IN THE HIGH COURT OF JUSTICE CHANCERY DIVISION PATENTS COURT

Claim Nos. <u>11P-2013-000001</u>24P-12-E0-1269 & <u>11P-2014-000001</u>44P-14-409007

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in EIP-3013-000001197-13F04260

(2) NOVO NORDISK A/S

Defendant in 11P-2014-00000111P-2-4A00002

ANNEX 1 TO AMENDED STATEMENT OF REASONS

CLAMISREQUEST 1

- 1. A method of modifying an endogenous immunoglobulin variable region gene locus in an isolated mouse embryonic stem (ES) cell by an *in situ* replacement of the endogenous locus with an orthologous human gene locus or by an *in situ* replacement of one or more V and J, or V, D, and J gene segments of the endogenous locus with orthologous human V and J, or V, D and J gene segments, to ereate a modified incumoglobulin locus that produces hybrid antibodics containing human variable regions and mouse constant regions, said method comprising:
 - a) obtaining a large cloned genomic fragment greater than 20kb containing orthologous human V and J, or V, D, and J gene segments;
 - b) using bacterial homologous recombination to genetically modify the cloned genomic fragment of (a) to create a large targeting vector for use in a mouse ES cell (LTVEC);
 - c) introducing the LTVEC of (b) into a mouse ES cell to replace said endogenous immunoglobulin variable gene locus or said one or more V and J, or V, D, and J segments thereof in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments; and
 - d) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (c) to identify a mouse ES cell in which said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D and J segments thereof have been replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.

2. The method of claim 1 further comprising:

e) obtaining a large cloned genomic fragment greater than 20kb containing
 V and J or V, D and J gene segments and that differs from the fragment of
 (a);

- using bacterial homologous recombination to genetically modify the cloned genomic fragment of (e) to create a second LTVEC;
- g) introducing the second LTVEC of (f) into the mouse ES cell identified in step (d) to replace said endogenous immunoglobulin variable gene locus or said one or more V and J, or V, D, and J segments thereof in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments; and
- h) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (g) to identify a mouse ES cell in which said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D and J segments thereof have been replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.
- 3. The method of claim 2 wherein steps (e) through (h) are repeated until the endogenous immunoglobulin variable region gene locus is replaced in whole with an orthologous human gene locus.
- 4. The method of any one of claims 1 to 3 wherein the immunoglobulin variable gene locus is a locus selected from the group consisting of:
 - a) a variable gene locus of the kappa light chain;
 - b) a variable gene locus of the lambda light chain; and
 - c) a variable gene locus of the heavy chain.
- 5. The method of any one of the preceding claims wherein the quantitative assay comprises quantitative PCR, FISH, comparative genomic hybridization, isothermic DNA amplification, or quantitative hybridization to an immobilized probe.
- 6. The method of claim 5 wherein the quantitative PCR comprises TaqMan® technology or quantitative PCR using molecular beacons.

- 7----The method of any-one of the proceeding claims—wherein the LTVEC comprises houselegy arms that total grooter than 20 kd;
- 57. The method of any one of the preceding claims wherein the large cloned genomic fragment is greater than 100 kb.
 - A method of modifying an endogenous immunoglobulin variable region gene locus by an in situ replacement of the endogenous locus with an orthologous human gene locus or by an in situ replacement of one or more V and J. or V, D and J gene segments of the endogenous locus with orthologous human V and J, or V, D and J gene segments, to create a modified incremoslobuling locus that produces hybrid antibodies containing human variable regions and manual constant regions, said method comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;
 - b) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region and a downstream homology arm within the variable gene locus;
 - c) introducing the LTVECs of (a) and (b) into a mouse ES cell;
 - d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (c) in which the sitespecific recombination sites flank the endogenous variable region gene locus;
 - e) creating a vector containing the site-specific recombination sequences flanking all or part of the orthologous <u>human</u> gene locus; and
 - f) introducing the vector of (e) into a mouse ES cell identified in step (d) such that, through recombination, said endogenous immunoglobulin

variable region gene locus or said one or more V and J, or V, D, and J segments thereof are replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.

- 409. The method of claim 98, wherein the site-specific recombination sites are selected from LoxP, Lox511 and Lox2272.
- 44— The mothed-of stain-9 or 10 a herein and LTVES cash semprise homelogy ones that total greater-than-204th-
- 1210. The method of may conservation 9 to 448 or 9, wherein said LTVECs are greater than 100 kb.
- 1311. A genetically modified hybrid immunoglobulin gene locus obtainable by the method of any one of the preceding claims.
- Fig. A genetically modified eukaryotic cell or a mouse comprising a genetically modified immunoglobulin variable region locus obtainable by the method of any one of the preceding claims in situ in place of the endogenous immunoglobulin variable region gene locus.
- 4313. A mouse embryonic stem (ES) cell containing a genetically modified immunoglobulin variable region gene locus obtainable by the method of any one of claims 1 to 43-10 in situ in place of the endogenous immunoglobulin variable region gene locus.
- region locus or one or more V, D and J gene segments thereof are replaced in situ with a human heavy chain variable gene locus or one or more V, D and J gene segments thereof; or the mouse kappa light chain variable region locus or one or more V and J gene segments thereof; or the mouse kappa light chain variable region locus or one or more V and J gene segments thereof are replaced in situ with a human kappa light chain variable region locus or one or more V and J gene segments thereof; or the mouse lambda light chain variable region locus or one or more V and J gene

segments thereof are replaced in situ with a human lambda light chain variable region locus or one or more V and J gene segments thereof.

- 4715. A mouse ES cell of claim 45-13 wherein the heavy and light chain variable region gene loci are replaced in situ with their human orthologs.
- #836. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous gene locus flanked downstream or upstream or both downstream and upstream by a site-specific recombination site comprising:
 - a) creating a LTVEC greater than 20 kb comprising the site-specific recombination site, a downstream homology arm containing a region that flanks the 3' end of the endogenous gene locus region and an upstream homology arm within the locus; and/or creating a LTVEC greater than 20 kb comprising the site-specific recombination site, an upstream homology arm containing a region that flanks the 5' end of the endogenous gene locus region and a downstream homology arm within the locus;
 - b) introducing the LTVEC or LTVECs of (a) into a mouse EO cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify a mouse ES cell in (b) in which the endogenous gene locus is flanked downstream or upstream or both downstream and upstream by the site-specific recombination site.
- 49: His method of dain-18, who win-sauld IVEC(s) comprise homology-arms that total-groups than 20 lds.
- The method of claim 18-or-1916 wherein said LTVEC(s) are greater than 100 kb.
- 413. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by a site-specific recombination site comprising:

- a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;
- b) introducing the LTVEC of (a) into a mouse ES cell; and
- c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (b) in which the sitespecific recombination site flanks the downstream end of the endogenous immunoglobulin variable gene locus.
- F2- The method of chim-11-whomin-seld-14 belonaprises he mology agains that total-properties 104b.
- 2319. The method of claim 24-01-2213, wherein said LTVEC is greater than 100 kb.
- 2420. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream homology arm within the locus;
 - b) introducing the LTVEC of (a) into a mouse ES cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (b) in which the sitespecific recombination sites flank the upstream end of the endogenous immunoglobulin variable region gene locus.

25.- The method of claim-24, wherein said-LTVEC comprises beautogy-amediated total-greater than 20 kb.

- 2621. The method of claim 24 or 2520, wherein said LTVEC is greater than 100 kb.
- A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region, and an upstream homology arm within the locus:
 - creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream arm within the locus;
 - c) introducing the LTVECs of (a) and (b) into a mouse ES cell; and
 - d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (c) in which the sitespecific recombination sites flank the endogenous immunoglobulin variable region gene locus.

38.--The method-of-claim-27.-wherein-oneh of said LEVECs egmpfise-homology cano-d-at-tenal-greater than 20-bla.

2923. The method of claim 27-61-2822, wherein said LTVECs are greater than 100 kb.

- 3024. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous gene locus flanked downstream or upstream or both downstream and upstream by a site-specific recombination site comprising:
 - a) creating a LTVEC greater than 20 kb comprising the site-specific recombination site, a downstream homology arm containing a region homologous to the 3'end of the endogenous gene locus region and an upstream homology arm within the locus; and or creating a LTVEC greater than 20 kb comprising the site-specific recombination site, an upstream homology arm containing a region homologous to the 5'end of the endogenous gene locus region and a downstream homology arm within the locus.
 - b) introducing the LTVEC or LTVECs of a) into a mouse ES cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify a mouse ES cell in b) in which the endogenous gene locus is flanked downstream or upstream or both downstream and upstream by a site-specific recombination site.
- 3425. The method of claim 3424, wherein the site-specific recombination site(s) are selected from LoxP, Lox511 and Lox2272.
- 32--- The method of claim 50 or 5 k-wherein suid-ESV ES-complise hemalogy-unus that deval-grower-than 304-b.

IN THE HIGH COURT OF JUSTICE CHANCERY DIVISION PATENTS COURT

Claim Nos. <u>HP-2013-000001</u>342-13404269 & <u>HP-2014-000001</u>447-14400967

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in HF-2013-000001107-1310-1269

(2) NOVO NORDISK A/S

<u>Defendant in 11P-2014-00000131P-43-400007</u>

ANNEX 2 TO AMENDED STATEMENT OF REASONS

GLAPASREQUEST 2

- 1. A method of modifying an endogenous immunoglobulin variable region gene locus in an isolated mouse embryonic stem (ES) cell by an *in situ* replacement of the endogenous locus with an orthologous human gene locus or by an *in situ* replacement of one or more V and J, or V, D, and J gene segments of the endogenous locus with orthologous human V and J, or V, D and J gene segments, to thereby operably link the orthologous human V and J, or V, D and J gene segments or orthologous human gene locus to an endogenous mouse constant region locus, said method comprising:
 - a) obtaining a large cloned genomic fragment greater than 20kb containing orthologous human V and J, or V, D, and J gene segments;
 - using bacterial homologous recombination to genetically modify the cloned genomic fragment of (a) to create a large targeting vector for use in a mouse ES cell (LTVEC);
 - c) introducing the LTVEC of (b) into a mouse ES cell to replace said endogenous immunoglobulin variable gene locus or said one or more V and J. or V. D. and J segments thereof in situ with the orthologous human gene locus or the orthologous human V and J. or V. D and J gene segments; and
 - d) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (c) to identify a mouse ES cell in which said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D and J segments thereof have been replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.

2. The method of claim 1 further comprising:

e) obtaining a large cloned genomic fragment greater than 20kb containing V and J or V, D and J gene segments and that differs from the fragment of (a);

- using bacterial homologous recombination to genetically modify the cloned genomic fragment of (e) to create a second LTVEC;
- g) introducing the second LTVEC of (f) into the mouse ES cell identified in step (d) to replace said endogenous immunoglobulin variable gene locus or said one or more V and J, or V, D, and J segments thereof in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments; and
- h) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (g) to identify a mouse ES cell in which said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D and J segments thereof have been replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.
- 3. The method of claim 2 wherein steps (e) through (h) are repeated until the endogenous immunoglobulin variable region gene locus is replaced in whole with an orthologous human gene locus.
- 4. The method of any one of claims 1 to 3 wherein the immunoglobulin variable gene locus is a locus selected from the group consisting of:
 - a) a variable gene locus of the kappa light chain;
 - b) a variable gene locus of the lambda light chain; and
 - c) a variable gene locus of the heavy chain.
- 5. The method of any one of the preceding claims wherein the quantitative assay comprises quantitative PCR, FISH, comparative genomic hybridization, isothermic DNA amplification, or quantitative hybridization to an immobilized probe.
- 6. The method of claim 5 wherein the quantitative PCR comprises TaqMan® technology or quantitative PCR using molecular beacons.

7--- The method of any one of the proceeding claims wherein the LTMEC comprises homelogy-arms that total greater than 20-kb.

3-7 The method of any one of the preceding claims wherein the large cloned genomic fragment is greater than 100 kb.

A method of modifying an endogenous immunoglobulin variable region gene locus by an *in situ* replacement of the endogenous locus with an orthologous language gene locus or by an *in situ* replacement of one or more V and I, or V, D and I gene segments of the endogenous locus with orthologous tanguage V and I, or V, D and I gene segments, to thereby operably link the orthologous human V and I, or V, D and I gene segments or orthologous human gene locus to an analogous process constant region locus, said method comprising:

- a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;
- b) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region and a downstream homology arm within the variable gene locus;
- c) introducing the LTVECs of (a) and (b) into a mouse ES cell;
- d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (c) in which the sitespecific recombination sites tlank the endogenous variable region gene locus;
- e) creating a vector containing the site-specific recombination sequences flanking all or part of the orthologous frames gene locus; and
- f) introducing the vector of (e) into a mouse ES cell identified in step (d) such that, through recombination, said endogenous immunoglobulin

variable region gene locus or said one or more V and J, or V, D, and J segments thereof are replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.

- 199. The method of claim 98, wherein the site-specific recombination sites are selected from LoxP, Lox511 and Lox2272.
- II. The mothed of chim-Ver-Wahrrobesald ETAECs each comprise hemology sime-that-tank greater than-20 kb.
- 1210. The method of any concept claim 4-to-118 of 9, wherein said LTVECs are greater than 100 kb.
- 1311. A genetically modified hybrid immunoglobulin gene locus obtainable by the method of any one of the preceding claims.
- 1-12. A genetically modified eukaryotic cell or a mouse comprising a genetically modified immunoglobulin variable region locus obtainable by the method of any one of the preceding claims *in situ* in place of the endogenous immunoglobulin variable region gene locus.
- 1513. A mouse embryonic stem (ES) cell containing a genetically modified immunoglobulin variable region gene locus obtainable by the method of any one of claims 1 to 44 10 in situ in place of the endogenous immunoglobulin variable region gene locus.
- 1614. A mouse ES cell of claim 15 13 wherein the mouse heavy chain variable region locus or one or more V, D and J gene segments thereof are replaced in situ with a human heavy chain variable gene locus or one or more V, D and J gene segments thereof; or the mouse kappa light chain variable region locus or one or more V and J gene segments thereof are replaced in situ with a human kappa light chain variable region locus or one or more V and J gene segments thereof; or the mouse lambda light chain variable region locus or one or more V and J gene

segments thereof are replaced in situ with a human lambda light chain variable region locus or one or more V and J gene segments thereof.

- 4715. A mouse ES cell of claim 45-13 wherein the heavy and light chain variable region gene loci are replaced *in situ* with their human orthologs.
- 1416. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous gene locus flanked downstream or upstream or both downstream and upstream by a site-specific recombination site comprising:
 - a) creating a LTVEC greater than 20 kb comprising the site-specific recombination site, a downstream homology arm containing a region that flanks the 3' end of the endogenous gene locus region and an upstream homology arm within the locus; and/or creating a LTVEC greater than 20 kb comprising the site-specific recombination site, an upstream homology arm containing a region that flanks the 5' end of the endogenous gene locus region and a downstream homology arm within the locus;
 - b) introducing the LTVEC or LTVECs of (a) into a mouse EO cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify a mouse ES cell in (b) in which the endogenous gene locus is flanked downstream or upstream or both downstream and upstream by the site-specific recombination site.
- 40- Hie-method of elaint-PS-vdrorein said-EEVEG(s) comprise homelagy arms that total-greater tion 20 kb.
- The method of claim 48-02-4016 wherein said LTVEC(s) are greater than 100 kb.
- 2413. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by a site-specific recombination site comprising:

- a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;
- b) introducing the LTVEC of (a) into a mouse ES cell; and
- c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (b) in which the sitespecific recombination site flanks the downstream end of the endogenous immunoglobulin variable gene locus.
- 2?-- The smillion of claim-21 and beroin said-LT-VIX-compaises homelogy-assas-time treal-access there-20-bbs
- 2319. The method of claim 24 or 2918, wherein said LTVEC is greater than 100 kb.
- 2420. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site. an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream homology arm within the locus;
 - b) introducing the LTVEC of (a) into a mouse ES cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (b) in which the sitespecific recombination sites flank the upstream end of the endogenous immunoglobulin variable region gene locus.

- 25.-- The method of claim-2-k-wherein-soid-LTA-RC comprises homology arms that total-greater than 20 leb.
- 262). The method of claim 240r-2520, wherein said LTVEC is greater than 100 kb.
- 2722. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region, and an upstream homology arm within the locus;
 - b) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream ann within the locus;
 - c) introducing the LTVECs of (a) and (b) into a mouse ES cell; and
 - d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (c) in which the sitespecific recombination sites flank the endogenous immunoglobulin variable region gene locus.
- 28. The method of claim-22, wherein each of said-ETVh Green priso boundary arms that total greater than 2006.
- 2023. The method of claim 37442322, wherein said LTVECs are greater than 100 kb.

- 3024. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous gene locus flanked downstream or upstream or both downstream and upstream by a site-specific recombination site comprising:
 - a) creating a LTVEC greater than 20 kb comprising the site-specific recombination site, a downstream homology arm containing a region homologous to the 3'end of the endogenous gene locus region and an upstream homology arm within the locus; and/or creating a LTVEC greater than 20 kb comprising the site-specific recombination site, an upstream homology arm containing a region homologous to the 5'end of the endogenous gene locus region and a downstream homology arm within the locus.
 - b) introducing the LTVEC or LTVECs of a) into a mouse ES cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify a mouse ES cell in b) in which the endogenous gene locus is flanked downstream or upstream or both downstream and upstream by a site-specific recombination site.
- 3425. The method of claim 3624, wherein the site-specific recombination site(s) are selected from LoxP, Lox511 and Lox2272.

3-2.— Phomothres betains 20-or 34; wherein said LEV 53; conquise homology urms that total greater shan 20-bb.

IN THE HIGH COURT OF JUSTICE CHANCERY DIVISION PATENTS COURT

Claim Nos. <u>IIP-2013-0000011IP-13-F04269 & IIP-2014-000001IIP-14-400007</u>

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in HP-2013-0000011H-13F04269

(2) NOVO NORDISK A/S

Defendant in 117-2014-000001112-1-1-000003

ANNEX 3 TO AMENDED STATEMENT OF REASONS

GLAIMSREQUEST 3

- 1. A method of modifying an endogenous immunoglobulin variable region gene locus in an isolated mouse embryonic stem (ES) cell by an in situ replacement of the endogenous locus with an orthologous human gene locus or by an in situ replacement of one or more V and J, or V, D, and J gene segments of the endogenous locus with orthologous human V and J, or V, D and J gene segments, to create a modified introduced by locus that produces having antibodies containing human variable regions and mouse constant regions, but that does not produce fully human applibadies, said method comprising:
 - a) obtaining a large cloned genomic fragment greater than 20kb containing orthologous human V and J, or V, D, and J gene segments;
 - using bacterial homologous recombination to genetically modify the cloned genomic fragment of (a) to create a large targeting vector for use in a mouse ES cell (LTVEC);
 - c) introducing the LTVEC of (b) into a mouse ES cell to replace said endogenous immunoglobulin variable gene locus or said one or more V and J, or V, D, and J segments thereof in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments; and
 - d) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (c) to identify a mouse ES cell in which said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D and J segments thereof have been replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.

2. The method of claim 1 further comprising:

e) obtaining a large cloned genomic fragment greater than 20kb containing
 V and J or V. D and J gene segments and that differs from the fragment of
 (a):

- using bacterial homologous recombination to genetically modify the cloned genomic fragment of (e) to create a second LTVEC;
- g) introducing the second LTVEC of (f) into the mouse ES cell identified in step (d) to replace said endogenous immunoglobulin variable gene locus or said one or more V and J, or V, D, and J segments thereof in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments; and
- h) using a quantitative assay to detect modification of allele (MOA) in the mouse ES cell of (g) to identify a mouse ES cell in which said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D and J segments thereof have been replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.
- 3. The method of claim 2 wherein steps (e) through (h) are repeated until the endogenous immunoglobulin variable region gene locus is replaced in whole with an orthologous human gene locus.
- 4. The method of any one of claims 1 to 3 wherein the immunoglobulin variable gene locus is a locus selected from the group consisting of:
 - a) a variable gene locus of the kappa light chain;
 - b) a variable gene locus of the lambda light chain; and
 - c) a variable gene locus of the heavy chain,
- 5. The method of any one of the preceding claims wherein the quantitative assay comprises quantitative PCR, FISH, comparative genomic hybridization, isothermic DNA amplification, or quantitative hybridization to an immobilized probe.
- 6. The method of claim 5 wherein the quantitative PCR comprises TaqMan® technology or quantitative PCR using molecular beacons.

7.— The method of any one of the proceding claims wherein the LTVEC comprises homology arms that total greater than 20-bb.

*7. The method of any one of the preceding claims wherein the large cloned genomic fragment is greater than 100 kb.

A method of modifying an endogenous immunoglobulin variable region gene locus by an *in situ* replacement of the endogenous locus with an orthologous framen gene locus or by an *in situ* replacement of one or more V and J, or V, D and J gene segments of the endogenous locus with orthologous framen V and J, or V, D and J gene segments, to create a modified immunoglobulin locus that produces by brid antibodies containing human variable regions and mouse constant regions, but that does not produce fully human antibodies, said method comprising:

- a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;
- b) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region and a downstream homology arm within the variable gene locus;
- c) introducing the LTVECs of (a) and (b) into a mouse ES cell;
- d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (c) in which the sitespecific recombination sites flank the endogenous variable region gene locus;
- e) creating a vector containing the site-specific recombination sequences flanking all or part of the orthologous <u>human</u> gene locus; and

- f) introducing the vector of (e) into a mouse ES cell identified in step (d) such that, through recombination, said endogenous immunoglobulin variable region gene locus or said one or more V and J, or V, D, and J segments thereof are replaced in situ with the orthologous human gene locus or the orthologous human V and J, or V, D and J gene segments.
- 400. The method of claim 95, wherein the site-specific recombination sites are selected from LoxP, Lox511 and Lox2272.
- i is The method of claim A or 10 wherein soid LTV Rice each comprise homelogy arms that two legicies as then 2014s.
- 4210. The method of way one of claim 9-to-148 or 9, wherein said LTVECs are greater than 100 kb.
- 1311. A genetically modified hybrid immunoglobulin gene locus obtainable by the method of any one of the preceding claims.
- A genetically modified eukaryotic cell or a mouse comprising a genetically modified immunoglobulin variable region locus obtainable by the method of any one of the preceding claims *in situ* in place of the endogenous immunoglobulin variable region gene locus.
- 1513. A mouse embryonic stem (ES) cell containing a genetically modified immunoglobulin variable region gene locus obtainable by the method of any one of claims 1 to 1410 in situ in place of the endogenous immunoglobulin variable region gene locus.
- region locus or one or more V, D and J gene segments thereof are replaced in situ with a human heavy chain variable gene locus or one or more V, D and J gene segments thereof; or the mouse kappa light chain variable region locus or one or more V and J gene segments thereof; or the mouse kappa light chain variable region locus or one or more V and J gene segments thereof are replaced in situ with a human kappa light

chain variable region locus or one or more V and J gene segments thereof; or the mouse lambda light chain variable region locus or one or more V and J gene segments thereof are replaced *in situ* with a human lambda light chain variable region locus or one or more V and J gene segments thereof.

- $\frac{1715}{1}$. A mouse ES cell of claim $\frac{15}{15}$ wherein the heavy and light chain variable region gene loci are replaced in situ with their human orthologs.
- 46 19. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous gene locus flanked downstream or upstream or both downstream and upstream by a site-specific recombination site comprising:
 - a) creating a LTVEC greater than 20 kb comprising the site-specific recombination site, a downstream homology arm containing a region that flanks the 3' end of the endogenous gene locus region and an upstream homology arm within the locus; and/or creating a LTVEC greater than 20 kb comprising the site-specific recombination site, an upstream homology arm containing a region that flanks the 5' end of the endogenous gene locus region and a downstream homology arm within the locus;
 - b) introducing the LTVEC or LTVECs of (a) into a mouse EO cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify a mouse ES cell in (b) in which the endogenous gene locus is flanked downstream or upstream or both downstream and upstream by the site-specific recombination site.
- 19---Floorethod-ef-eloin-18--Aberoù-sal-LFWEC(s) compelso-brandlaguerans-urg tatul-grenor-tinn-20-kls.
- The method of claim 18-65-1916 wherein said LTVEC(s) are greater than 100 kb.

- 2416. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by a site-specific recombination site comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region and an upstream homology arm within the variable gene locus;
 - b) introducing the LTVEC of (a) into a mouse ES cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (b) in which the sitespecific recombination site flanks the downstream end of the endogenous immunoglobulin variable gene locus.
- 92.—The medical collection 24- wherein hald-IFVEC-comprises homelogy arms that total greater than 30 libs
- 23/9. The method of claim 24-or-2213, wherein said LTVEC is greater than 100 kb.
- 2420. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream homology arm within the locus;
 - b) introducing the LTVEC of (a) into a mouse ES cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (b) in which the site-

specific recombination sites flank the upstream end of the endogenous immunoglobulin variable region gene locus.

- 45 The method of chain-24-wherein said LTVEC comprises handlegy amos that total-greater than-20 like
- 2621. The method of claim 24 or 2520, wherein said LTVEC is greater than 100 kb.
- A method of creating, in a mouse embryonic stem (ES) cell, an endogenous immunoglobulin variable gene locus flanked by site-specific recombination sites comprising:
 - a) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, a downstream homology arm containing the region immediately adjacent to, but not including, the J segments of the immunoglobulin variable gene locus region, and an upstream homology arm within the locus:
 - b) creating a LTVEC greater than 20 kb comprising a site-specific recombination site, an upstream homology arm containing the region adjacent to the most distal V gene segment, but not containing any V gene segments of the immunoglobulin variable gene locus region, and a downstream arm within the locus;
 - c) introducing the LTVECs of (a) and (b) into a mouse ES cell; and
 - d) using a quantitative assay to detect modification of allele (MOA) in the variable gene locus to identify a mouse ES cell in (c) in which the sitespecific recombination sites flank the endogenous immunoglobulin variable region gene locus.
- 38.- -- Humuthind-of alaba 27.; wkeroja-osob-of-sajd 4,4MCCs eempriso hemejogy arms-that-total-grees arthat-20-kb;
- 2023. The method of claim 27-or-2822, wherein said LTVECs are greater than 100 kb.

- 2023. A method of creating, in a mouse embryonic stem (ES) cell, an endogenous gene locus flanked downstream or upstream or both downstream and upstream by a site-specific recombination site comprising:
 - a) creating a LTVEC greater than 20 kb comprising the site-specific recombination site, a downstream homology arm containing a region homologous to the 3'end of the endogenous gene locus region and an upstream homology arm within the locus; and/or creating a LTVEC greater than 20 kb comprising the site-specific recombination site, an upstream homology arm containing a region homologous to the 5'end of the endogenous gene locus region and a downstream homology arm within the locus.
 - b) introducing the LTVEC or LTVECs of a) into a mouse ES cell; and
 - c) using a quantitative assay to detect modification of allele (MOA) in the endogenous gene locus to identify a mouse ES cell in b) in which the endogenous gene locus is flanked downstream or upstream or both downstream and upstream by a site-specific recombination site.
- 34.25. The method of claim 30.24, wherein the site-specific recombination site(s) are selected from LoxP, Lox511 and Lox2272.
- 32— The maked of elabor-14 or 3 k-a teroin said-let-billis compaso homology-ands then with ground than-20 des

Claim Nos. <u>11P-2013-0000914IP-13F04269</u> & <u>11P-2014-0000014IP-14A40007</u>

IN THE HIGH COURT OF JUSTICE CHANCERY DIVISION PATENTS COURT

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in HP-2013-000001112-13F04269

(2) NOVO NORDISK A/S

Defendant in HP-2634-060001138-14-100002

AMENDED STATEMENT OF REASONS

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