IN THE HIGH COURT OF JUSTICE
CHANCERY DIVISION
PATENTS COURT

Claim Nos. HP-2013-000001~~HP-1350-1269~~ &
HP-2014-000001~~HP-14A05007~~

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in HP-2013-000001~~HP-1350-1269~~

(2) NOVO NORDISK A/S

Defendant in HP-2014-000001~~HP-14A05007~~

ANNEX 1 TO AMENDED STATEMENT OF REASONS

~~CLAIMS~~REQUEST 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 create a modified immunoglobulin locus that produces hybrid antibodies 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);

f) 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 preceding claims wherein the LTVEC comprises homology arms that total greater than 20 kb.~~

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

68. 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 immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse 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 site-specific 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 human 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.

~~11. The method of claim 9 or 10 wherein said LTVECs each comprise homology arms that total greater than 20 kb.~~

1210. The method of ~~any one of~~ claim ~~9 to 11~~ 98 or 9, wherein said LTVECs are greater than 100 kb.

1211. A genetically modified hybrid immunoglobulin gene locus obtainable by the method of any one of the preceding claims.

1412. 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 ~~12~~ 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.

~~17~~15. A mouse ES cell of claim ~~15~~13 wherein the heavy and light chain variable region gene loci are replaced *in situ* with their human orthologs.

~~18~~16. 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. The method of claim 18, wherein said LTVEC(s) comprise homology arms that total greater than 20 kb.~~

~~20~~17. The method of claim ~~18 or 19~~16 wherein said LTVEC(s) are greater than 100 kb.

~~21~~18. 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 site-specific recombination site flanks the downstream end of the endogenous immunoglobulin variable gene locus.

~~22. The method of claim 21, wherein said LTVEC comprises homology arms that total greater than 30 kb.~~

~~23~~19. The method of claim ~~21 or 22~~18, wherein said LTVEC is greater than 100 kb.

~~24~~20. 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.

~~25. The method of claim 24, wherein said LTVEC comprises homology arms that total greater than 20 kb.~~

2621. The method of claim ~~24 or 25~~20, wherein said LTVEC is greater than 100 kb.

~~27~~22. 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 site-specific recombination sites flank the endogenous immunoglobulin variable region gene locus.

~~28. The method of claim 27, wherein each of said LTVECs comprises homology arms that total greater than 20 kb.~~

2923. The method of claim ~~27 or 28~~22, 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.

3125. The method of claim 3024, wherein the site-specific recombination site(s) are selected from LoxP, Lox511 and Lox2272.

32. The method of claim 30 or 31, wherein said LTVEC comprises homology arms that total greater than 20 kb.

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Claim Nos. HP-2013-000031HP-13C04269 &
HP-2014-000001HP-14A00007

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in HP-2013-000031HP-13C04269

(2) NOVO NORDISK A/S

Defendant in HP-2014-000001HP-14A00007

ANNEX 2 TO AMENDED STATEMENT OF REASONS

~~CLAIMS~~REQUEST 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;

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);

f) 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 preceding claims wherein the LTVEC comprises homology arms that total greater than 20 kb.~~

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

9. 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 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) 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 site-specific 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 human 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.

109. The method of claim 98, wherein the site-specific recombination sites are selected from LoxP, Lox511 and Lox2272.

~~11. The method of claim 9 or 10, wherein said LTVECs each comprise homology arms that are greater than 20 kb.~~

1210. The method of ~~any one of~~ claim ~~9 to 11~~ 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.

1412. 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 ~~12~~ 10 *in situ* in place of the endogenous immunoglobulin variable region gene locus.

1614. A mouse ES cell of claim ~~13~~ 12 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 13 wherein the heavy and light chain variable region gene loci are replaced *in situ* with their human orthologs.

4816. 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. The method of claim 48 wherein said LTVEC(s) comprise homology arms that total greater than 20 kb.

5017. The method of claim 48 or 4916 wherein said LTVEC(s) are greater than 100 kb.

5118. 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 site-specific recombination site flanks the downstream end of the endogenous immunoglobulin variable gene locus.

~~22. The method of claim 21, wherein said LTVEC comprises homology arms that each are greater than 20 kb.~~

~~23~~22. The method of claim ~~21 or 22~~18, wherein said LTVEC is greater than 100 kb.

~~24~~23. 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.

~~25. The method of claim 24, wherein said LTVEC comprises homology arms that total greater than 20 kb.~~

2621. The method of claim ~~24 or 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 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 site-specific recombination sites flank the endogenous immunoglobulin variable region gene locus.

~~28. The method of claim 27, wherein each of said LTVECs comprise homology arms that total greater than 20 kb.~~

2923. The method of claim ~~27 or 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.

3125. The method of claim 3024, wherein the site-specific recombination site(s) are selected from LoxP, Lox511 and Lox2272.

32. The method of claim 30 or 31, wherein said LTVECs comprise homology arms that total greater than 20kb.

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Claim Nos. HP-2013-000001HP-13F04269 &
HP-2014-000001HP-14A00007

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in HP-2013-000001HP-13F04269

(2) NOVO NORDISK A/S

Defendant in HP-2014-000001HP-14A00007

ANNEX 3 TO AMENDED STATEMENT OF REASONS

CLAIMSREQUEST 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 immunoglobulin locus that produces hybrid antibodies containing human variable regions and mouse constant regions, but that does not produce fully human antibodies, 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);

f) 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 preceding claims wherein the LTVEC comprises homology arms that total greater than 20 kb.~~

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

~~48.~~ 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 immunoglobulin locus that produces hybrid 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 site-specific 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 human 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.

~~109~~. The method of claim ~~98~~, wherein the site-specific recombination sites are selected from LoxP, Lox511 and Lox2272.

~~110~~. The method of claim ~~9~~ wherein said LTVECs each comprise homology arms that total greater than 20 kb.

~~1210~~. The method of ~~any one of~~ claim ~~9 to 115~~ 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.

~~1412~~. 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 ~~14~~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.

1715. A mouse ES cell of claim 15-13 wherein the heavy and light chain variable region gene loci are replaced *in situ* with their human orthologs.

1816. 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. The method of claim 18 wherein said LTVEC(s) comprise homologous arms and total greater than 20 kb.

2017. The method of claim 18-or-1916 wherein said LTVEC(s) are greater than 100 kb.

~~218~~. 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 site-specific recombination site flanks the downstream end of the endogenous immunoglobulin variable gene locus.

~~22. The method of claim 21, wherein said LTVEC comprises homology arms that total greater than 20 kb.~~

~~219~~. The method of claim ~~21-or-218~~, wherein said LTVEC is greater than 100 kb.

~~220~~. 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.

~~25. The method of claim 24, wherein said LTVEC comprises homology arms that total greater than 20 kb.~~

2621. The method of claim ~~24 or 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 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 site-specific recombination sites flank the endogenous immunoglobulin variable region gene locus.

~~28. The method of claim 27, wherein each of said LTVECs comprise homology arms that total greater than 20 kb.~~

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

3023. 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.

3124. The method of claim 3023, wherein the site-specific recombination site(s) are selected from LoxP, Lox511 and Lox2272.

3225. The method of claim 3023, wherein the LTVEC comprises homology arms that total greater than 20 kb.

Claim Nos. HP-2013-000001HP-13F04269 &
HP-2014-000001HP-14A00007

IN THE HIGH COURT OF JUSTICE
CHANCERY DIVISION
PATENTS COURT

BETWEEN:

REGENERON PHARMACEUTICALS, INC.

Claimant

- and -

(1) KYMAB LIMITED

Defendant in HP-2013-000001HP-13F04269

(2) NOVO NORDISK A/S

Defendant in HP-2014-000001HP-14A00007

AMENDED STATEMENT OF REASONS

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