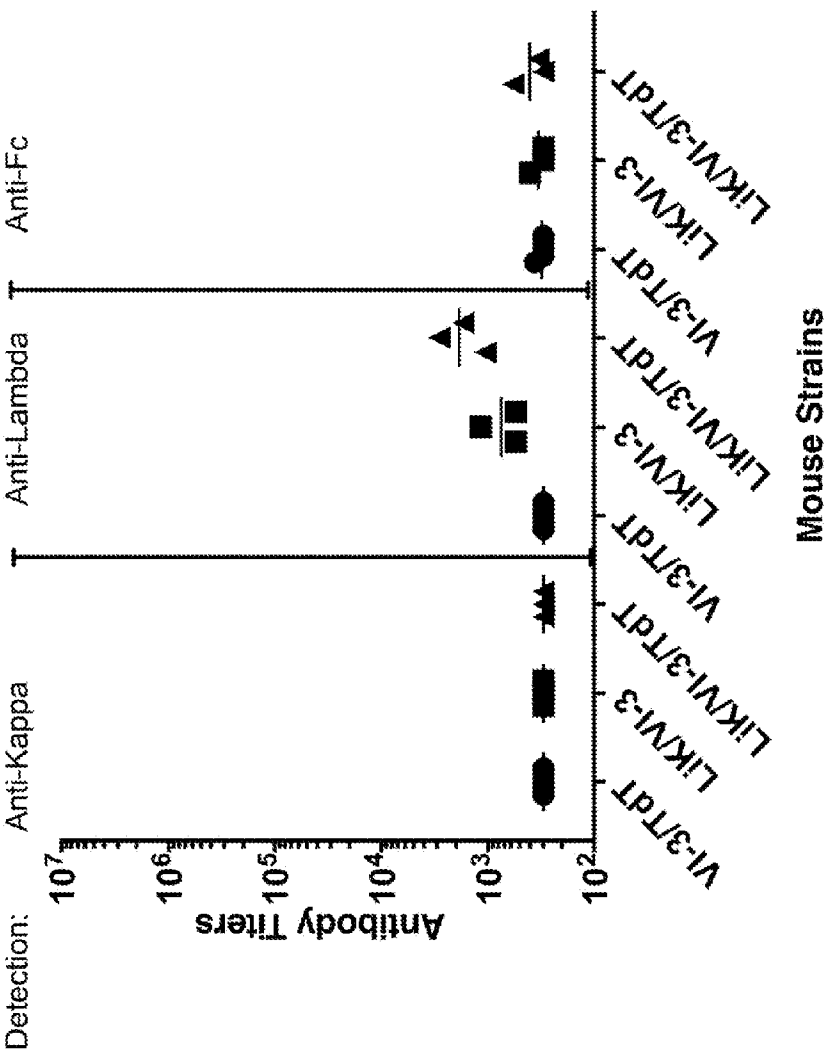

Detection:
Anti-Kappa
Anti-Lambda
Anti-Fc
Antibody Titers
10^7
10^6
10^5
10^4
10^3
10^2
Vl-3/TdT
LiKVl-3
Vl-3/TdT
LiKVl-3
Vl-3/TdT
LiKVl-3
Vl-3/TdT
LiKVl-3
Mouse Strains

Figure 19

Portion of Endogenous Human λ Light Chain Locus

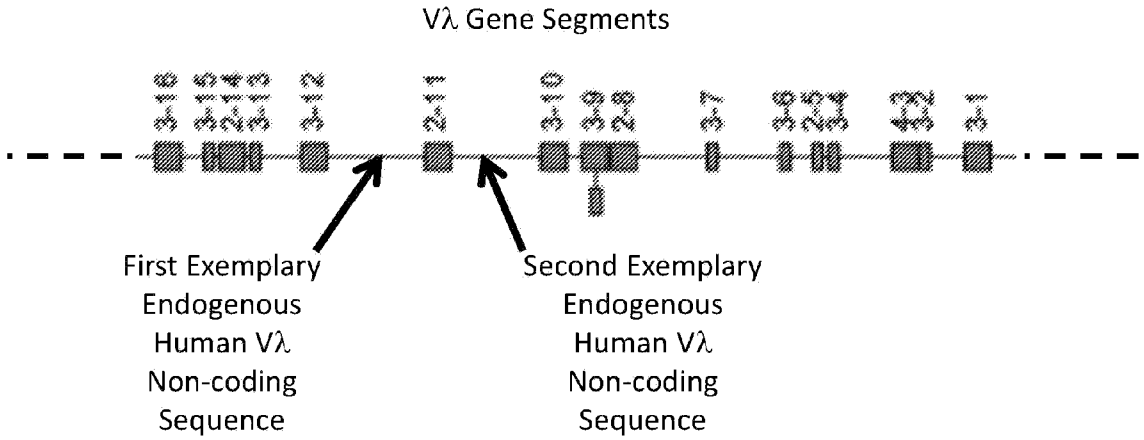


Figure 20

Portion of Endogenous Human κ Light Chain Locus

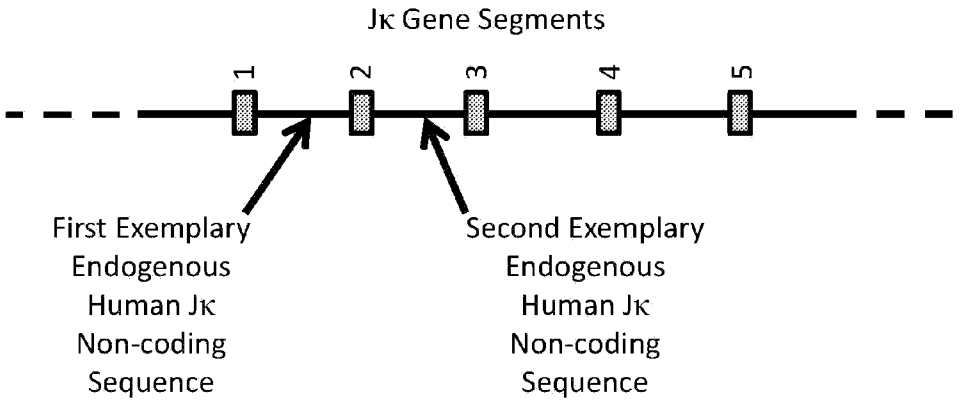


Figure 21

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.