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Platzer et al.

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(54) **HUMANIZED IMMUNOGLOBULIN LOCI**

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(21) Appl. No.: **15/019,852**

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C12N 15/67	(2006.01)
C07K 16/00	(2006.01)
C07K 16/46	(2006.01)
C12N 15/85	(2006.01)
A01K 67/027	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/67*** (2013.01); ***A01K 67/0278*** (2013.01); ***C07K 16/00*** (2013.01); ***C07K 16/461*** (2013.01); ***C12N 15/8509*** (2013.01); *A01K 2207/15* (2013.01); *A01K 2217/00* (2013.01); *A01K 2227/105* (2013.01); *A01K 2227/107* (2013.01); *A01K 2267/01* (2013.01); *C07K 2317/24* (2013.01)

(58) **Field of Classification Search**

CPC A01K 67/0278; C07K 16/00
USPC 435/326, 328; 800/14
See application file for complete search history.

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Primary Examiner — Padmashri Ponnaluri

(57) **ABSTRACT**

The present invention concerns methods and means to produce humanized antibodies from transgenic non-human animals. The invention specifically relates to novel immunoglobulin heavy and light chain constructs, recombination and transgenic vectors useful in making transgenic non-human animals expressing humanized antibodies, transgenic animals, and humanized immunoglobulin preparations.

3 Claims, 49 Drawing Sheets

Specification includes a Sequence Listing.

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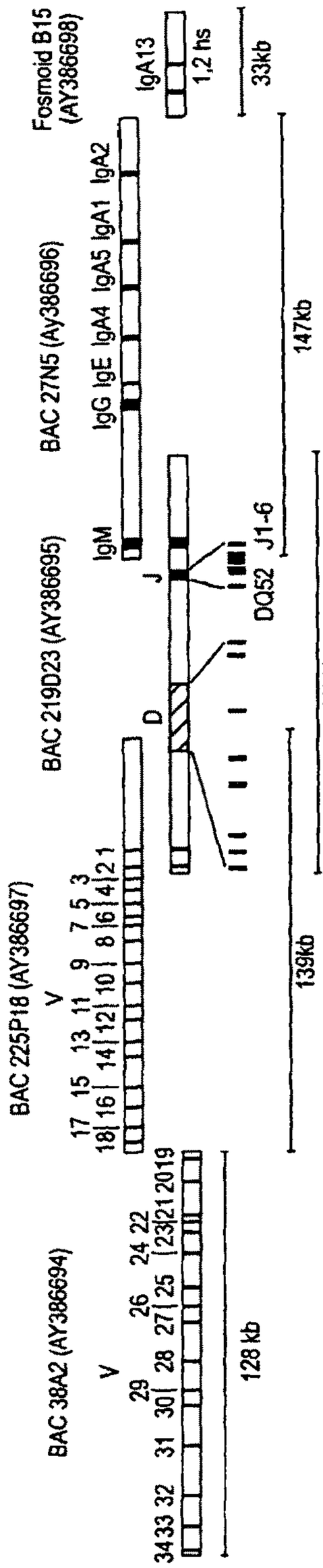
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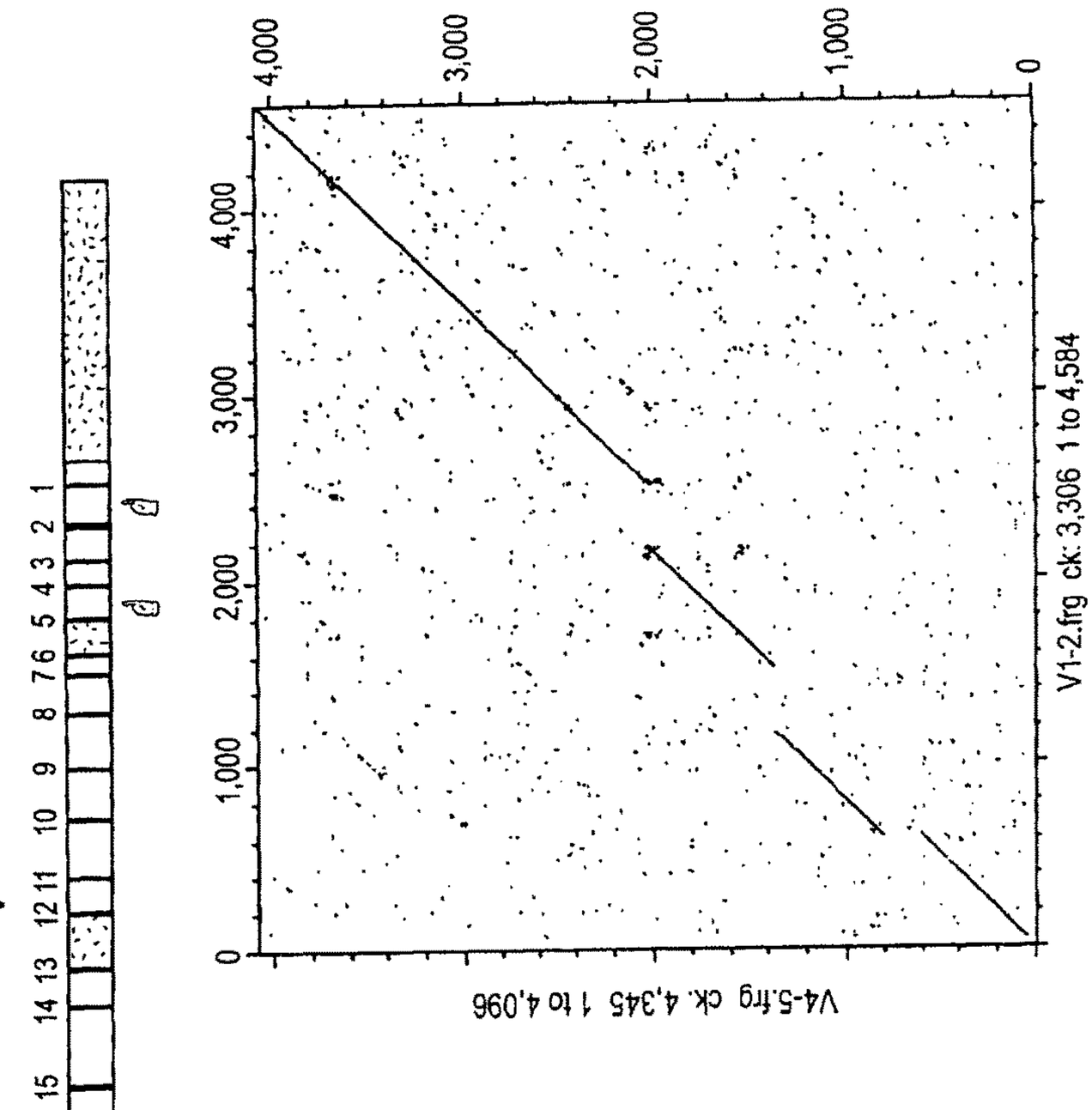
Figure 1

Figure 2



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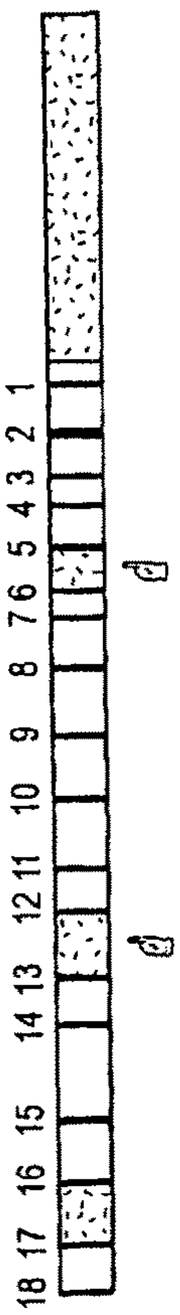

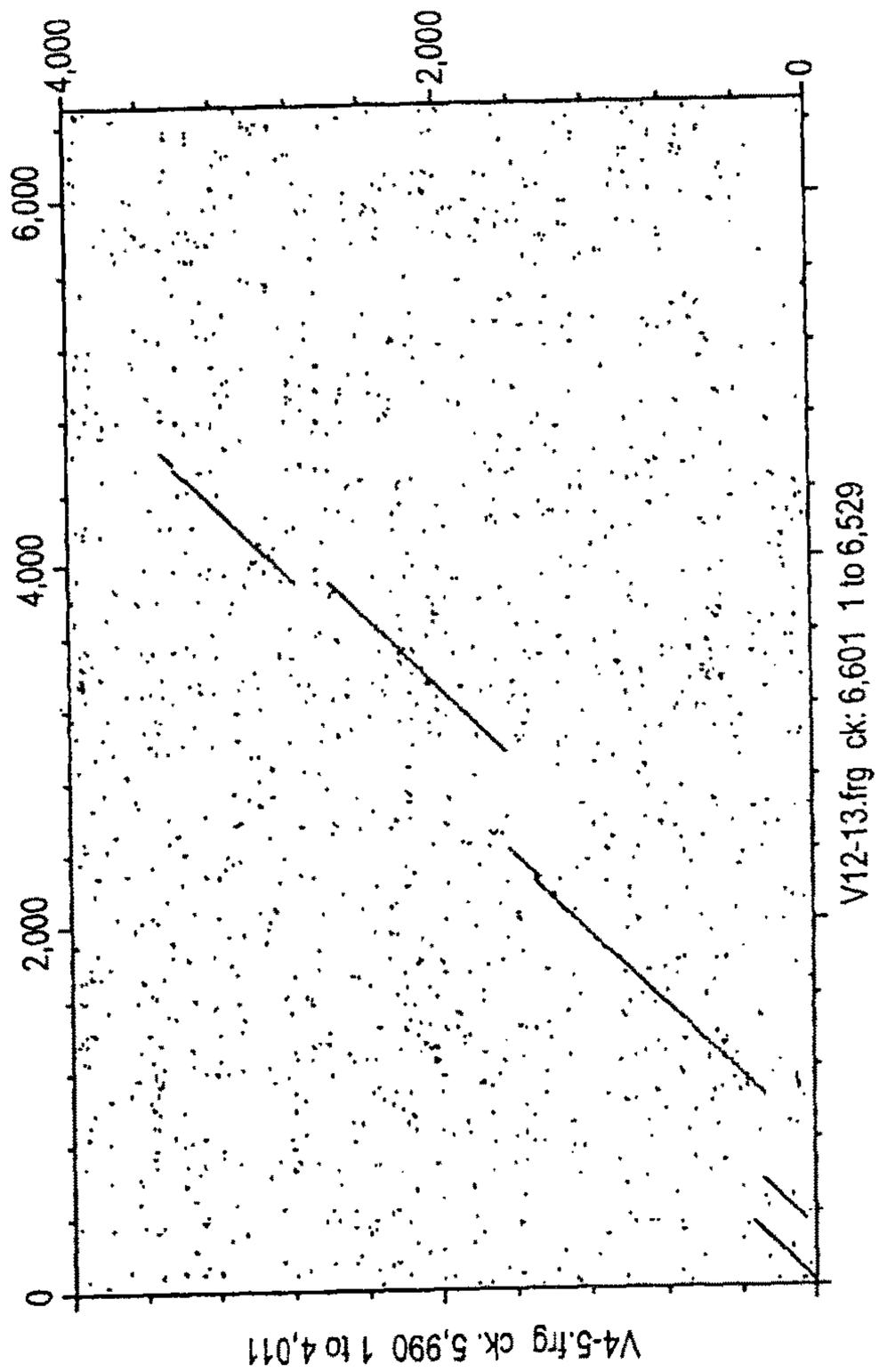

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V1-2	4584 bp
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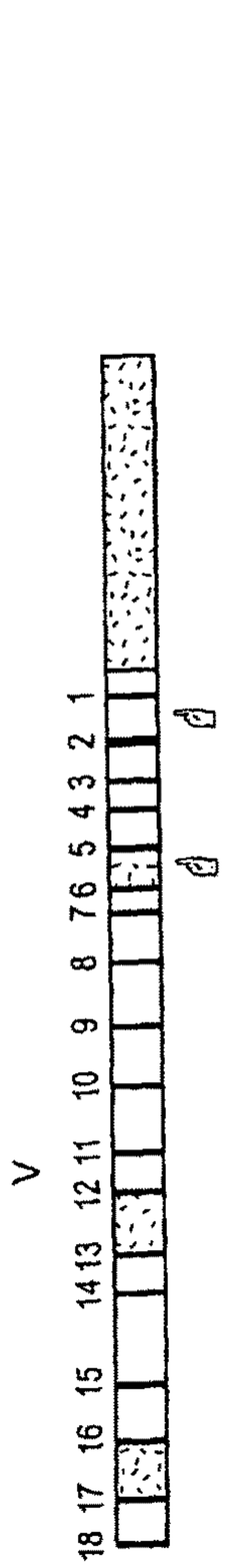

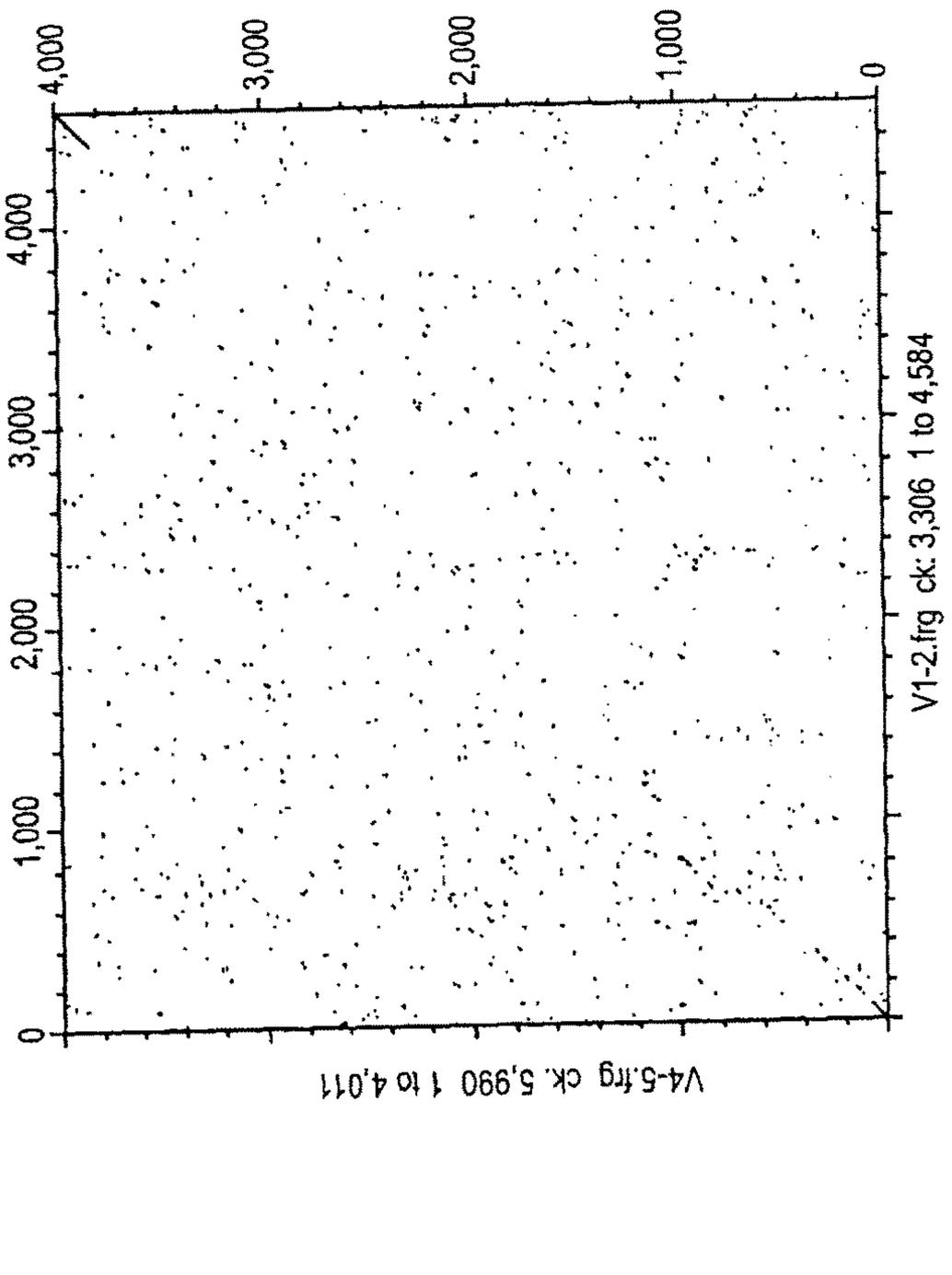

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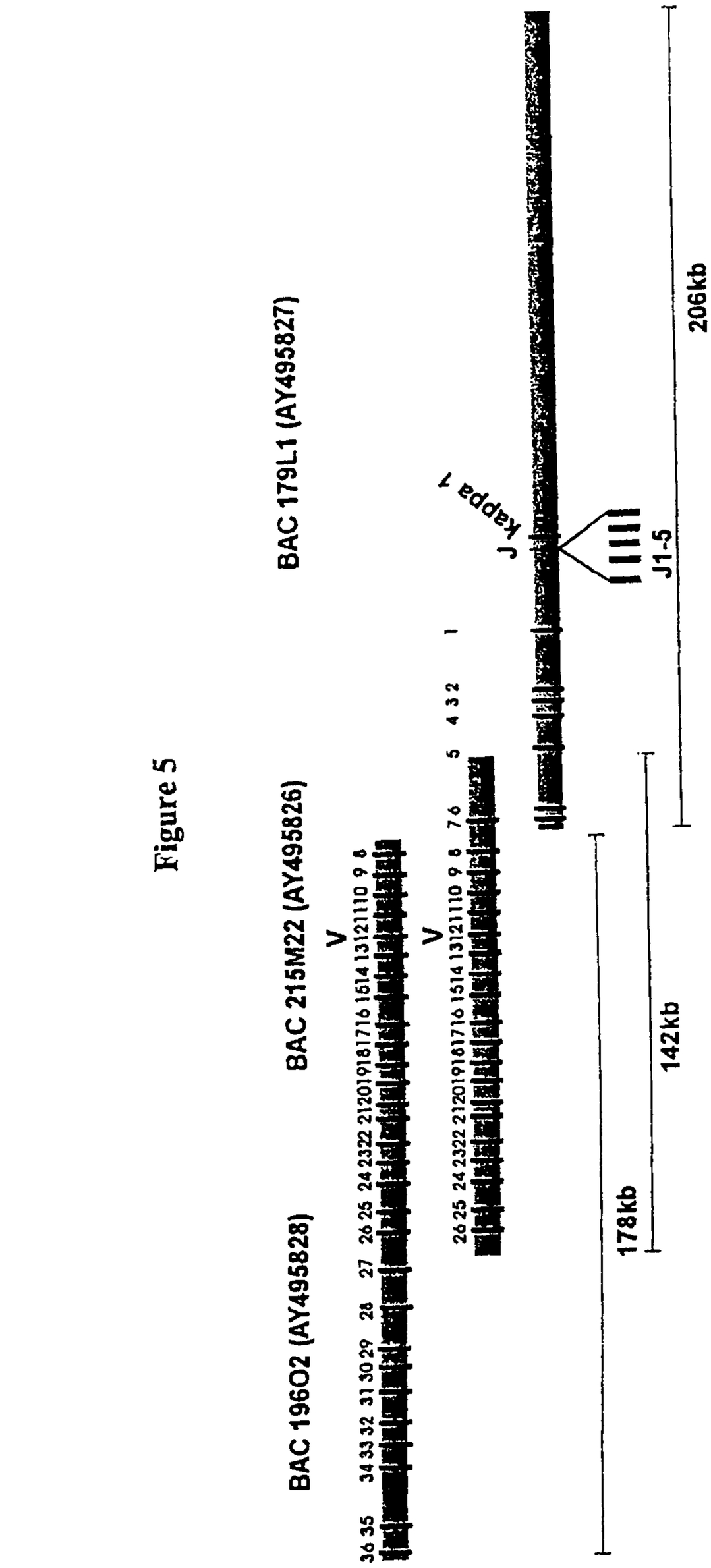
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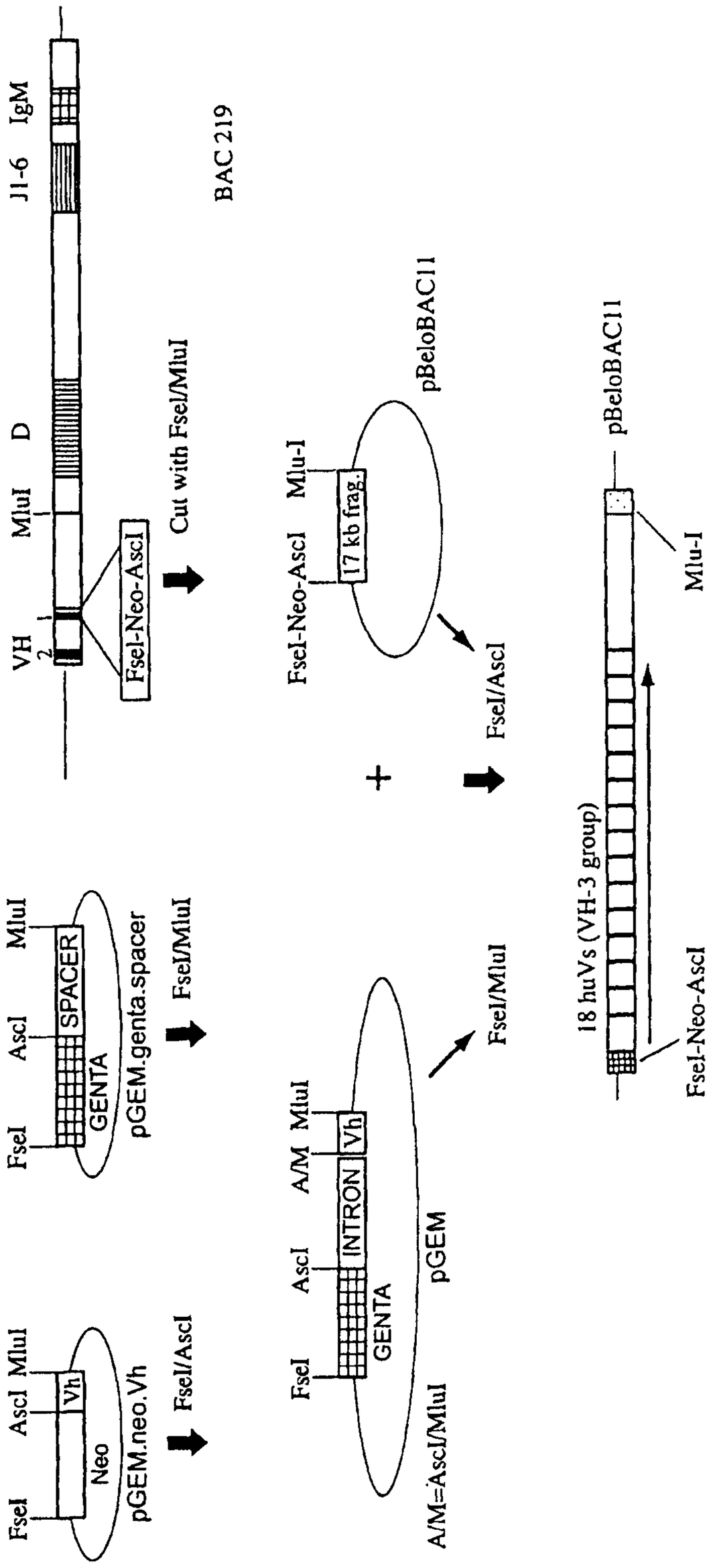
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FseI-Neo-AscI
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A/M
MluI
Vh
INTRON
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Mlu-I
FseI-Neo-AscI
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AscI
MluI
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Figure 7

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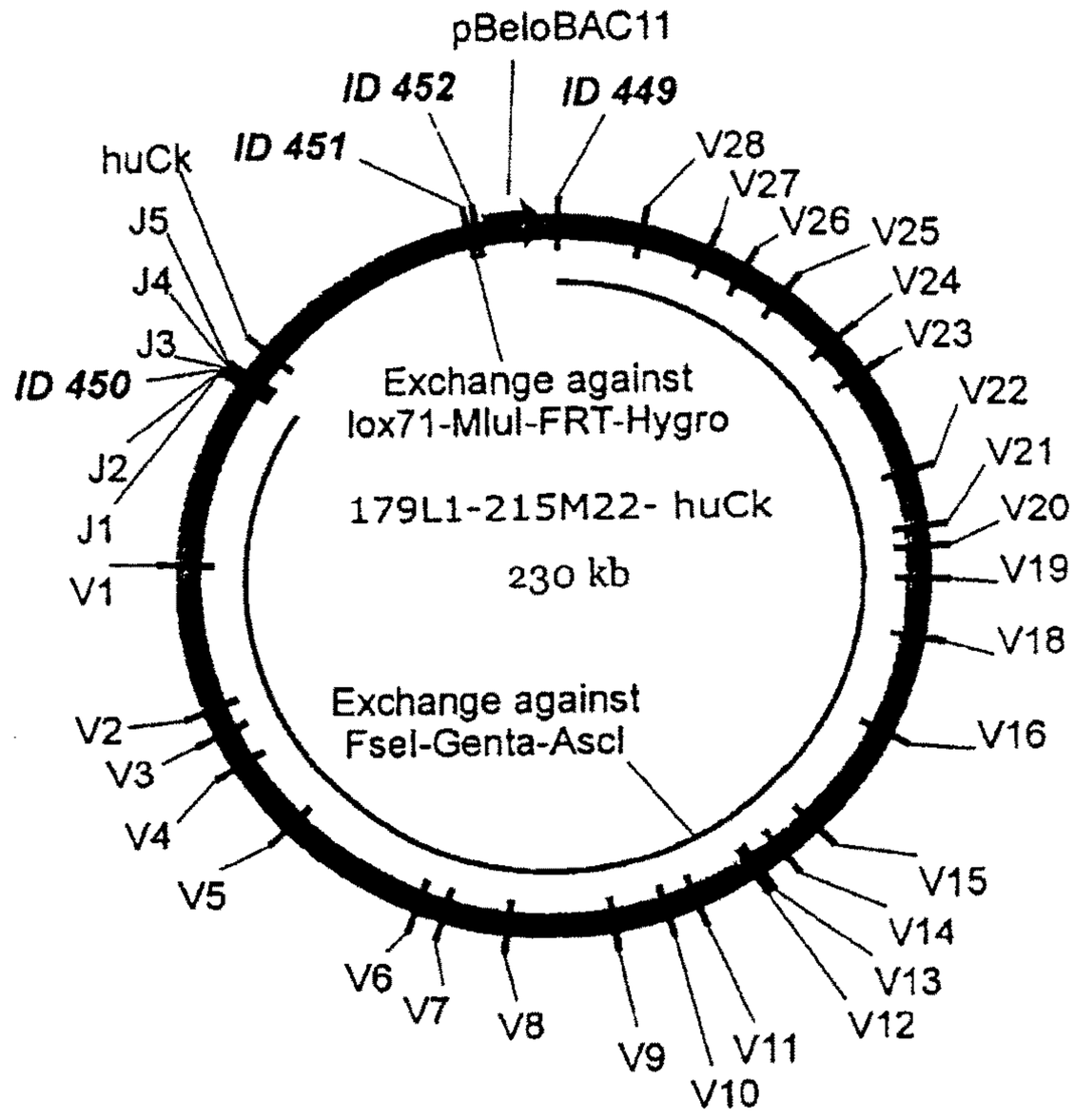
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V6
V5
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V3
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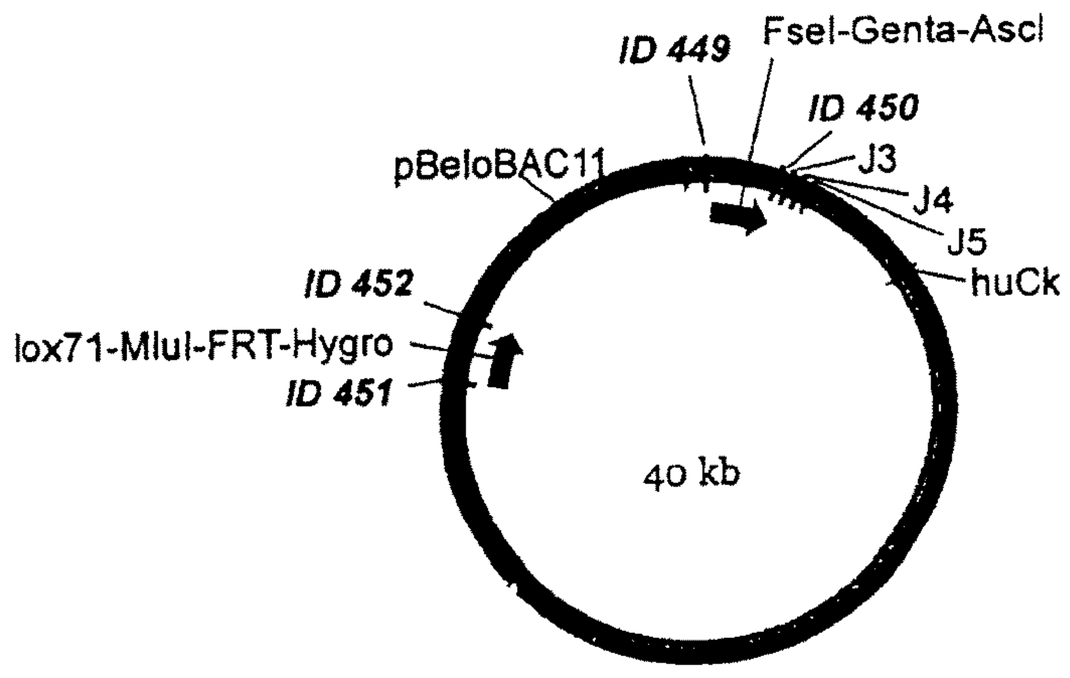
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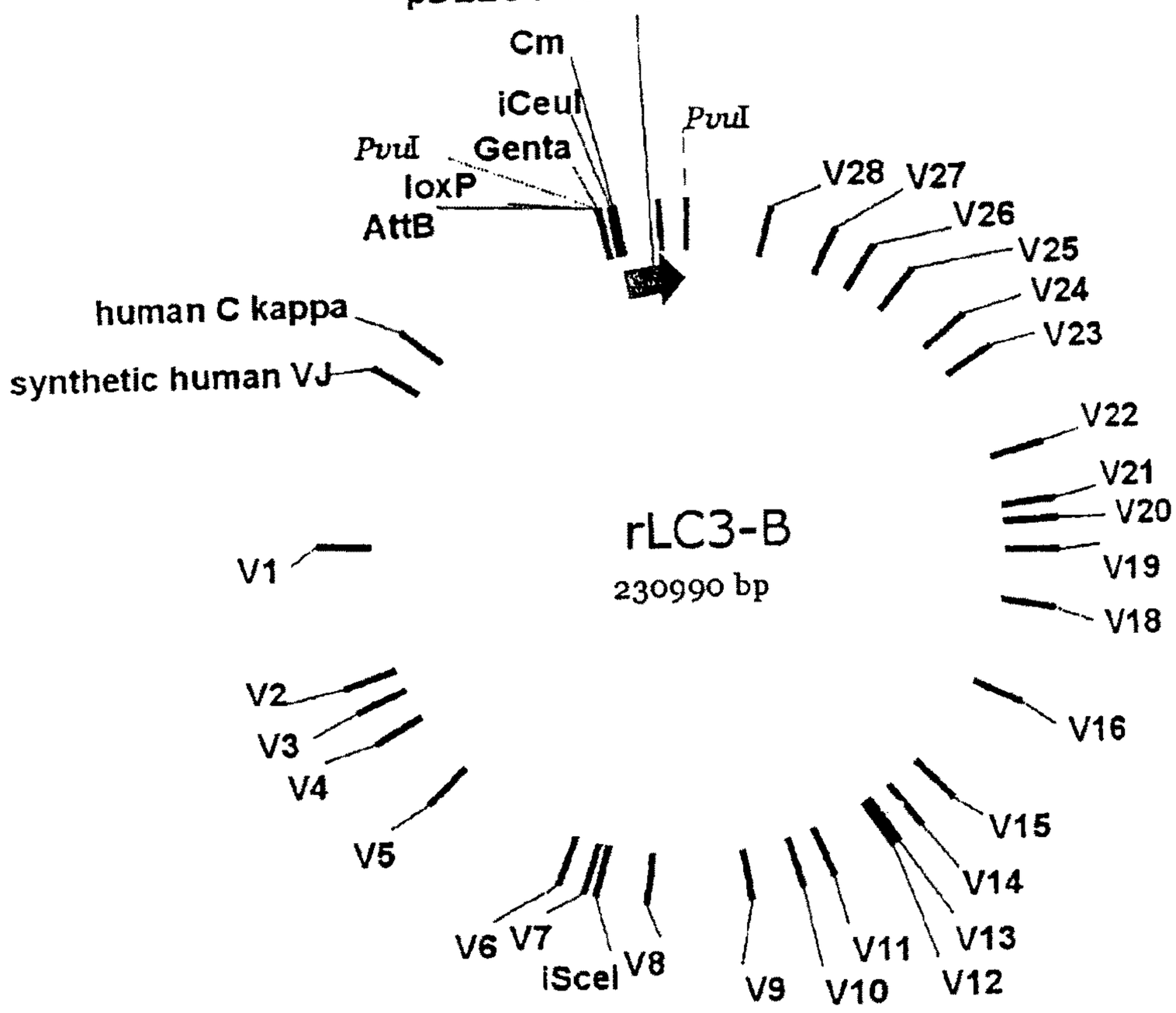
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Figure 9a

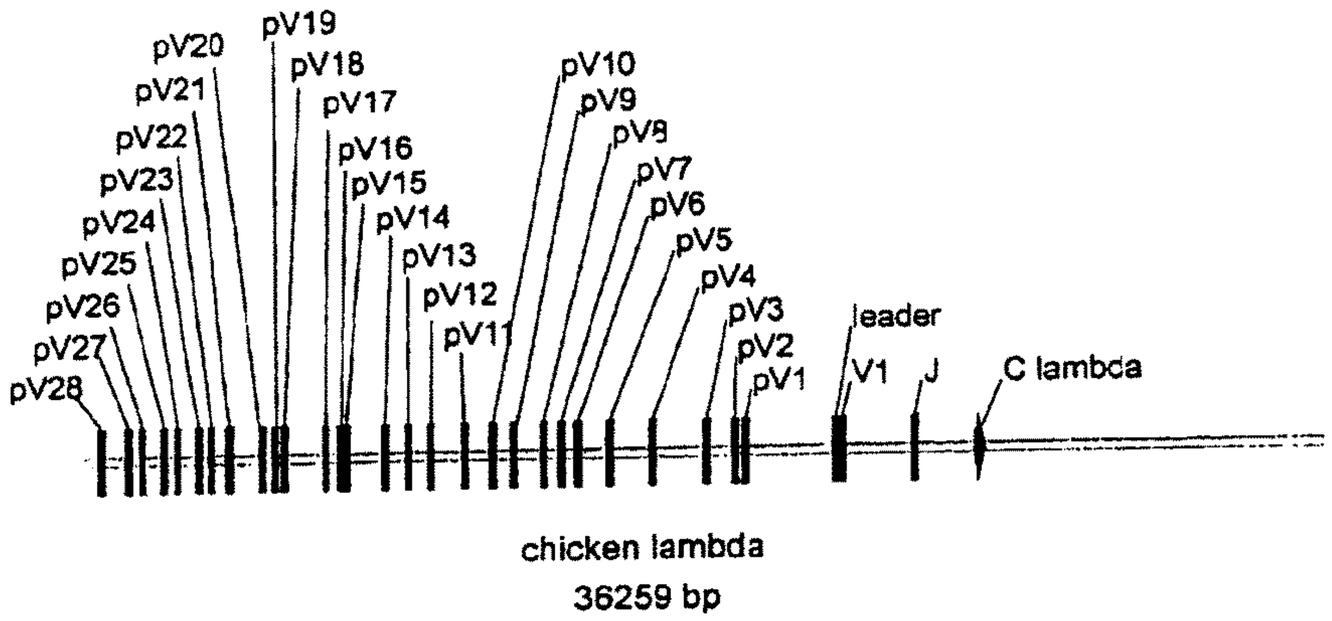
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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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Figure 9b Seq ID 186 (con't)

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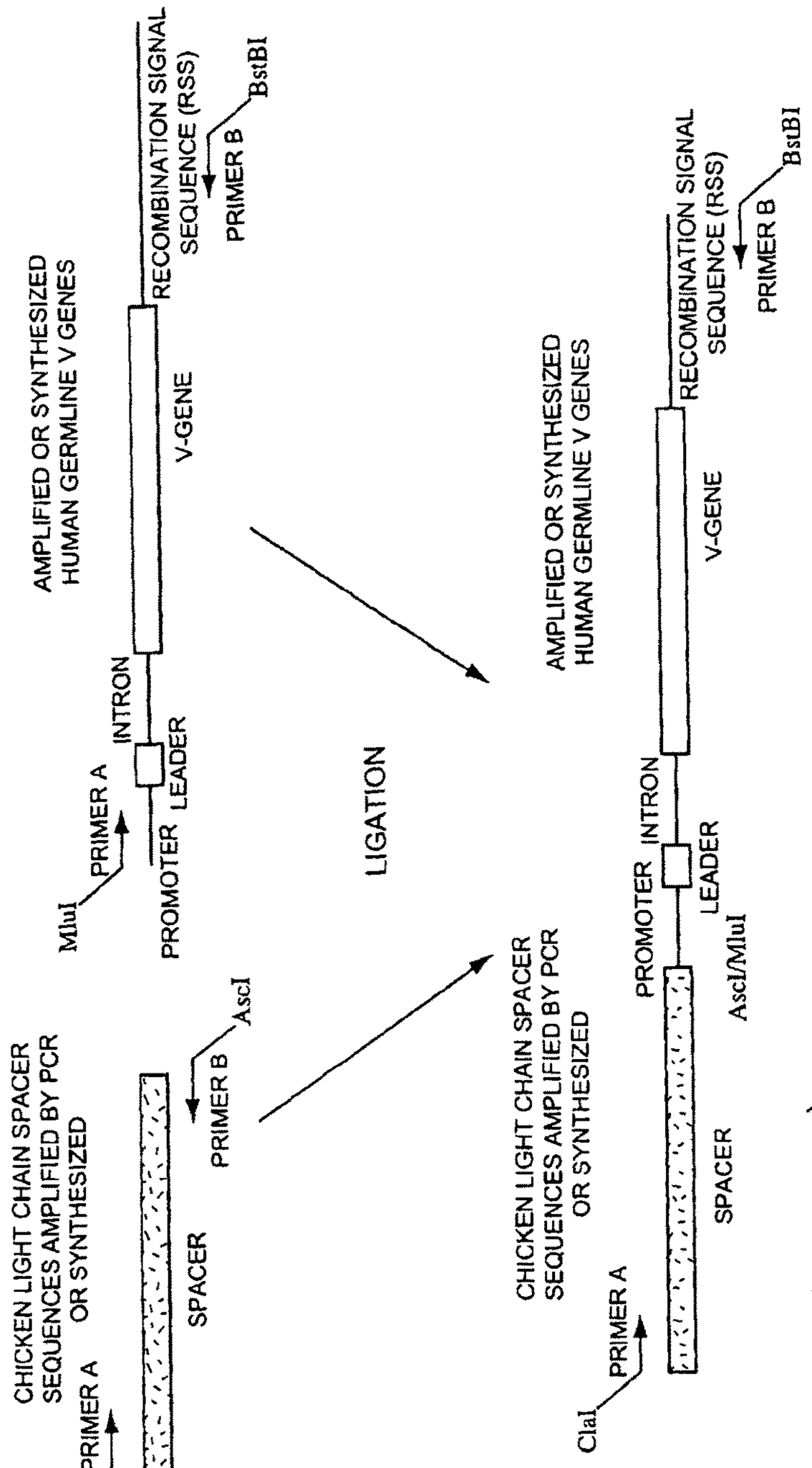
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RECOMBINATION SIGNAL SEQUENCE (RSS)
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BstBI
MluI
PRIMER A
INTRON
LEADER
PROMOTER
V-GENE
LIGATION
CHICKEN LIGHT CHAIN SPACER SEQUENCES AMPLIFIED BY PCR OR SYNTHESIZED
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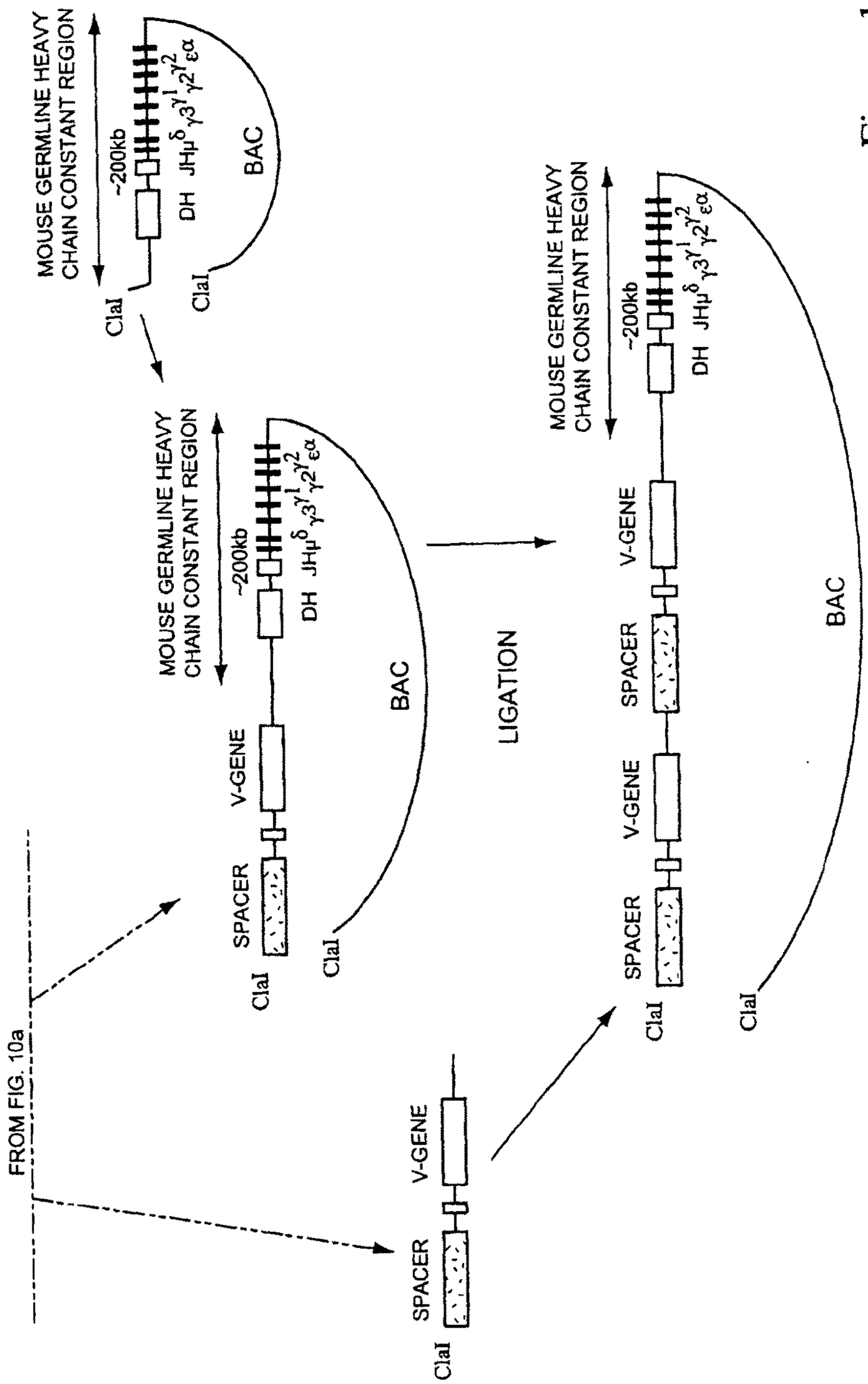
ClaI
SPACER
PRIMER B
AscI
TO FIG. 10b

Figure 10a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BAC
ClaI
ClaI
FROM FIG. 10a
SPACER
V-GENE
ClaI
LIGATION
MOUSE GERMLINE HEAVY CHAIN CONSTANT REGION
~200kb
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SPACER
V-GENE
BAC
ClaI

Figure 10b

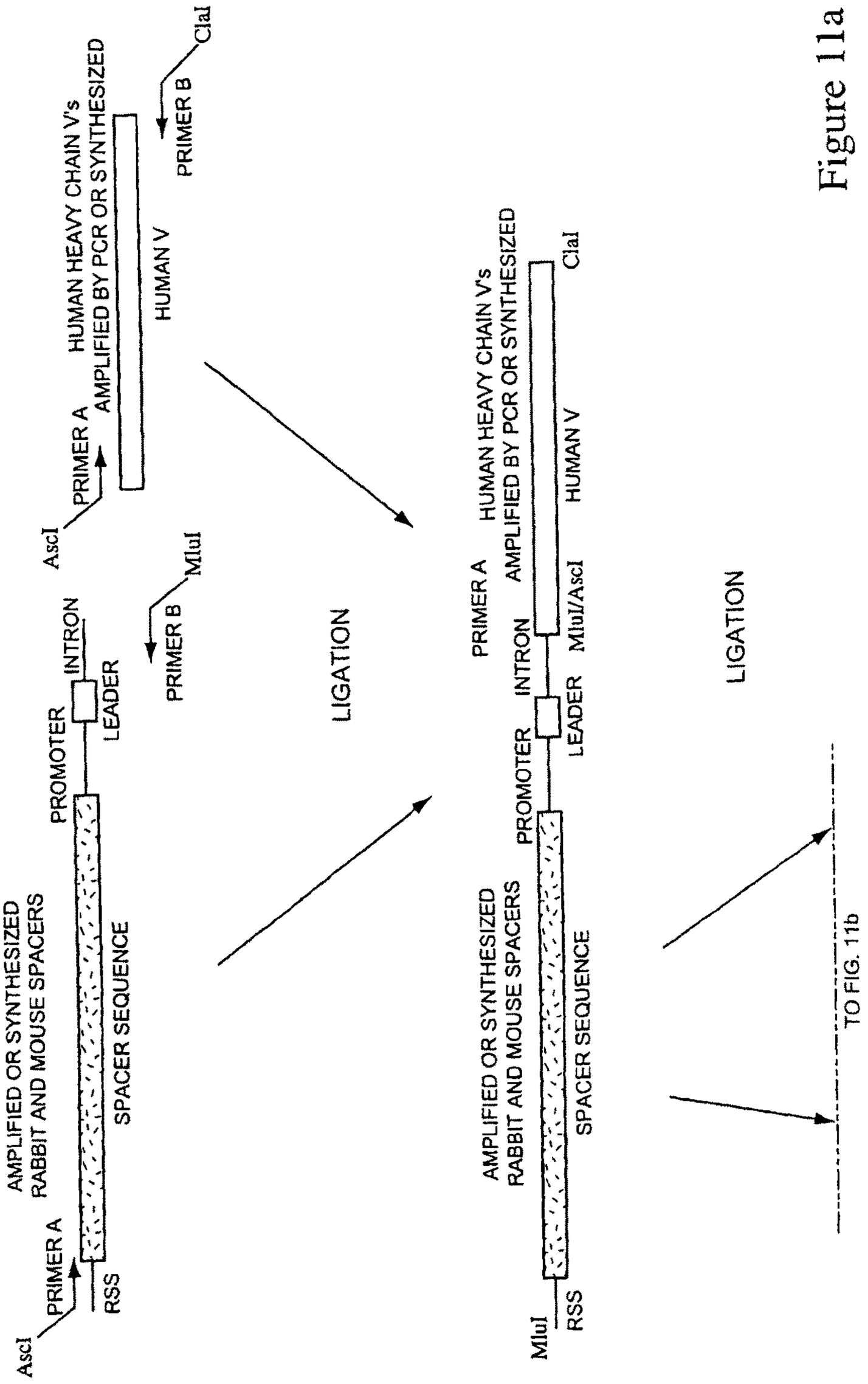
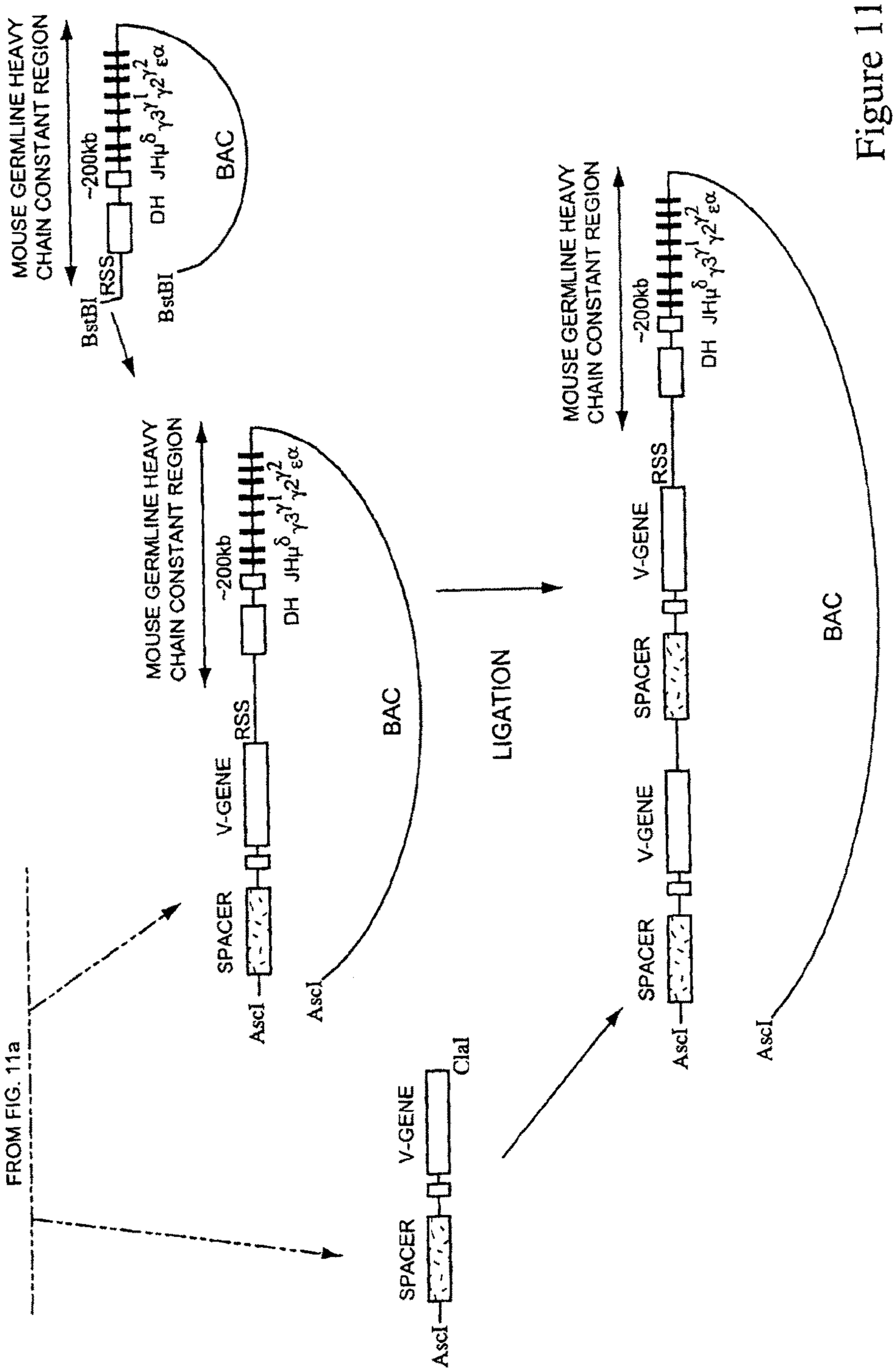

AMPLIFIED OR SYNTHESIZED
RABBIT AND MOUSE SPACERS
PRIMER A
AscI
RSS
SPACER SEQUENCE
PROMOTER
INTRON
LEADER
PRIMER B
MluI
HUMAN HEAVY CHAIN V's
AMPLIFIED BY PCR OR SYNTHESIZED
HUMAN V
ClaI
LIGATION
TO FIG. 11b

Figure 11a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BstBI
RSS
BstBI
BAC
FROM FIG. 11a
MOUSE GERMLINE HEAVY CHAIN CONSTANT REGION
~200kb
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RSS
V-GENE
SPACER
AscI
AscI
BAC
LIGATION
SPACER
V-GENE
ClaI
AscI
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~200kb
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RSS
V-GENE
SPACER
AscI
AscI
BAC

Figure 11b

Figure 12a Unit1 Seq ID 187

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Figure 12a Unit1 Seq ID 187 (con't)

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Figure 12a Unit1 Seq ID 187 (con't)

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Figure 12a Unit1 Seq ID 187 (con't)

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Figure 12b Unit2 Seq Id 188

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Figure 12b Unit2 Seq Id 188 (con't)

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Figure 12b Unit2 Seq Id 188 (con't)

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Figure 12b Unit2 Seq Id 188 (con't)

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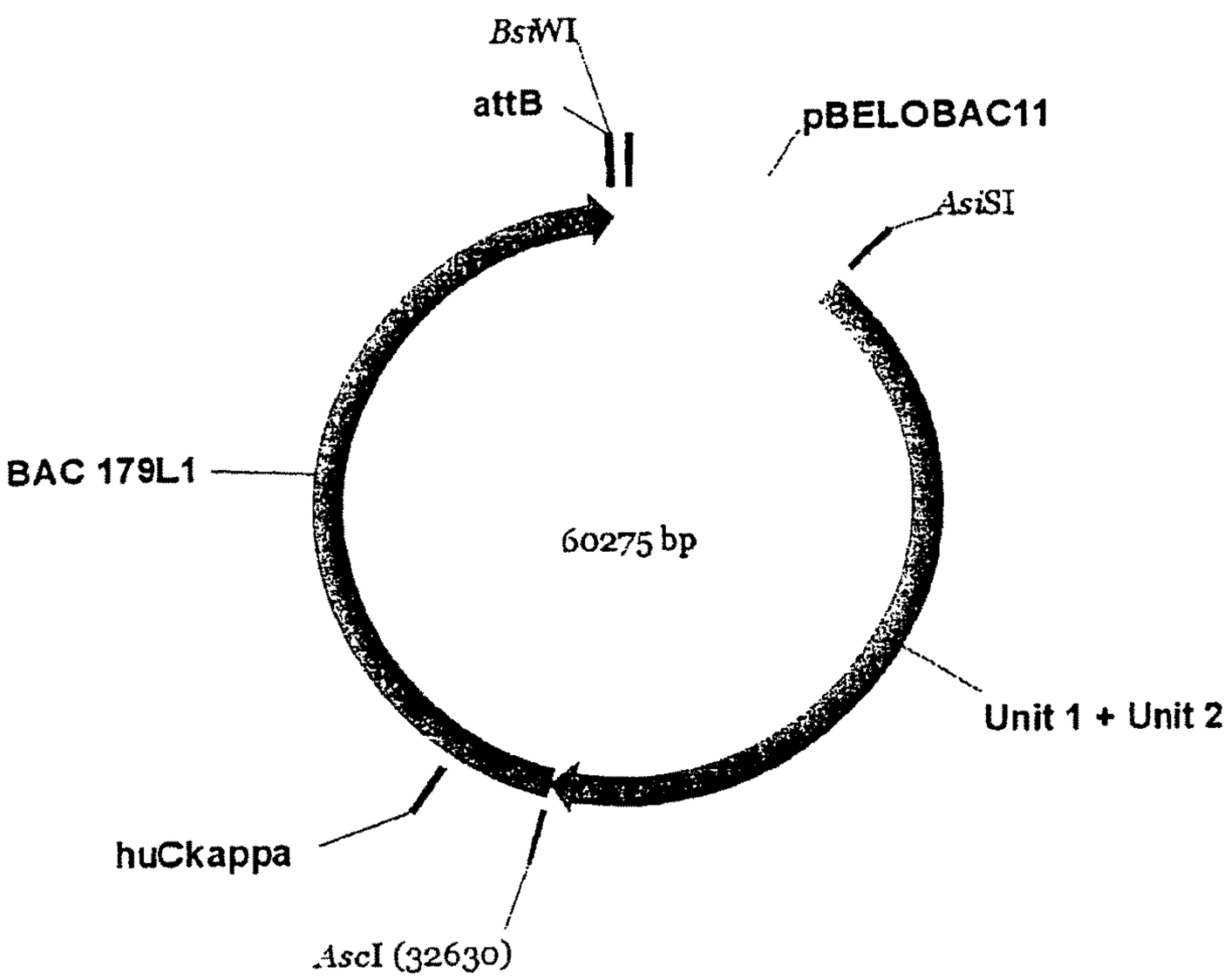
Figure 12c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Unit 1 + Unit 2
huCkappa
AscI (32630)

Figure 13a Unit 1 6607bp Seq ID 189

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Figure 13a Unit 1 6607bp Seq ID 189 (con't)

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Figure 13b Unit 2 15699bp Seq ID 190

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Figure 13b Unit 2 15699bp Seq ID 190 (con't)

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Figure 13b Unit 2 15699bp Seq ID 190 (con't)

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Figure 13b Unit 2 15699bp Seq ID 190 (con't)

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Figure 13b Unit 2 15699bp Seq ID 190 (con't)

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Figure 13c Unit 3 13971bp Seq ID 191

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Figure 13c Unit 3 13971bp Seq ID 191 (con't)

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Figure 13c Unit 3 13971bp Seq ID 191 (con't)

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Figure 13c Unit 3 13971bp Seq ID 191 (con't)

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Figure 13d Unit 4 15445bp Seq ID 192

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Figure 13d Unit 4 15445bp Seq ID 192 (con't)

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Figure 13d Unit 4 15445bp Seq ID 192 (con't)

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cactttctgcagaatgtggccacctttaatcctggaacagtctgtggtccctgtgtgaacgtcacagcactcaggaatgtacttgca
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accatgctgtacagtagatcctcagtgtatggatcctgttggactggaactctgaccttgctccaacgtctcctctgctcctcccttg

Figure 13d Unit 4 15445bp Seq ID 192 (con't)

ctcagacgttggtcttcaccatcgtacttcctgcctctgtgacttcatccttttatcccatgtatccagcatcacgtattatttgtcttcttg
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ctcattctaacaggaaggatgggatgtctcattgtgcatctattagggacatgcatgatgggcccaatatatcattttatatgttgcctt
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tattcactggttcacttcgtgaatggctgcaaaggctgggatggctgagcctgaagccaggagcctggaacttcccccagtttcc
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gactgcatgtataactaatgtcacatactatgtgtccacatgtacctgcattatttctgttagcatattatgctccaggctgaccaatga
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aatggatgaataaggaaacaaaacatagttaacctaaaaagaatccacttgcagtcttttatctgtttaaaagtgggaggcaggcat
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ttctgataatgcatttttggaaaactgtagatgacactacaagtgaatgagcccctaccactgacgtgggaaacaaatttgcagttttg
cgctccatgttttagaaaggctaagtcctcggtgttgtaggcacttgggatgtttggggctgtcaatcaaaggtggaaatctccctttt
gtcacctttctgtccatctctacatttctctttctctcacccttctctcactgtcactattccaatgttattcaaatagttaaaaatgaacc
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tgtccagtgaggtgcagctggtggagtctgggggaggcttagttcagcctgggggtccctgagactctcctgtgcagcctctg
gattcaccttcagtagctactggatgcactgggtccgccaagctccagggaaggggctggtgtgggtctcacgtattaatagtga
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gcagagggaaatgagccctggtttcctgtcagtgatttgtggtttgttctacagttttgcggcaagaattctacttcatgattctgtctg
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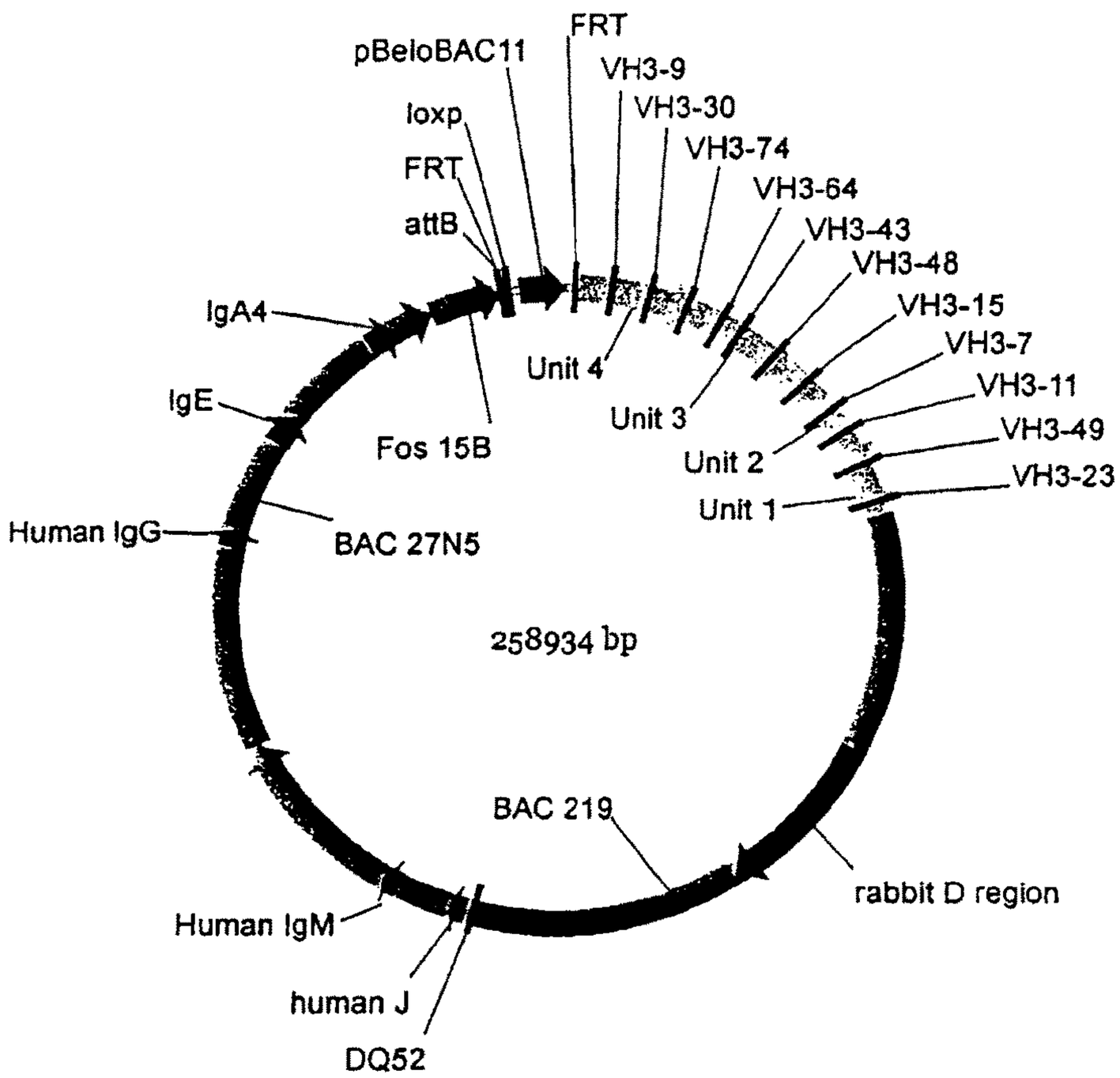
ggtcaccagggtcagagatgtgagacaatcaggggacctcgtgagtgttgtccagtctgactcaggacagcgttccaggacact
gccgactataagacaattttagttttccttcaaacccagagagagccgagtcagagcattgatttttcaccttaattatataagtcatg
acacatcctgtcggagtagcctgtgtgctgcactgaaccaactgtcttattttaaactgtgggtgttctgcattcactttctacagactt

Figure 13d Unit 4 15445bp Seq ID 192 (con't)

0tataccccatctccatttccctttcatcctggaacagtctgtggtccctgtgtgaacgtcacagcactcaggaatgtactgcagctc
agttgtgtctcaggctgagcttctgcaggcacctgtaccgttcgtatagcatacattatacgaagttatggccggccacgcgt

Figure 13e


pBeloBAC11
FRT
VH3-9
VH3-30
VH3-74
VH3-64
VH3-43
VH3-48
VH3-15
VH3-7
VH3-11
VH3-49
VH3-23
loxp
FRT
attB
IgA4
IgE
Human IgG
Human IgM
human J
DQ52
Unit 4
Unit 3
Unit 2
Unit 1
Fos 15B
BAC 27N5
258934 bp
BAC 219
rabbit D region

HUMANIZED IMMUNOGLOBULIN LOCI

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional application of U.S. application Ser. No. 12/511,188 filed Jul. 29, 2009, which is a divisional application of U.S. application Ser. No. 10/893,483 filed Jul. 15, 2004 (now U.S. Pat. No. 7,585,668) which claims priority under 35 U.S.C. Section 119(e) and the benefit of U.S. Provisional Application Ser. No. 60/487,733 filed Jul. 15, 2003, the entire disclosures of which are incorporated herein by reference in their entireties.

In accordance with 37 CFR 1.821(e), we hereby expressly incorporate herein by reference, in its entirety, the last-filed (filed Apr. 4, 2005) computer readable Sequence Listing, saved as "39691-0007A saved Apr. 4, 2005.txt" date of creation Apr. 4, 2005, size 1,489 KB, submitted in U.S. application Ser. No. 10/893,483, filed Jul. 15, 2004.

FIELD OF THE INVENTION

The present invention concerns methods and means to produce humanized antibodies from transgenic non-human animals. The invention specifically relates to novel immunoglobulin heavy and light chain constructs, recombination and transgenic vectors useful in making transgenic non-human animals expressing humanized antibodies, transgenic animals, and humanized immunoglobulin preparations. The transgenic vectors contain humanized immunoglobulin loci, which are capable of undergoing gene rearrangement, gene conversion and hypermutation in transgenic non-human animals to produce diversified humanized antibodies, while leaving the endogenous regulatory and antibody production machinery of the non-human animals essentially intact. The humanized antibodies obtained have minimal immunogenicity to humans and are appropriate for use in the therapeutic treatment of human subjects.

BACKGROUND OF THE INVENTION

Antibodies are an important class of pharmaceutical products that have been successfully used in the treatment of various human diseases and conditions, such as cancer, allergic diseases, prevention of transplant rejection and host-versus-graft disease.

A major problem of antibody preparations obtained from animals is the intrinsic immunogenicity of non-human immunoglobulins in human patients. In order to reduce the immunogenicity of non-human antibodies, it has been shown that by fusing animal variable (V) region exons with human constant (C) region exons, a chimeric antibody gene can be obtained.

Humanized monoclonal antibodies have also been developed and are in clinical use. Humanized monoclonal antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in non-human animal, e.g. rodent, antibodies. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321: 522 (1986); Riechmann et al., Nature, 332: 323 (1988); Verhoeyen et al., Science, 239: 1534 (1988)), by substituting non-human animal, e.g. rodent, CDRs or CDR sequences for the corresponding sequences of a human monoclonal antibody.

It has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90: 2551 (1993); Jakobovits et al., Nature, 362: 255 (1993); Bruggemann et al., Year in Immunol., 7: 33 (1993). While this genetic engineering approach resulted in the expression of human immunoglobulin polypeptides in genetically engineered mice, the level of human immunoglobulin expression is low. This may be due to species-specific regulatory elements in the immunoglobulin loci that are necessary for efficient expression of immunoglobulins. As demonstrated in transfected cell lines, regulatory elements present in human immunoglobulin genes may not function properly in non-human animals.

Indeed, several regulatory elements in immunoglobulin genes have been described. Of particular importance are enhancers downstream (3') of heavy chain constant regions and intronic enhancers in light chain genes. In addition, other, yet to be identified, control elements may be present in immunoglobulin genes. Studies in mice have shown that the membrane and cytoplasmic tail of the membrane form of immunoglobulin molecules play an important role in expression levels of human-mouse chimeric antibodies in the serum of mice homozygous for the human Cγ1 gene. Therefore, for the expression of heterologous immunoglobulin genes in animals it is desirable to replace sequences that contain enhancer elements and exons encoding transmembrane (M1 exon) and cytoplasmic tail (M2 exon) with sequences that are normally found in the animal in similar positions.

In addition to the issues raised by the potential immunogenicity of the non-human antibodies, the use of monoclonal antibodies in general, whether chimeric, humanized or human, is further limited by the fact that devastating diseases, such as cancer and infections with virulent pathogens, are difficult to treat by attacking one target, due to their complexity, multifactorial etiology and adaptivity. Monoclonal antibodies directed against singularly defined targets fail when those targets change, evolve and mutate. Thus, malignancies may gain resistance to standard monoclonal antibody therapies. A solution to this problem is the use of polyclonal antibodies, which have the ability to target and attack a plurality of evolving targets linked with complex diseases. Polyclonal antibodies also have the ability to neutralize bacterial and viral toxins, and direct immune responses to kill and eliminate pathogens.

Accordingly, there is a great clinical need for a new approach suitable for the large-scale production of high-titer, high-affinity, humanized poly- and monoclonal antibodies.

The introduction of human immunoglobulin genes into the genome of mice resulted in expression of a diversified human antibody repertoire in genetically engineered mice. In both mice and humans, primary antibody diversity is generated by gene rearrangement. This process results in the generation of many different recombined V(D)J segments encoding a large number of antibody molecules with different antigen binding sites. However, in other animals, like

rabbits, pigs, cows and birds, primary antibody diversity is generated by substantially different mechanisms, namely templated mutations or gene conversion and non-templated mutations or hypermutation. For example, it is well established that in rabbit and chicken, VDJ rearrangement is very limited (almost 90% of immunoglobulin is generated with the 3' proximal VH1 element) and antibody diversity is generated by gene conversion and hypermutation. In contrast, mouse and human gene conversion occurs very rarely, if at all. Therefore, it is expected that in animals that diversify their primary antibody repertoire by gene conversion and hypermutation a genetic engineering approach based on gene rearrangement will result in animals with low antibody titers and limited antibody diversity. Thus, the genetic engineering of large animals for the production of non-immunogenic antibody preparations for human therapy requires alternative genetic engineering strategies.

The production of humanized antibodies in transgenic non-human animals is described in PCT Publication No. WO 02/12437, published on Feb. 14, 2002, the disclosure of which is hereby expressly incorporated by reference in its entirety. WO 02/12437 describes genetically engineered non-human animals containing one or more humanized immunoglobulin loci which are capable of undergoing gene rearrangement and gene conversion in transgenic non-human animals, including animals in which antibody diversity is primarily generated by gene conversion to produce diversified humanized antibodies. The humanized antibodies obtained have minimal immunogenicity to humans and are appropriate for use in the therapeutic treatment of human subjects. It further describes novel nucleotide sequences from the 5' and 3' flanking regions of immunoglobulin heavy chain constant region segments of various non-human mammalians, such as chickens, cows, sheep, and rabbits. Recombinant vectors in which human immunoglobulin heavy chain gene segments are flanked by sequences homologous to such 5' and 3' sequences are shown to be useful for replacing an immunoglobulin heavy chain gene segment of a non-human animal with the corresponding human immunoglobulin heavy chain gene segment.

SUMMARY OF THE INVENTION

In one aspect, the present invention concerns an isolated nucleic acid molecule comprising a human immunoglobulin gene segment, flanked by nucleotide sequences, wherein the flanking sequences are identical or different, and comprise at least about 20 contiguous nucleotides of a spacer sequence from an immunoglobulin heavy or light chain gene of an animal generating antibody diversity primarily by gene conversion and/or hypermutation, or from a consensus sequence of two or more of the spacer sequences.

In another aspect, the invention concerns an isolated nucleic acid molecule comprising a human immunoglobulin heavy or light chain constant region (C) gene segment, flanked by nucleotide sequences, wherein the flanking sequences are identical or different, and comprise at least about 20 contiguous nucleotides of a spacer sequence from an immunoglobulin heavy or light chain gene of a non-primate animal, or from a consensus sequence of two or more of the spacer sequences.

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a human immunoglobulin heavy or light chain gene segment, flanked by nucleotide sequences, wherein the flanking sequences are identical or different, and comprise at least about 20 contiguous nucleotides of a spacer sequence selected from the group consisting of SEQ ID NOS: 1 to 185 (Table 1), or from a consensus sequence of two or more of the spacer sequences.

In one embodiment, the flanking sequences comprise at least about 50 contiguous nucleotides of a spacer sequence.

In another embodiment, the human immunoglobulin gene segment is a heavy chain V, D, or J segment, where the V fragment may, for example be a member of the VH3, VH1, VH5, or VH4 family.

In a further embodiment, the human immunoglobulin gene segment is a light chain V or J segment, where the V segment may, for example be a κ light chain gene segment, such as Vκ1, Vκ3, or Vκ4, or a λ light chain segment, e.g. Vλ1, Vλ2 or Vλ3.

In a further embodiment, the non-primate animal which generates antibody diversity primarily by gene conversion and/or somatic hypermutation is, for example, rabbit, pig, bird, e.g. chicken, turkey, duck, or goose, sheep, goat, cow, horse or donkey, however, other non-primate animals, e.g. rodents are also specifically included within the scope of the invention.

In another aspect, the invention concerns a recombination vector comprising any of the foregoing nucleic acid molecules.

In yet another aspect, the invention concerns a transgenic vector comprising a humanized immunoglobulin (Ig) locus, wherein the humanized Ig locus is derived from an Ig locus or a portion of an Ig locus of a non-human animal, comprising multiple Ig segments wherein

(a) at least one of the gene segments is a human Ig gene segment flanked by nucleotide sequences comprising at least about 20 contiguous nucleotides from a spacer sequence, or from a consensus sequence or two or more of such spacer sequences;

(b) the gene segments are juxtaposed in an unrearranged, partially rearranged or fully rearranged configuration, and

(c) the humanized Ig locus is capable of undergoing gene rearrangement, if necessary, as well as gene conversion and/or hypermutation, if the non-human animal is a gene converting animal, and producing a repertoire of humanized immunoglobulins in the non-human animal.

In a further embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 5 to 100 V gene segments, with at least one human V gene segment. In a specific embodiment, the humanized Ig heavy chain locus comprises more than one human V gene segments.

In another embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 5 to 25 D gene segments, In a specific embodiment, the humanized Ig heavy chain locus comprises one or several human D gene segments.

In yet another embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 1 to 10 J gene segments, with at least one human J gene segment. In a specific embodiment, the humanized Ig heavy chain locus comprises more than one human J gene segments.

In another embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises about 1-25 C region segments, with at least one human C region segment. In a specific embodiment, the humanized Ig heavy chain locus present in the transgenic vector comprises more than one human C gene segment.

In a still further embodiment, the humanized Ig locus present in the transgenic vector is a light chain locus of a non-human animal, and it comprises at least one V gene segment, at least one J gene segment and at least one constant (C) region gene segment, where at least one gene

segment is selected from the group of human light chain V and J segments and human light chain C region segments. In a specific embodiment, the constant region gene segment is a human light chain constant region gene segment, which can, for example, be a Cλ or Cκ gene segment. In another embodiment, the humanized Ig light chain locus comprises two or more segments selected from human V and J segments and human C region segments. In a further embodiment, the humanized Ig light chain locus comprises at least one human V segment, at least one human J segment, and at least one human C region segment.

In a further embodiment, the humanized Ig light chain locus present in the transgenic vector comprises about 5-100 V gene segments, with at least one human V gene segment, wherein the human V gene segment is placed downstream to the 5-100 V gene segments of the non-human animal. In a specific embodiment, the human V gene segment is placed immediately 5' to a J gene segment in a rearranged configuration. In another embodiment, the humanized Ig light chain locus present in the transgenic vector comprises more than one human V gene segment.

In a still further embodiment, the humanized Ig light chain locus present in the transgenic vector comprises about 1-10 J gene segments, with at least one human J gene segment. In a specific embodiment, the humanized Ig light chain locus present in the transgenic vector comprises more than one human J gene segment.

In another embodiment, the humanized Ig light chain locus present in the transgenic vector comprises about 1-25 C region segments, with at least one human C region segment. In a specific embodiment, the humanized Ig light chain locus present in the transgenic vector comprises more than one human C gene segment.

In a still further embodiment, the humanized Ig locus present in the transgenic vector is a light chain locus of a non-human animal, and it comprises at least one V gene segment, at least one J gene segment and at least one constant (C) region gene segment, where at least one gene segment is selected from the group of human light chain V and J segments and human light chain C region segments. In a specific embodiment, the constant region gene segment is a human light chain constant region gene segment, which can, for example, be a Cλ or Cκ gene segment. In another embodiment, the humanized Ig light chain locus comprises two or more segments selected from human V and J segments and human C region segments. In a further embodiment, the humanized Ig light chain locus comprises at least one human V segment, at least one human J segment, and at least one human C region segment.

In a different aspect, the invention concerns a nucleic acid molecule comprising two or more units consisting of, from 5' to 3' direction, a 5' nucleotide sequence, a human immunoglobulin sequence, and a 3' nucleotide sequence, wherein the 5' and 3' nucleotide sequences are identical or different, and comprise at least about 20 contiguous nucleotides from a spacer sequence separating the coding regions in a non-primate animal immunoglobulin heavy or light chain gene, or from a consensus sequence of two or more of the spacer sequences. In a specific embodiment, the spacer sequences are selected from within SEQ ID NOS: 1 to 185 (Table 1). In another particular embodiment, the 5' and/or 3' nucleotide sequences in all repetitive units of the nucleic acid molecule are identical. In another particular embodiment, the repetitive units of the nucleic acid molecule comprise at least two different 5' and/or 3' sequences. In a further embodiment, the 5' and 3' nucleotide sequence are different from each other, but all 5' and all 3' nucleotide sequences are identical.

In a further aspect, the invention concerns a method for making a transgenic vector comprising a humanized immunoglobulin (Ig) locus capable of producing a functional repertoire of humanized antibodies in a non-human animal, comprising:

(a) obtaining a DNA fragment comprising an Ig locus or a portion thereof from the non-human animal which comprises at least one V gene segment, at least one J gene segment and at least one constant region gene segment; and

(b) integrating at least one human Ig gene segment into the DNA fragment of step (a) to produce a humanized Ig locus, wherein the human Ig gene segment flanked by nucleotide sequences comprising at least about 20 contiguous nucleotides from a spacer sequence separating the coding regions in a non-primate animal immunoglobulin heavy or light chain gene, or from a consensus sequence or two or more of such spacer sequences; wherein (i) the gene segments are juxtaposed in an unrearranged, partially rearranged or fully rearranged configuration, and (ii) the humanized Ig locus is capable of undergoing gene rearrangement, if necessary, and producing a repertoire of humanized immunoglobulins in the non-human animal.

The humanized Ig locus can be a humanized Ig heavy chain or light chain locus. In the case of a humanized Ig heavy chain locus the DNA fragment obtained in step (a) additionally comprises at least one D gene segment.

In another aspect, the invention concerns a transgenic animal comprising a humanized immunoglobulin locus described above, and methods for making such transgenic animals. In one embodiment, the transgenic animal comprises both a humanized immunoglobulin heavy chain locus and a humanized immunoglobulin light chain locus. In another embodiment, only one of the heavy and light chain loci present in the transgenic animal is humanized. In another embodiment, all of the V, D, J and C regions of at least one of the animal's immunoglobulin loci are humanized. In yet another embodiment, all of the V, D, J, and C region of the transgenic animals endogenous immunoglobulin loci are humanized.

In a further aspect, the invention concerns a B cell from the transgenic animals produced in accordance with the present invention.

In a still further aspect, the invention concerns a humanized immunoglobulin produced using a transgenic animal of the present invention, and an antibody preparation or a pharmaceutical composition comprising the humanized immunoglobulin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic depiction of the rabbit immunoglobulin gene heavy chain locus.

FIGS. **2-4** show a comparison of rabbit heavy chain spacer sequences.

FIG. **5** is a schematic depiction of the rabbit immunoglobulin light chain locus.

FIG. **6** illustrates the building of an immunoglobulin gene V locus using human V_H and rabbit spacer elements.

FIGS. **7** (a) and (b): Insertion of two cassettes by homologous recombination using the redϵβγ-system. FIG. **7**(a) shows that the upper cassette contains two restriction sites (FseI and AscI) flanking a gentamycin cassette. FIG. **7**(b) shows that the lower cassette contains an inverted (i) and mutated (71) loxP-site, a FRT-site and a MluI-restriction site. After modification the BAC is digested with FseI and AscI.

FIG. **8** shows a humanized rabbit light chain locus (rLC3-B) based on the rabbit K1 light chain locus. Rabbit Cκ1 was replaced with human Cκ. A human rearranged human VκJκ was inserted. The synthetic human VκJκ shares more than 80% sequence homology with rabbit Vκ elements

FIG. **9**a shows the sequence of a BAC clone comprising a chicken light chain genomic locus whose nucleotide sequence is shown in FIG. **9**b (SEQ ID NO: 186).

FIGS. **10**a and **10**b illustrate an outline showing the construction of a humanized immunoglobulin locus using chicken immunoglobulin spacer sequences and human V elements.

FIGS. **11**a and **11**b illustrate an outline showing the construction of a humanized immunoglobulin locus using mouse or rabbit immunoglobulin spacer sequences and human V elements.

FIGS. **12**a to **12**c show a humanized light chain locus. A synthetic sequence (FIG. **12**a, Unit 1, 12,235 bp, SEQ ID NO: 187) containing 17 human V pseudogenes and 18 chicken spacer sequences is shown in (a). A second synthetic sequence (FIG. **12**b, Unit 2, 13,283 bp, SEQ ID NO: 188) containing a functional rearranged human VkJk gene fragment, 11 human V pseudogenes, 12 chicken spacer sequences and 2 introns is shown in (b). Units 1 and 2 were combined with a fragment derived from BAC 179L1 containing human Ck and rabbit intron and spacer sequences (FIG. **12**c).

FIG. **13**a-e show a humanized heavy chain locus. Four synthetic DNA fragments (Unit 1-4, FIG. **13**a-d, SEQ ID NOS: 189, 190, 191, 192) consisting of human VH3 gene fragments and rabbit spacer and intron sequences were combined with parts of BAC 219D23, 27N5 and Fos15B as shown in (FIG. **13**e).

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

"Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

"Native antibodies and immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by covalent disulfide bond(s), while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Clothia et al., J. Mol. Biol. 186:651 (1985); Novotny and Haber, Proc. Natl. Acad. Sci. U.S.A. 82:4592 (1985)).

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, connected by three CDRs. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "monoclonal antibody" is used to refer to an antibody molecule synthesized by a single clone of B cells.

The term "polyclonal antibody" is used to refer to a population of antibody molecules synthesized by many clones of B cells. In a specific embodiment, polyclonal antibodies recognize several epitopes.

The terms "a humanized antibody" and "a humanized immunoglobulin", as used herein, mean an immunoglobulin molecule comprising at least a portion of a human immunoglobulin polypeptide sequence (or a polypeptide sequence encoded by a human immunoglobulin gene segment). The humanized immunoglobulin molecules of the present invention can be isolated from a transgenic non-human animal engineered to produce humanized immunoglobulin molecules. Such humanized immunoglobulin molecules are less immunogenic to primates, especially humans, relative to non-humanized immunoglobulin molecules prepared from the animal or prepared from cells derived from the animal.

The term "non-human animal" as used herein includes, but is not limited to mammals, and includes, for example, non-human primates, rabbits, pigs, birds (e.g., chickens, turkeys, ducks, geese and the like), sheep, goats, cows, horses, and rodents (e.g. mice and rats). Preferred non-human animals are those animals which create antibody diversity substantially by gene conversion and/or somatic hypermutation, e.g., rabbit, pigs, birds (e.g., chicken, turkey, duck, goose and the like), sheep, goat, and cow. Particularly preferred non-human animals are rabbit and chicken.

The term "non-primate animal" as used herein includes, but is not limited to, mammals other than primates, including those listed above.

The phrase "animals which create antibody diversity substantially by gene conversion and/or hypermutation" is used to refer to animals in which the predominant mechanism of antibody diversification is gene conversion and/or hypermutation as opposed to gene rearrangement.

The term "Ig gene segment" as used herein refers to segments of DNA encoding various portions of an Ig molecule, which are present in the germline of animals and humans, and which are brought together in B cells to form rearranged Ig genes. Thus, Ig gene segments as used herein include V gene segments, D gene segments, J gene segments and C region gene segments.

The term "human Ig gene segment" as used herein includes both naturally occurring sequences of a human Ig

gene segment, degenerate forms of naturally occurring sequences of a human Ig gene segment, as well as synthetic sequences that encode a polypeptide sequence substantially identical to the polypeptide encoded by a naturally occurring sequence of a human Ig gene segment. By "substantially" is meant that the degree of amino acid sequence identity is at least about 85%-95%. In a particular embodiment, the human Ig gene segment renders the immunoglobulin molecule non-immunogenic in humans.

A specific humanized immunoglobulin molecule of the present invention contains at least a portion of a human heavy or light chain variable region polypeptide sequence. Another specific immunoglobulin molecule contains at least a portion of a human heavy or light chain variable region polypeptide sequence, and at least a portion of a human constant domain polypeptide sequence.

By "a preparation of humanized antibodies" or "a humanized antibody preparation" is meant an isolated antibody product or a purified antibody product prepared from a transgenic non-human animal (e.g., serum, milk, or egg yolk of the animal) or from cells derived from a transgenic non-human animal (e.g., a B-cell or a hybridoma cell).

A humanized antibody preparation can be a preparation of polyclonal antibodies, which includes a repertoire of humanized immunoglobulin molecules. A humanized antibody preparation can also be a preparation of a monoclonal antibody.

The terms "antibody diversity" and "antibody repertoire" are used interchangeably, and refer to the total of all antibody specificities that an organism is capable of expressing.

The term "spacer sequence" is used herein to refer to any non-coding nucleotide sequence present in an immunoglobulin heavy or light chain gene. Thus, the term specifically includes intron sequences and any other non-coding sequences separating the coding regions within the V, D, J segments and C region segments in an immunoglobulin heavy chain gene, intron sequences and any other non-coding sequences separating the coding regions within the V and J segments an C region segments in an immunoglobulin light chain gene, as well as non-coding sequence flanking regulatory elements, such as enhancers, in an immunoglobulin heavy or light chain gene. In addition, non-coding sequences between exons encoding parts of a membrane-spanning helix and heavy and light chain enhancers are specifically included.

An Ig locus having the capacity to undergo gene rearrangement and gene conversion is also referred to herein as a "functional" Ig locus, and the antibodies with a diversity generated by a functional Ig locus are also referred to herein as "functional" antibodies or a "functional" repertoire of antibodies.

B. Relevant Literature

Regulatory elements in immunoglobulin genes have been described by Bradley et al. (1999), Transcriptional enhancers and the evolution of the IgH locus; Lauster, R. et al., Embo J 12: 4615-23 (1993); Volgina et al., J Immunol 165:6400 (2000); Hole et al., J Immunol 146:4377 (1991).

Antibody diversification by gene conversion in chicken and rabbit has been described by Bucchini et al., Nature 326: 409-11 (1987); Knight et al., Advances in Immunology 56: 179-218 (1994); Langman et al., Res Immunol 144: 422-46 (1993). The generation of mice expressing human-mouse chimeric antibodies has been described by Pluschke et al., Journal of Immunological Methods 215: 27-37 (1998). The generation of mice expressing human-mouse chimeric antibodies with mouse derived membrane and cytoplamic tails has been described by Zou et al., Science 262: 1271-1274 (1993); Zou et al. Curr Biol 4: 1099-1103. The generation of mice expressing human immunoglobulin polypeptides has been described by Bruggemann et al. Curr Opin Biotechnol 8(4): 455-8 (1997); Lonberg et al. Int Rev Immunol 13(1): 65-93 (1995); Neuberger et al., Nature 338: 350-2 (1989). Generation of transgenic mice using a BAC clone has been described by Yang et al., Nat Biotechnol 15: 859-65 (1997). The generation of cows expressing human antibodies has been described by Kuroiwa et al., Nature Biotech 20(9): 889-894 (2002).

The generation of transgenic rabbits has been described by Fan, J. et al., Pathol Int 49: 583-94 (1999); Brem et al., Mol Reprod Dev 44: 56-62 (1996). Rabbits with impaired immunoglobulin expression have been described by McCartney-Francis et al., Mol Immunol 24: 357-64 (1987); Allegrucci, et al., Eur J Immunol 21: 411-7 (1991).

The production of transgenic chicken has been described by Sherman et al., Nature Biotech 16:1050-1053 (1998); Etches et al., Methods in Molecular Biology 62: 433-450; Pain et al., Cells Tissues Organs 165(3-4): 212-9 (1999); Sang, H., "Transgenic chickens—methods and potential applications", Trends Biotechnol 12:415 (1994); and in WO2004003157, "Gene regulation in transgenic animals using a transposon based vector"; and in WO 200075300, "Introducing a nucleic acid into an avian genome, useful for transfecting avian blastodermal cells for producing transgenic avian animals with the desired genes, by directly introducing the nucleic acid into the germinal disc of the egg".

A gammaglobulinemic chicken have been described by Frommel et al., J Immunol 105(1): 1-6 (1970); Benedict et al., Adv Exp Med Biol 1977; 88(2): 197-205.

The cloning of animals from cells has been described by T. Wakayama et al., Nature 1998; 394:369-374; J. B. Cibelli et al., Science 280:1256-1258 (1998); J. B. Cibelli et al., Nature Biotechnology 1998; 16:642-646; A. E. Schnieke et al., Science 278: 2130-2133 (1997); K. H. Campbell et al., Nature 380: 64-66 (1996), Kuroiwa et al., Nature Genetics 2004, Jun. 6. Nuclear transfer cloning of rabbits has been described by Stice et al., Biology of Reproduction 39: 657-664 (1988), and Challah-Jacques et al., Cloning and Stem Cells 8(4):295-299 (2003).

Production of antibodies from transgenic animals is described in U.S. Pat. No. 5,814,318, No. 5,545,807 and No. 5,570,429. Homologous recombination for chimeric mammalian hosts is exemplified in U.S. Pat. No. 5,416,260. A method for introducing DNA into an embryo is described in U.S. Pat. No. 5,567,607. Maintenance and expansion of embryonic stem cells is described in U.S. Pat. No. 5,453, 357.

The mechanisms involved in the diversification of the antibody repertoire in pigs, sheep and cows are reviewed in Butler, J. E. (1998), "Immunoglobulin diversity, B-cell and antibody repertoire development in large farm animals", Rev Sci Tech 17:43. Antibody diversification in sheep is described in Reynaud, C. A., C. Garcia, W. R. Hein, and J. C. Weill (1995), "Hypermutation generating the sheep immunoglobulin repertoire is an antigen-independent process", Cell 80:115; and Dufour, V., S. Malinge, and F. Nau. (1996), "The sheep Ig variable region repertoire consists of a single VH family," J Immunol 156:2163.

C. Detailed Description

Immunoglobulin heavy and light chain genes comprise several segments encoded by individual genes and separated

by intron sequences. Thus genes for the human immunoglobulin heavy chain are found on chromosome 14. The variable region of the heavy chain (VH) comprises three gene segments: V, D and J segments, followed by multiple genes coding for the C region. The V region is separated from the C region by a large spacer, and the individual genes encoding the V D and J segments are also separated by spacers.

There are two types of immunoglobulin light chains: κ and λ. Genes for the human κ light chain are found on chromosome 2 and genes for the human λ light chain are found on chromosome 22. The variable region of antibody light chains includes a V segment and a J segment, encoded by separate gene segments. In the germline configuration of the κ light chain gene, there are approximately 100-200 V region genes in linear arrangement, each gene having its own leader sequence, followed by approximately 5 J gene segments, and C region gene segment. All V regions are separated by introns, and there are introns separating the V, J and C region gene segments as well.

The immune system's capacity to protect against infection rests in a genetic machinery specialized to create a diverse repertoire of antibodies. Antibody-coding genes in B cells are assembled in a manner that allows to countless combinations of binding sites in the variable (V) region. It is estimated that more than 10^{12} possible binding structures arise from such mechanisms. In all animals, including humans, the antibody-making process begins by recombining variable (V), diversity (D) and joining (J) segments of the immunoglobulin (Ig) locus. Following this step, depending on the animal species, two general mechanisms are used to produce the diverse binding structures of antibodies.

In some animals, such as human and mouse, there are multiple copies of V, D and J gene segments on the immunoglobulin heavy chain locus, and multiple copies of V and J gene segments on the immunoglobulin light chain locus. Antibody diversity in these animals is generated primarily by gene rearrangement, i.e., different combinations of gene segments to form rearranged heavy chain variable region and light chain variable region. In other animals (e.g., rabbit, birds, e.g., chicken, goose, and duck, sheep, goat, and cow), however, gene rearrangement plays a smaller role in the generation of antibody diversity. For example, in rabbit, only a very limited number of the V gene segments, most often the V gene segments at the 3' end of the V-region, is used in gene rearrangement to form a contiguous VDJ segment. In chicken, only one V gene segment (the one adjacent to the D region, or "the 3' proximal V gene segment"), one D segment and one J segment are used in the heavy chain rearrangement; and only one V gene segment (the 3' proximal V segment) and one J segment are used in the light chain rearrangement. Thus, in these animals, there is little diversity among initially rearranged variable region sequences resulting from junctional diversification. Further diversification of the rearranged Ig genes is achieved by gene conversion, a process in which short sequences derived from the upstream V gene segments replace short sequences within the V gene segment in the rearranged Ig gene. Additional diversification of antibody sequences may be generated by hypermutation.

Immunoglobulins (antibodies) belong into five classes (IgG, IgM, IgA, IgE, and IgD, each with different biological roles in immune defense. The most abundant in the blood and potent in response to infection is the IgG class. Within the human IgG class, there are four sub-classes (IgG1, IgG2, IgG3 and IgG4 isotypes) determined by the structure of the heavy chain constant regions that comprise the Fc domain. The F(ab) domains of antibodies bind to specific sequences (epitopes) on antigens, while the Fc domain of antibodies recruits and activates other components of the immune system in order to eliminate the antigens.

Antibodies have been used successfully as therapeutics since the 1890s when it was found that polyclonal antiserum taken from animals could treat life-threatening infections in humans. A significant advance in antibody research occurred with the development of methods for the recombinant production of antibodies, followed by the development of antibody humanization techniques and method for making fully human monoclonal antibodies in non-human animals.

As a result, chimeric, humanized and human monoclonal antibodies have recently emerged as an important class of pharmaceutical products. While monoclonal antibody-based drugs are very effective in treating diseases when blocking a particular target (e.g. receptor or ligand) certain devastating diseases, such as cancer and infections with virulent pathogens, may be difficult to treat due to their complexity, multifactoral etiology and adaptivity. Monoclonal antibodies address singularly defined targets that change, evolve and mutate during the spread of diseases throughout a population or within an individual. Such adaptive evolution is the bane of mono-specific drugs (e.g. monoclonal antibodies), which are quickly circumvented by resistant strains. Examples abound of bacterial and viral resistance to high-potency antibiotics, and malignant cancers that develop resistance to standard anticancer drugs, such as monoclonal antibody therapies.

In contrast, polyclonal antibodies have the ability to bind and eliminate a plurality of evolving targets linked with complex diseases. By binding multiple antigens, polyclonal antibodies saturate the target and retain activity even in the event of antigen mutation. Following this, through a cascade of signals, polyclonal antibodies induce a potent immune response to eliminate the target antigen, pathogen or cell. These properties make polyclonal antibodies ideal for treating infectious diseases and cancer.

So far, the use of polyclonal antibodies has been severely limited by either supply problems or unwanted reactions to non-human proteins.

The present invention provides a new humanization approach, based on selective humanization the immunoglobulin-coding elements of the immunoglobulin (Ig) translocus. The creation of such human-animal translocus allows for the creation of transgenic animals that express diversified, high-affinity humanized (polyclonal) antibodies in high yields.

As a first step, the genomic loci for non-human, including non-primate, immunoglobulin heavy and light chains are identified and sequenced. For example, as part of the present invention, genomic sequences for rabbit and chicken immunoglobulin heavy and light chains were determined, and are shown in FIGS. **1**, **5**, and **9**.

Analysis of the rabbit Ig heavy chain genomic locus has shown that the immunoglobulin heavy chain variable region (Vh) contains numerous genes, including functional genes and non-functional pseudogenes. Alignment of 18 Vh genes has revealed a high degree (80-90%) sequence identity among rabbit heavy chain variable region gene sequences (Vh1-Vh18). The rabbit heavy chain variable region genes have been found to share highest homology with the Vh3 group of the human heavy chain variable region genes. Specifically, sequence comparison of the rabbit Vh1-a2 gene with the human Vh3-23 sequences has revealed 72.8% sequence identity.

In addition, the non-coding (e.g. intron) sequences separating the rabbit heavy chain variable region gene sequences were analyzed. FIGS. **2-4** show a comparison of rabbit heavy chain intron sequences. It has been found that such intron sequences fall into two groups, and are highly conserved. Especially members of the Group 1 introns show a surprisingly high (80-90%) sequence identity.

Similar findings were made by analysis of rabbit immunoglobulin light chain variable region genomic sequences. In particular, analysis of the rabbit immunoglobulin light chain locus has shown that the light chain variable region (V1) region contains numerous gene segments, which show a high degree (80-90% sequence identity). It has further been found that the rabbit light chain variable region (Vκ) exhibits high homology with the Vκ1 group of the human light chain variable region gene sequences. Most Vκ sequences have been found to be functional and highly conserved. Unlike in the rabbit heavy chain variable region genes, in the rabbit light chain variable region genes the intron sequences have been found to be heterogeneous.

Similar studies with chicken immunoglobulin heavy and light chain genomic sequences provide analogous results.

In one aspect, the present invention provides spacer sequences, which separate the coding regions in a non-primate animal heavy or light chain gene. In one embodiment, the present invention provides spacer sequences from the heavy and light chain genes of animals which create antibody diversity substantially by gene conversion, including, for example, rabbit and chicken. Such spacer sequences are then used to flank human immunoglobulin heavy or light chain gene segments used in the process of creating a humanized immunoglobulin locus.

The spacer sequences typically comprise at least about 20 nucleotides, or at least about 30 nucleotides, or at least about 40 nucleotides, or at least about 50 nucleotides, and typically are between about 20 and about 10000 nucleotides in length. The spacer sequences may contain a contiguous stretch of nucleotides of appropriate length from a naturally occurring intron sequence in a non-human (e.g. non-primate) animal, or may include an artificial sequence, which may, for example, be a consensus sequence of two or more naturally occurring intron sequences.

The spacer sequences may comprise at least about 20 (30, 40, 50, etc. up to 1000 in 10-nucleotide increments) contiguous nucleotides from a sequence selected from the group consisting of SEQ ID NOS 1 to 185 (Table 1), or from a consensus sequence of two or more of such sequences. It is possible, but not necessary, to separate human heavy or light chain sequences (e.g. V, D, J, C region sequences) used for humanization by spacer sequences that separate the corresponding regions within the genomic sequence of the non-human (non-primate) animal the immunoglobulin of which is humanized.

In general, the humanization of an immunoglobulin (Ig) locus in a non-human animal involves the integration of one or more human Ig gene segments into the animal's genome to create humanized immunoglobulin loci. Thus, creation of a humanized Ig heavy chain locus involves the integration of one or more V and/or D and/or J segments, and/or C region segments into the animal's genome. Similarly, the creation of a humanized Ig light chain locus involves the integration of one or more V and/or J segments, and/or C region segments into the animal's genome.

Depending upon the approach used, the human Ig gene segment(s) can be integrated at the chromosomal location where the endogenous Ig locus of the animal ordinarily resides, or at a different locus of the animal. Regardless of the chromosomal location, the humanized Ig locus of the present invention has the capacity to undergo gene rearrangement and gene conversion and hypermutation in the non-human animal, thereby producing a diversified repertoire of humanized Ig molecules. An Ig locus having the capacity to undergo gene rearrangement and gene conversion is also referred to as a "functional" Ig locus and the antibodies with a diversity generated by a functional Ig locus are also referred to as "functional" antibodies or a "functional" repertoire of antibody molecules.

In a further aspect, the invention provides nucleic acid molecules comprising a human Ig gene segment, flanked by nucleotide sequences which comprise at least bout 20 contiguous nucleotides from a spacer sequence separating the coding regions in a non-primate animal Ig heavy or light chain gene, or from a consensus sequence of two or more of such spacer sequences. The flanking sequences just as the spacer sequence-derived sections within the flanking sequences can be identical or different. The contiguous nucleotides derived from a spacer sequence or from a consensus sequence of two or more spacer sequences can be fused directly to the human Ig gene segment. Alternatively, there might be an intervening sequence between the human Ig gene segment and at least one of the spacer-originating nucleotide sequences. Thus, for example, a flanking sequence at the 5' end of a human V gene segment may include a promoter region, which is linked directly to the human V gene segment, and separates it from the spacer-sequence derived nucleotide stretch of at least 20 nucleotides.

In yet another aspect, the invention concerns a humanized Ig heavy chain locus in which human heavy chain V, D and/or J gene segments and/or C region segments are present in the same configuration as in the original non-human animal immunoglobulin gene, and separated by sequences including at least about 20 contiguous nucleotides from an intron sequence separating the coding regions in a non-primate animal Ig heavy or light chain gene. In another embodiment, the present invention provides a humanized light chain locus in which human light chain C region segments and/or J gene segments and/or V region segments are separated by non-human animal (e.g. non-primate) intron sequences in the same configuration as in the original non-human animal immunoglobulin gene. In a particular embodiment, the spacer sequences are designed based on non-coding, e.g. intron sequences of the non-human (non-primate) animal. In one embodiment, the spacers may retain the appropriate non-coding sequences from the non-human (non-primate) animal. Alternatively, in order to simplify the construct, a consensus sequence, designed based upon the highly homologous non-coding (intron) sequences may be designed, and used as a uniform spacer sequence for the preparation of multiple human heavy or light chain gene segments.

The invention specifically provides isolated nucleic acid sequences and vectors useful in the construction of humanized immunoglobulin loci.

In one embodiment, DNA fragments containing an Ig locus to be humanized are isolated from animals which generate antibody diversity by gene conversion, e.g., rabbit and chicken. Such large DNA fragments can be isolated by screening a library of plasmids, cosmids, yeast artificial chromosomes (YACs) or bacterial artificial chromosomes (BACs), and the like, prepared from the genomic DNA of the non-human, e.g. non-primate animal. An entire animal C-region can be contained in one plasmid or cosmid clone which is subsequently subjected to humanization. YAC

clones can carry DNA fragments of up to 2 megabases, thus an entire animal heavy chain locus or a large portion thereof can be isolated in one YAC clone, or reconstructed to be contained in one YAC clone. BAC clones are capable of carrying DNA fragments of smaller sizes (about 150-450 kb). However, multiple BAC clones containing overlapping fragments of an Ig locus can be separately humanized and subsequently injected together into an animal recipient cell, wherein the overlapping fragments recombine in the recipient animal cell to generate a continuous Ig locus.

Human Ig gene segments can be integrated into the Ig locus on a vector (e.g., a BAC clone) by a variety of methods, including ligation of DNA fragments, or insertion of DNA fragments by homologous recombination. Integration of the human Ig gene segments is done in such a way that the human Ig gene segment is operably linked to the host animal sequence in the transgene to produce a functional humanized Ig locus, i.e., an Ig locus capable of gene rearrangement and gene conversion and hypermutation which lead to the production of a diversified repertoire of humanized antibodies.

In one embodiment, human Ig gene segments can be integrated into the Ig locus by homologous recombination. Homologous recombination can be performed in bacteria, yeast and other cells with a high frequency of homologous recombination events. For example, a yeast cell is transformed with a YAC containing an animal's Ig locus or a large portion thereof. Subsequently, such yeast cell is further transformed with a recombination vector as described hereinabove, which carries a human Ig gene segment linked to a 5' flanking sequence and a 3' flanking sequence. The 5' and the 3' flanking sequences in the recombination vector are homologous to those flanking sequences of the animal Ig gene segment on the YAC. As a result of a homologous recombination, the animal Ig gene segment on the YAC is replaced with the human Ig gene segment. Alternatively, a bacterial cell such as E. coli is transformed with a BAC containing an animal's Ig locus or a large portion thereof. Such bacterial cell is further transformed with a recombination vector which carries a human Ig gene segment linked to a 5' flanking sequence and a 3' flanking sequence. The 5' and the 3' flanking sequences in the recombination vector mediate homologous recombination and exchange between the human Ig gene segment on the recombination vector and the animal Ig gene segment on the BAC. Humanized YACs and BACs can be readily isolated from the cells and used in making transgenic animals.

In a further aspect of the present invention, methods of making transgenic animals capable of producing humanized immunoglobulins are provided.

According to the present invention, a transgenic animal capable of making humanized immunoglobulins are made by introducing into a recipient cell or cells of an animal one or more of the transgenic vectors described herein above which carry a humanized Ig locus, and deriving an animal from the genetically modified recipient cell or cells.

The recipient cells may, for example, be from non-human animals which generate antibody diversity by gene conversion and/or hypermutation, e.g., bird (such as chicken), rabbit, cows and the like. In such animals, the 3'proximal V gene segment is preferentially used for the production of immunoglobulins. Integration of a human V gene segment into the Ig locus on the transgene vector, either by replacing the 3'proximal V gene segment of the animal or by being placed in close proximity of the 3'proximal V gene segment, results in expression of human V region polypeptide sequences in the majority of immunoglobulins. Alternatively, a rearranged human V(D)J segment may be inserted into the J locus of the immunoglobulin locus on the transgene vector.

The transgenic vectors containing a humanized Ig locus is introduced into the recipient cell or cells and then integrated into the genome of the recipient cell or cells by random integration or by targeted integration.

For random integration, a transgenic vector containing a humanized Ig locus can be introduced into an animal recipient cell by standard transgenic technology. For example, a transgenic vector can be directly injected into the pronucleus of a fertilized oocyte. A transgenic vector can also be introduced by co-incubation of sperm with the transgenic vector before fertilization of the oocyte. Transgenic animals can be developed from fertilized oocytes. Another way to introduce a transgenic vector is by transfecting embryonic stem cells and subsequently injecting the genetically modified embryonic stem cells into developing embryos. Alternatively, a transgenic vector (naked or in combination with facilitating reagents) can be directly injected into a developing embryo. Ultimately, chimeric transgenic animals are produced from the embryos which contain the humanized Ig transgene integrated in the genome of at least some somatic cells of the transgenic animal.

In a particular embodiment, a transgene containing a humanized Ig locus is randomly integrated into the genome of recipient cells (such as fertilized oocyte or developing embryos) derived from animal strains with an impaired expression of endogenous immunoglobulin genes. The use of such animal strains permits preferential expression of immunoglobulin molecules from the humanized transgenic Ig locus. Examples for such animals include the Alicia and Basilea rabbit strains, as well as Agammaglobinemic chicken strain, as well as immunoglobulin knock-out mice. Alternatively, transgenic animals with humanized immunoglobulin transgenes or loci can be mated with animal strains with impaired expression of endogenous immunoglobulins. Offspring homozygous for an impaired endogenous Ig locus and a humanized transgenic Ig locus can be obtained.

For targeted integration, a transgenic vector can be introduced into appropriate animal recipient cells such as embryonic stem cells or already differentiated somatic cells. Afterwards, cells in which the transgene has integrated into the animal genome and has replaced the corresponding endogenous Ig locus by homologous recombination can be selected by standard methods See for example, Kuroiwa et al, Nature Genetics 2004, Jun. 6. The selected cells may then be fused with enucleated nuclear transfer unit cells, e.g. oocytes or embryonic stem cells, cells which are totipotent and capable of forming a functional neonate. Fusion is performed in accordance with conventional techniques which are well established. Enucleation of oocytes and nuclear transfer can also be performed by microsurgery using injection pipettes. (See, for example, Wakayama et al., Nature (1998) 394:369). The resulting egg cells are then cultivated in an appropriate medium, and transferred into synchronized recipients for generating transgenic animals. Alternatively, the selected genetically modified cells can be injected into developing embryos which are subsequently developed into chimeric animals.

Further, according to the present invention, a transgenic animal capable of producing humanized immunoglobulins can also be made by introducing into a recipient cell or cells, one or more of the recombination vectors described herein above, which carry a human Ig gene segment, linked to 5' and 3' flanking sequences that are homologous to the flanking sequences of the endogenous Ig gene segment, selecting

cells in which the endogenous Ig gene segment is replaced by the human Ig gene segment by homologous recombination, and deriving an animal from the selected genetically modified recipient cell or cells.

Similar to the target insertion of a transgenic vector, cells appropriate for use as recipient cells in this approach include embryonic stem cells or already differentiated somatic cells. A recombination vector carrying a human Ig gene segment can be introduced into such recipient cells by any feasible means, e.g., transfection. Afterwards, cells in which the human Ig gene segment has replaced the corresponding endogenous Ig gene segment by homologous recombination, can be selected by standard methods. These genetically modified cells can serve as nuclei donor cells in a nuclear transfer procedure for cloning a transgenic animal. Alternatively, the selected genetically modified embryonic stem cells can be injected into developing embryos which can be subsequently developed into chimeric animals.

Transgenic animals produced by any of the foregoing methods form another embodiment of the present invention. The transgenic animals have at least one, i.e., one or more, humanized Ig loci in the genome, from which a functional repertoire of humanized antibodies is produced.

In a specific embodiment, the present invention provides transgenic rabbits having one or more humanized Ig loci in the genome. The transgenic rabbits of the present invention are capable of rearranging and gene converting the humanized Ig loci, and expressing a functional repertoire of humanized antibodies.

In another specific embodiment, the present invention provides transgenic chickens having one or more humanized Ig loci in the genome. The transgenic chickens of the present invention are capable of rearranging and gene converting the humanized Ig loci, and expressing a functional repertoire of humanized antibodies In another specific embodiment, the present invention provides transgenic mice with one or more humanized V regions in the genome. The humanized V region comprises at least two human V gene segments flanked by non-human spacer sequences. The transgenic mice are capable of rearranging the human V elements and expressing a functional repertoire of antibodies.

Once a transgenic non-human animal capable of producing diversified humanized immunoglobulin molecules is made, humanized immunoglobulins and humanized antibody preparations against an antigen can be readily obtained by immunizing the animal with the antigen. A variety of antigens can be used to immunize a transgenic host animal. Such antigens include, without limitation, microorganisms, e.g. viruses and unicellular organisms (such as bacteria and fungi), alive, attenuated or dead, fragments of the microorganisms, or antigenic molecules isolated from the microorganisms.

Exemplary bacterial antigens for use in immunizing an animal include purified antigens from Staphylococcus aureus such as capsular polysaccharides type 5 and 8, recombinant versions of virulence factors such as alpha-toxin, adhesin binding proteins, collagen binding proteins, and fibronectin binding proteins. Exemplary bacterial antigens also include an attenuated version of S. aureus, Pseudomonas aeruginosa, enterococcus, enterobacter, and Klebsiella pneumoniae, or culture supernatant from these bacteria cells. Other bacterial antigens which can be used in immunization include purified lipopolysaccharide (LPS), capsular antigens, capsular polysaccharides and/or recombinant versions of the outer membrane proteins, fibronectin binding proteins, endotoxin, and exotoxin from Pseudomonas aeruginosa, enterococcus, enterobacter, and Klebsiella pneumoniae.

Exemplary antigens for the generation of antibodies against fungi include attenuated version of fungi or outer membrane proteins thereof, which fungi include, but are not limited to, Candida albicans, Candida parapsilosis, Candida tropicalis, and Cryptococcus neoformans.

Exemplary antigens for use in immunization in order to generate antibodies against viruses include the envelop proteins and attenuated versions of viruses which include, but are not limited to respiratory synctial virus (RSV) (particularly the F-Protein), Hepatitis C virus (HCV), Hepatits B virus (HBV), cytomegalovirus (CMV), EBV, and HSV.

Therapeutic antibodies can be generated for the treatment of cancer by immunizing transgenic animals with isolated tumor cells or tumor cell lines; tumor-associated antigens which include, but are not limited to, Her-2-neu antigen (antibodies against which are useful for the treatment of breast cancer); CD19, CD20, CD22 and CD53 antigens (antibodies against which are useful for the treatment of B cell lymphomas), (3) prostate specific membrane antigen (PMSA) (antibodies against which are useful for the treatment of prostate cancer), and 17-1A molecule (antibodies against which are useful for the treatment of colon cancer).

The antigens can be administered to a transgenic host animal in any convenient manner, with or without an adjuvant, and can be administered in accordance with a predetermined schedule.

After immunization, serum or milk from the immunized transgenic animals can be fractionated for the purification of pharmaceutical grade polyclonal antibodies specific for the antigen. In the case of transgenic birds, antibodies can also be made by fractionating egg yolks. A concentrated, purified immunoglobulin fraction may be obtained by chromatography (affinity, ionic exchange, gel filtration, etc.), selective precipitation with salts such as ammonium sulfate, organic solvents such as ethanol, or polymers such as polyethyleneglycol.

For making a monoclonal antibody, spleen cells are isolated from the immunized transgenic animal and used either in cell fusion with transformed cell lines for the production of hybridomas, or cDNAs encoding antibodies are cloned by standard molecular biology techniques and expressed in transfected cells. The procedures for making monoclonal antibodies are well established in the art. See, e.g., European Patent Application 0 583 980 A1 ("Method For Generating Monoclonal Antibodies From Rabbits"), U.S. Pat. No. 4,977,081 ("Stable Rabbit-Mouse Hybridomas And Secretion Products Thereof"), WO 97/16537 ("Stable Chicken B-cell Line And Method of Use Thereof"), and EP 0 491 057 B1 ("Hybridoma Which Produces Avian Specific Immunoglobulin G"), the disclosures of which are incorporated herein by reference. In vitro production of monoclonal antibodies from cloned cDNA molecules has been described by Andris-Widhopf et al., "Methods for the generation of chicken monoclonal antibody fragments by phage display", J Immunol Methods 242:159 (2000), and by Burton, D. R., "Phage display", Immunotechnology 1:87 (1995), the disclosures of which are incorporated herein by reference.

Cells derived from the transgenic animals of the present invention, such as B cells or cell lines established from a transgenic animal immunized against an antigen, are also part of the present invention.

In a further aspect of the present invention, methods are provided for treating a disease in a primate, in particular, a

human subject, by administering a purified humanized antibody composition, preferably, a humanized polyclonal antibody composition, desirable for treating such disease.

In another aspect of the present invention, purified monoclonal or polyclonal antibodies are admixed with an appropriate pharmaceutical carrier suitable for administration in primates especially humans, to provide pharmaceutical compositions. Pharmaceutically acceptable carriers which can be employed in the present pharmaceutical compositions can be any and all solvents, dispersion media, isotonic agents and the like. Except insofar as any conventional media, agent, diluent or carrier is detrimental to the recipient or to the therapeutic effectiveness of the antibodies contained therein, its use in the pharmaceutical compositions of the present invention is appropriate. The carrier can be liquid, semi-solid, e.g. pastes, or solid carriers. Examples of carriers include oils, water, saline solutions, alcohol, sugar, gel, lipids, liposomes, resins, porous matrices, binders, fillers, coatings, preservatives and the like, or combinations thereof.

The humanized polyclonal antibody compositions used for administration are generally characterized by containing a polyclonal antibody population, having immunoglobulin concentrations from 0.1 to 100 mg/ml, more usually from 1 to 10 mg/ml. The antibody composition may contain immunoglobulins of various isotypes. Alternatively, the antibody composition may contain antibodies of only one isotype, or a number of selected isotypes.

In most instances the antibody composition consists of unmodified immunoglobulins, i.e., humanized antibodies prepared from the animal without additional modification, e.g., by chemicals or enzymes. Alternatively, the immunoglobulin fraction may be subject to treatment such as enzymatic digestion (e.g. with pepsin, papain, plasmin, glycosidases, nucleases, etc.), heating, etc, and/or further fractionated.

The antibody compositions generally are administered into the vascular system, conveniently intravenously by injection or infusion via a catheter implanted into an appropriate vein. The antibody composition is administered at an appropriate rate, generally ranging from about 10 minutes to about 24 hours, more commonly from about 30 minutes to about 6 hours, in accordance with the rate at which the liquid can be accepted by the patient. Administration of the effective dosage may occur in a single infusion or in a series of infusions. Repeated infusions may be administered once a day, once a week once a month, or once every three months, depending on the half-life of the antibody preparation and the clinical indication. For applications on epithelial surfaces the antibody compositions are applied to the surface in need of treatment in an amount sufficient to provide the intended end result, and can be repeated as needed. In addition, antibodies can, for example, be administered as an intramuscular bolus injection, which may, but does not have to, be followed by continuous administration, e.g. by infusion.

The antibody compositions can be used to bind and neutralize antigenic entities in human body tissues that cause disease or that elicit undesired or abnormal immune responses. An "antigenic entity" is herein defined to encompass any soluble or cell-surface bound molecules including proteins, as well as cells or infectious disease-causing organisms or agents that are at least capable of binding to an antibody and preferably are also capable of stimulating an immune response.

Administration of an antibody composition against an infectious agent as a monotherapy or in combination with chemotherapy results in elimination of infectious particles. A single administration of antibodies decreases the number of infectious particles generally 10 to 100 fold, more commonly more than 1000-fold. Similarly, antibody therapy in patients with a malignant disease employed as a monotherapy or in combination with chemotherapy reduces the number of malignant cells generally 10 to 100 fold, or more than 1000-fold. Therapy may be repeated over an extended amount of time to assure the complete elimination of infectious particles, malignant cells, etc. In some instances, therapy with antibody preparations will be continued for extended periods of time in the absence of detectable amounts of infectious particles or undesirable cells. Similarly, the use of antibody therapy for the modulation of immune responses may consist of single or multiple administrations of therapeutic antibodies. Therapy may be continued for extended periods of time in the absence of any disease symptoms.

The subject treatment may be employed in conjunction with chemotherapy at dosages sufficient to inhibit infectious disease or malignancies. In autoimmune disease patients or transplant recipients, antibody therapy may be employed in conjunction with immunosuppressive therapy at dosages sufficient to inhibit immune reactions. The invention is further illustrated, but by no means limited, by the following examples.

EXAMPLE 1

Isolation and Sequencing of BAC Clones Containing Rabbit Immunoglobulin Loci

High molecular weight DNA was isolated from a2b5 male rabbits. The rabbits were euthanized, spleen and kidneys were removed and rinsed in ice-cold PBS. Fat and connecting tissues were removed and processed separately. The organs were cut into pieces and homogenized in a pre-cooled Dounce homogenizer. The supernatant was transferred into cooled 50 ml falcon tubes, mixed with cold PBS and large tissue debris was allowed to sink to the bottom for 2 minutes. Cells in the supernatant were pelleted at 200 g for 10 min at 4° C., washed once with PBS, resuspended in 1 ml PBS and counted. Sets of 5×10^6, 5×10^7 and 5×10^8 cells were embedded in agarose plugs using the CHEF Mammalian Genomic DNA Plug Kit (BIORAD) To optimize conditions for partial digestion with HindIII, plugs were cut into 5 equal pieces and digested with 1 to 10 units of HindIII for various times and temperatures. Best results were obtained with 2 units HindIII at 4° C. for 3 hrs or 37° C. for 25 min. Digested DNA was double size fractioned on a Pulse Field Gel Electrophoresis (PFGE) apparatus using the following parameters: 6 hr backwards, 15 s switch times; 6 hr forwards, 15 s switch times; 20 hr forwards, 90 s switch times; 200V 14° C. The area of the gel with the desired size of partial digested DNA was cut and DNA was isolated using gelase. 11 ng of insert was ligated with 1 ng of HindIII digested pBELOBAC 11 and electroporated into DH10B cells. 1% of the resulting colonies was sized using NotI and revealed an average insert size of 124 kb. 1×10^5 clones were spotted on Nylon filters and screened by hybridization with specific probes.

Probes for screening were amplified by PCR using genomic DNA from rabbits, cloned into pBlueScript, and verified by sequencing. Primer pairs (SEQ ID NO: 193-208, Table 2) were designed according to published sequences. Several BACs representing rabbit heavy and light chain immunoglobulin loci were isolated and mapped (FIGS. **1** and **5**). BACs 219D23 219D23 (GenBank Acc. No.

AY386695), 225P18 (GenBank Acc. No. AY386697), 27N5 (GenBank Acc. No. AY386696), 38A2 (GenBankAcc. No. AY386694), 179L1 (GenBank Acc. No. AY495827), 215M22 (GenBank Acc. No. AY495826), 19 (GenBankAcc. No. AY495828) and Fosmid Fos15B (GenBank Acc. No. AY3866968) were sequenced. Shotgun libraries for sequencing were constructed in pCR-Blunt with an insert size of 1.5-2 kb. For sequence analysis the STADEN package (Roger Staden, Cambridge, UK) was used. The software modules pregap and gap4 were used for assembly and gap closure. For the quality clipping of sequences PHRED (Washington University) and the STADEN package was coupled.

EXAMPLE 2

Construction of a Humanized Rabbit Immunoglobulin Heavy Chain Locus

BAC and fosmid clones containing rabbit immunoglobulin heavy chain locus sequences were isolated from genomic DNA libraries using probes specific for the constant, variable, and joining gene segments or the 3' enhancer region. Isolated BACs (FIG. **1**) 27N5 (GenBank Acc. No. AY386696), 219D23 (GenBankAcc. No. AY386695), 225P18 (GenBank Acc. No. AY386697), 38A2 (GenBank Acc. No. AY386694) and fosmid Fos15B (GenBank Acc. No. AY3866968) were sequenced (Ros et al., Gene 330, 49-59).

Selected immunoglobulin coding sequences were exchanged with corresponding human counterparts by homologous recombination in E. Coli by ET cloning (E-Chiang Lee et al., Genomics 73, 56-65 (2001); Daiguan Yu et al., PNAS 97, 5978-5983 (2000); Muyrers et al., Nucleic Acids Research 27, 1555-1557 (1999); Zhang et al., Nature Biotechnology 18, 1314-1317 (2000)).

Alternatively, DNA fragments were recombined by ligation in vitro and subsequent transformation of E. coli. BACs and/or Fos15B or parts thereof were combined by in vitro ligation and transformation, ET cloning, or by Cre recombinase mediated integration.

For ET cloning, vectors containing target sequence were transformed into a streptomycin resistant E. coli strain containing the inducible lambda phage recombination enzymes Redα, Redβ and γ. These recombination proteins were expressed either from a co-transfected plasmid (DH10B E. coli cells with plasmid pSC101) or from a genomic integrated lambda prophage (DY380 E. coli strain). The ET cloning procedure encompassed two homologous recombination steps.

In a first step the target locus was replaced by a selection-counter selection cassette (e.g. neo-rpsL which confers resistance to neomycin (neo) and sensitivity to streptomycin (rpsL)). After isolation of neo-resistant colonies, insertion of the selection cassette by homologous recombination was confirmed by restriction enzyme analysis and partial sequencing.

In a second step, the rpsL-neo selection cassette was exchanged with a new sequence. Streptomycin resistant clones were analyzed by restriction analysis and sequencing. Fragments used for the ET cloning procedure had flanking sequences of 20 to 50 bp length, which were identical to target sequences. Sequences used for ligation had appropriate restriction enzyme sites at their 3' and 5' ends. These sites were either naturally occurring sites or they were introduced by PCR using primers containing appropriate sites.

Alternatively, sequences were generated synthetically.

A humanized heavy chain was constructed by replacement of rabbit J_H, Cμ in BAC 219D23 and Cγ in BAC 27N5 with their corresponding human counterparts by ET cloning. Human sequences used for the ET cloning procedures were amplified by PCR from human genomic DNA.

Human Cμ, Cγ and J_H gene segments was amplified using primers (SEQ ID Nos: 209-214, Table 2) with 50 bp homologies to rabbit target sequences.

After ligation of BAC clone 225P18 with clone 219D23 and BAC 27N5 with Fosmid 15B, the ligated constructs were transformation into E. coli and connected by Cre recombinase mediated insertion. This resulted in a functional locus consisting of 18 rabbit variable genes, rabbit D region, human J region, human Cμ, human Cγ, rabbit Cϵ, rabbit Cα4 and the 3'enhancer element.

For the generation of transgenic animals the humanized BAC clones were coinjected either separately as three overlapping BACs (225P18 and 219D23 and BAC 27N5) or two overlapping combined BACs (225P18-219D23 and BAC 27N5-Fosmid 15B) or as one BAC (225P18-219D23-27N5-Fosmid 15B). Founder animals with transgenes were identified by PCR.

EXAMPLE 3

Construction of a Humanized Immunoglobulin Heavy Chain Locus Using Synthetic Fragments

Four fragments denoted Unit1, Unit2, Unit3, and Unit 4 (FIG. **13**, SEQ ID Nos: 189-192) with human V sequences and rabbit spacers were chemically synthesized. Each fragment was flanked 5' by an AscI restriction endonuclease recognition sequence, 3' by a lox71 Cre recombinase recognition sequence followed by Fse I and MluI restriction enzyme recognition sequences. Unit 1 consisted of human V_H3-49, V_H3-11, V_H3-7 and V_H3-15 variable genes separated by rabbit spacers I29-30, I3-4, I2-3 and the 3' half of I1-2 (I1-2B). Unit 2 consisted of human V_H3-48, V_H3-43 and V_H3-64 separated by rabbit spacers I1-2A (5' half of I1-2), I7-8, I6-7 and the 3' half of I4-5 (I4-5B). Unit 3 consisted of human V_H3-74, V_H3-30, and V_H3-9 separated by the rabbit spacer sequences I4-5B, I26-27, I11-12 and I17-18.

Unit 3 had in addition to the afore mentioned upstream flanks an Flp recombinase recognition target (FRT) sequence, followed by a Sglf I restriction endonuclease recognition sequence preceding the already mentioned Asc I site.

Unit 4 had the human V_H3-23 gene 5' flanked by the rabbit spacer I1-2, a lox66 Cre recombinase target sequence and an AscI endonuclease recognition sequence, and 3' flanked by IV-C (5' half) rabbit spacer sequence followed by a MluI endonuclease recognition sequence.

A gentamycin selection cassette was PCR-amplified, using primers SEQ ID NOs 215 and 216 (Table 2) containing AscI and FseI sites and ligated into a pGEM vector with a modified cloning site including AscI, FseI, and MluI endonuclease recognition sites (pGEM.Genta modified by PCR using SEQ ID NOs 217 and 218, Table 2).

Units 1, 2 and 3 were cloned into pGEM.Genta (Promega) vectors.

Unit 4 was sub-cloned into a customized pBELOBAC11 (NEB) vector linearized with Hind III, and PCR-amplified. The forward primer (SEQ ID NO: 219, Table 2) had restriction sites for HindIII, PacI and AatII, and the reverse primer (SEQ ID NO: 220) sites for Bam HI, MluI and AscI. The

primers were designed in such a way that the pBELOBAC11 Chloramphenicol selection cassette was deleted. Furthermore, a Neomycin selection cassette was PCR-amplified with primers SEQ ID NOs 221 and 222 (Table 2) carrying Bam HI and Hind III restriction sites, and ligated to the modified pBELOBAC11 vector (pBB11.1).

Units 1-4 were assembled by cre-mediated recombination as described (Mejia et al, Genomics 70(2) 165-70 (2000)). First Unit 1 was cloned into the customized pgem.Genta vector, digested with Fse I and subsequently recircularized by ligation. This vectorless construct was transformed into E. coli containing pBB11.1.Unit4 and p706-Cre plasmid. Following recombination of Unit 1 with PBB11.1 unit 4, positive clones (Unit4/1) were selected on kanamycin and gentamycin containing media. Clones were characterized by restriction analyses using various enzymes.

For recombination of Unit 2, the Unit4/1 insert was excised by double digestion with AscI and PacI, and cloned into pBELOBAC11 with a modified linker (pBB11.2: modified by PCR using primers SEQ ID NOs 223 and 224, Table 2).

pBELOBAC11 was linearized with HindIII and PCR-amplified with a forward primer encoding PacI and AatII endonuclease recognition sites and a reverse primer encoding MluI and NotI endonuclease recognition sites and a lox66 Cre recombinase target site. For ligation with Unit1/4 the pBB11.2 vector was opened with MluI and PacI. pGEM-.Genta.Unit2 was converted into a circular vectorless construct as described for pGEM.Genta.Unit1 and connected with pBB11.2.Unit4/1 by in vivo Cre mediated recombination. Subsequently, the resulting construct pBB11.2.Unit4/1/2 is prepared for Cre mediated recombination with Unit 3 by replacing the wild type loxp site with a lox66 target site by ET-cloning (Muyrers et al., Nucleic Acids Research 27, 1555-1557 (1999); Muyrers et at Trends Biochem. Sci. 26(5):325-31 (2001)). A chloramphenicol selection cassette was amplified by PCR with primers (SEQ ID NOs 225 and 226, Table 2) containing 50 bp sequences homologous to the BAC target sequence. The reverse primer included a lox66 site. The gel-purified PCR product was transformed into cells carrying the target BAC as well as the pSC101 plasmid, required for homologous recombination. Positive clones were selected with chloramphenicol and confirmed by restriction analysis and sequencing. pGEM.Genta.Unit 3 was prepared for in vivo recombination as described above for Unit1 and 2 and transformed into cells carrying the receptor BAC, as well as the p706-Cre plasmid. Positive clones pBB11.2.Unit4/1/2/3 were selected with gentamycin and confirmed by restriction analysis. pBB11.2.Unit4/1/2/3 was further modified by ET-cloning to generate a lox 71 target site. Subsequently, pBB11.2.Unit4/1/2/3 was connected to fragments from BACs 219D23, 27N5 and Fos15B.

EXAMPLE 4

Construction of a Humanized Immunoglobulin Heavy Chain Locus Using PCR Amplified Fragments

Human V_H elements were amplified using genomic DNA (ClonTech) and primers SEQ ID NOs 227-248 (Table 2). PCR products were analyzed by gel-electrophoresis and gel purified using the GENECLEAN kit (Q-Biogen). Subsequently, amplification products were sub-cloned into Zero-Blunt TOPO™ (Invitrogen), according to the manufacturer's instructions. The sequences of all amplified V elements were confirmed. For the construction of the humanized V region, V elements were amplified using plasmid DNA as template and primers SEQ ID NOs 249-270 (Table 2). Forward primers contained an AscI site, followed by a rabbit splice site. Reverse primers contained a rabbit recombination signal sequence (RSS) and a MluI restriction site. PCR products were gel purified using the GENECLEAN kit.

Human V_Hs, V3-33, V3-74, V3-49, V3-21, V3-48, V3-73, V3-7, and V3-D could not be isolated by PCR and were synthesized chemically (BlueHeron, Bothel, Wash.). Restriction sites and rabbit regulatory sequences were added during synthesis.

Rabbit spacer sequences were amplified using BACs 38A2 and 225P18 as templates and primers SEQ ID NOs 271-288 (Table 2). BAC 225P18 was double digested with NheI and a 41 kb fragment was gel purified. This fragment served as a template for the amplification of spacers V1-2, V2-3, V3-4, V4-5, and V5-6.

BAC 225P18 was digested with BstBI and the template for spacers V6-7 and V7-8 was gel-purified. A double digestion of BAC 38A2 with PacI and RsrII allowed gel purification of the template for spacers V21-22, and V22-23.

Amplified spacer sequences were gel-purified, and sub-cloned into XL-PCR-TOPO™ (Invitrogen) according to the manufacturer's instructions.

V_H elements and rabbit spacer sequences were sub-cloned into modified pGEM (Promega) and pBS (Strategene) vectors. The pGEM vector was cut with NotI and Hind III and ligated with chemically synthesized oligonucleotide sequences containing FseI, AscI and MluI sites (Oligo1: SEQ ID NO: 289; Oligo2: SEQ ID NO: 290; Table 2). Vector pBS was cut with SacI and KpnI and ligated with a chemically synthesized oligonucleotide sequence containing the restriction sites FseI, AscI and MluI (Oligo1: (SEQ ID NO: 291; Oligo2: SEQ ID NO: 292, Table 2).

Gentamycin and neomycin selection cassettes were amplified using primers (SEQ ID NOs: 293-296, Table 2) with Fse I or AscI sites and ligated into the modified pGEM and pBS-vectors.

The final construct is built in a modified pBeloBAC II vector. The pBeloBAC II vector was opened with BamHI and HindIII and the cloning sites were modified to contain FseI, AscI, MluI sites using a chemically synthesized oligonucleotide sequence (Oligo1: SEQ ID NO: 297; Oligo2: SEQ ID NO: 298, Table 2).

BAC 219D23 was modified by introduction of restriction sites using ET-cloning (Muyrers et al., Nucleic Acids Research 27, 1555-1557 (1999); Muyers et at Trends Biochem. Sci. 26(5):325-31 (2001)). The Neomycin selection cassette was amplified with primers SEQ ID NO: 299 and SEQ ID NO: 300 (Table 2). The forward primer contained an FseI site, the reverse primer an AscI site.

The purified PCR product was transformed into E. coli cells carrying BAC 219D23 and plasmid pSC101 necessary for homologous recombination. After homologous recombination of the cassette and the target sites in the BAC, introduced restriction sites were confirmed by restriction analysis. Subsequently, the modified BAC 219D23 was digested with FseI and MluI and the resulting 17 kb fragment (containing the FseI-Neo-AscI cassette) was separated by PFGE and purified by electro-elution. This purified fragment was ligated with the modified pBeloBAC II vector opened with FseI and MluI.

A purified DNA fragment encoding a human V_H element is ligated with the modified pGEM.neo vector opened with AscI and MluI. Similarly a spacer sequence is sub-cloned into the modified pGEM.genta vector. Subsequently, the pGEM.genta vector carrying the spacer sequence is cut with

FseI and MluI and the insert is ligated with pGEM.neo.V_H vector opened with FseI and AscI. This step is repeated several times to build a fragment consisting of several spacer and V_H segments. Such fragments are excised with FseI and MluI and ligated with the modified pBeloBAC II vector linearized with FseI and AscI. These processes are repeated to build a large immunoglobulin V region (FIG. **6**). The humanized heavy chain locus is used for the generation of transgenic animals.

EXAMPLE 5

Construction of a Humanized Rabbit Light Chain Locus Containing Humanized Ck and Humanized Rearranged VJ

Screening of a rabbit genomic BAC libraries resulted in the identification of three BACs (215M22, 179L1 and 196O2; Gene Bank Accession Nos: AY495826, AY495827, and AY495828, respectively) containing rabbit light chain K1 gene segments. Rabbit Cκ□ was exchanged with human Cκ□ allotype Km3 by ET cloning as described (E-Chiang Lee et al., Genomics 73, 56-65 (2001); Daiguan Yu et al., PNAS 97, 5978-5983 (2000); Muyrers et al., Nucleic Acids Research 27, 1555-1557 (1999); Zhang et al., Nature Biotechnology 18, 1314-1317 (2000)). Human Cκ (allotype Km3) was amplified by PCR with primers (SEQ ID Nos 301 and 302, Table 2) containing 50 bp sequences homologous to target sequences. Homology arms were designed based on the published sequence of rabbit germline kappa (b5; GenBank Accession No. K01363) and matched the intron-exon boundary of Cκ. The exchange of rabbit Cκagainst the human Cκ in BAC 179L1 was verified by sequencing.

BAC 179L1-huCk was modified by two ET cloning. A neomycin selection cassette was amplified with primers (SEQ ID NOs 303 and 304, Table 2) containing 50 bp sequences homologous to BAC 179L1. The forward primer additionally had an i-CeuI meganuclease site. The PCR product was used for ET cloning. Positive clones were selected with neomycin and checked for correctness by restriction enzyme digests and sequencing. A zeocin selection cassette was amplified with primers (SEQ ID NOs 305 and 306, Table 2) containing 50 bp sequences homologous to BAC 179L1. The forward primer additionally had an i-SceI meganuclease site. The PCR product was used for ET cloning. Positive clones were selected with zeozin and checked for correctness by restriction enzyme digests and sequencing.

BAC 215M22 was modified by one ET cloning. A gentamycin resistance gene was amplified with primers (SEQ ID NOs 307 and 308, Table 2) containing 50 bp sequences homologous to BAC215M22. The forward primer additionally had an i-CeuI Meganuclease site and the reverse primer an i-SceI meganuclease site. The PCR product was used for ET cloning. Resulting clones were selected with gentamycin and analyzed by restriction enzyme digests and sequencing.

Modified BAC179L1 and 225M22 were cut with i-CeuI and i-SceI. Fragments of 98 kb and 132 kb were purified and ligated. Resulting clones were selected with kanamycin and chloramphenicol and checked for correctness by restriction enzyme digests, PCR of the regions containing i-SceI and i-CeuI restriction sites, and sequencing. The resulting BAC was termed 179-215-huCk.

Rabbit Jκ1 and Jκ2 of BAC 179-215-huCk were replaced by ET cloning with a synthetic human rearranged VκJκ gene. A DNA fragment with rabbit promoter, rabbit leader, rabbit intron and human VκJκ gene was synthesized chemically. The codon usage of the synthetic human VJ was optimised to achieve highest DNA sequence homology to rabbit V kappa genes.

The synthetic human VJ was PCR amplified with a forward primer (SEQ ID NO 309, Table 2) containing 50 bp sequences homologous to BAC 179L1 and a reverse primer (SEQ ID NO 310, Table 2) containing a sequence homologous to the gentamycin resistance gene and a FRT site. A gentamycin resistance gene was amplified with a forward primer (SEQ ID NO 311, Table 2) containing a FRT site and a reverse primer (SEQ ID NO 312, Table 2) with 50 bp homology to BAC 179L1 and a FRT site. The human synthetic human VJ and the gentamycin resistance gene were combined by overlap extension PCR using the forward primer for the synthetic human VJ gene and the reverse primer for the gentamycin resistance gene. The resulting fragment was used for ET cloning. Positive clones were selected with gentamycin and checked for correctness by restriction enzyme digests and sequencing.

The gentamycin resistance gene was removed by site specific recombination via expression of Flp recombinase. After recombination one FRT was left. The FRT site was deleted by ET cloning. A 232 bp fragment from the synthetic human VJ was amplified by PCR (using primers SEQ ID NOs 313 and 314, Table 2) and used for ET cloning. Resulting colonies were screened by PCR (using primers SEQ ID NOs 315 and 316, Table 2) for loss of the FRT site and confirmed by sequencing.

The neomycin resistance gene of BAC179-215-huCk was replaced by ET cloning. A gentamycin resistance (pRep-Genta; Genebridges) gene was amplified by PCR with primers (SEQ ID NOs 317 and 318, Table 2) containing 50 bp sequences homologous to BAC 179-215-huCk. The forward primer additionally had a loxP site, an attB site and a PvuI restriction site. Resulting clones were selected with gentamycin and checked for correctness by restriction enzyme digests and sequencing.

The resulting BAC (rLC3-B; FIG. **8**) was used for the generation of transgenic animals.

EXAMPLE 6

Construction of a Humanized Rabbit Light Chain Locus Containing Multipe Human Vk Elements, Chicken Spacer Elements and a Rearranged Human VJ

Screening of a rabbit genomic BAC libraries resulted in the identification of three BACs (215M22, 179L1 and 196O2; Gene Bank Accession Nos: AY495826, AY495827, and AY495828, respectively) containing rabbit light chain K1 gene segments. Rabbit Cκ□0 was exchanged with human Cκ□ allotype Km3 by ET cloning as described (E-Chiang Lee et al., Genomics 73, 56-65 (2001); Daiguan Yu et al., PNAS 97, 5978-5983 (2000); Muyrers et al., Nucleic Acids Research 27, 1555-1557 (1999); Zhang et al., Nature Biotechnology 18, 1314-1317 (2000)). Human Cκ allotype Km3) was amplified by PCR with primers (SEQ ID Nos 301 and 302, Table 2) containing 50 bp sequences homologous to target sequences. Homology arms were designed based on the published sequence of rabbit germline kappa (b5; GenBank Accession No. K01363) and matched the intron-exon boundary of Cκ. The exchange of rabbit Cκ against the human Cκ in BAC 179L1 was verified by sequencing.

Two DNA fragments, Unit1 (12,235 bp, FIG. **12**a, SEQ ID NO 187), containing 17 human V pseudogenes and 18

chicken spacer sequences and Unit 2 (13,283 bp, FIG. **12**b, SEQ ID NO 188) containing one functional rearranged human kappa VJ gene with leader, 11 human V pseudogenes, 12 chicken spacer sequences and intron 1 and parts of intron 2 were synthesized chemically and cloned into vector pBR322.

Units 1 and 2 were digested with the restriction enzyme NgoMIV and AsiSI or NgoMIV and AscI respectively and ligated into pBELOBAC11 with a modified linker by three fragment ligation. The modified linker contained a BsiWI restriction site, a FRT5-site, a rpsL-Neo-cassette, a AscI site and a AsiSI-site. The linker fragment was amplified with High fidelity polymerase (Roche), primers CE_1_001_012904 (SEQ ID NO 319, Table 2) and CE_1_on005_013004 (SEQ ID NO 320, Table 2) and plasmid pRpsL-Neo (Genebridges) as template. Subsequently, the amplified product was ligated into BamHI and HindIII sites of pBELOBAC11. For ligation with Unit 1 and 2 the modified pBELOBAC11 was opened with AsiSI and AscI. Positive clones (pBELOBAC11 Unit1/2) were checked by restriction enzyme digests.

BAC 179L1 (GENBANK Acc. No. AY495827) was modified by insertion of two modified selection cassettes by ET cloning. Cassette 1 was a gentamycin resistance gene amplified with primers (SEQ ID Nos 321 and 322, Table 2) containing 50 bp sequences homologous to BAC 179L1 and an AscI site in the reverse primer. Cassette 2 was a rpsl-Neo selection cassette amplified with primers (SEQ ID Nos 323 and 324, Table 2) containing 50 bp sequences homologous to BAC 179L1 and an attB site, a FRT5 site and a BsiWI site in the forward primer.

The purified PCR products were transformed into E. coli cells carrying the BAC and plasmid pSC101 necessary for homologous recombination. After homologous recombination successful modification of the BAC was confirmed by restriction digest analyses, Southern Blot and sequencing.

Modified BAC 179L1 was cut with the restriction enzymes AscI and BsiWI. The fragment containing the human Cκ was purified and ligated with pBELOBAC11 Unit1/2 opened with the same restriction enzymes. Positive clones were checked by restriction enzyme digests. The final construct (FIG. **12**c) is used for the generation of transgenic animals

EXAMPLE 7

Construction of a Humanized Rabbit Light Chain Locus Containing Multiple Human Vκ Elements, Chicken Spacers and an Unrearranged Human J Kappa Locus

The construct described in example 6 was modified by ET cloning as follows: an. The rearranged functional VJ sequence was exchanged with a functional V1 flanked by a functional recombination signal sequence (RSS). The RSS was PCR amplified from BAC179L1 with a forward primer (SEQ ID NO 325, Table 2) containing a 50 bp sequence homologous to V1 of pBELOBAC11 Unit1/2 and a reverse primer (SEQ ID NO 326, Table 2) containing an AscI restriction enzyme site and homology to the gentamycin resistance gene. A gentamycin resistance gene was amplified with a forward primer (SEQ ID NO 327, Table 2) containing a sequence homologous to the reverse primer used for RSS amplification and a reverse primer (SEQ ID NO 328, Table 2) containing a 50 bp sequence homologous to pBELOBAC11 Unit1/2 and a BsiWI restriction enzyme site.

The RSS and the gentamycin resistance gene were combined by overlap extension PCR using the forward primer for RSS amplification and the reverse primer for Gentamycin resistance gene amplification. The resulting fragment was used to modify pBELOBAC11 Unit1/2 by ET cloning. Positive clones were selected with gentamycin and analyzed by restriction enzyme digests and sequencing.

BAC 179L1 with human Cκ was further modified by ET cloning. A kanamycin selection cassette was amplified with a primers (SEQ ID NO 329 and 330, Table 2) containing 50 bp sequences homologous to BAC 179L1. The reverse primer contained also an AscI restriction enzyme site and a FRT site. The PCR product was used for ET cloning. An ampicillin selection cassette was amplified with primers (SEQ ID Nos 331 and 332, Table 2) containing 50 bp sequences homologous to BAC 179L1. The forward primer contained also an attB site, an AsiSI restriction enzyme site and a FRT5 site The reverse primer contained a BsiWI restriction enzyme site and a FRT site. The PCR product was used for ET cloning. The human J region was amplified from human genomic DNA with primers (SEQ ID Nos 333 and 334, Table 2) containing 50 bp sequences homologous to BAC 179L1. The PCR product was used for ET cloning. The resulting clones were analyzed by restriction enzyme digest and sequencing.

A positive clone was cut with AscI and BsiWI. The resulting fragment was purified and ligated into the modified pBELOBAC11 Unit1/2 cut with the same enzymes. Positive clones were selected with ampicillin and analyzed by restriction enzyme digests and sequencing. Correct clones are used to generate transgenic animals.

EXAMPLE 8

Construction of a Humanized Rabbit Light Chain Locus Containing Multiple Human Vk Elements

Screening of a rabbit genomic BAC libraries resulted in the identification of three BACs (215M22, 179L1 and 196O2; Gene Bank Accession Nos: AY495826, AY495827, and AY495828, respectively) containing rabbit light chain K1 gene segments. Rabbit Cκ1 was exchanged with human Cκ□ allotype Km3 by ET cloning as described (E-Chiang Lee et al., Genomics 73, 56-65 (2001); Daiguan Yu et al., PNAS 97, 5978-5983 (2000); Muyrers et al., Nucleic Acids Research 27, 1555-1557 (1999); Zhang et al., Nature Biotechnology 18, 1314-1317 (2000)). Human Cκ allotype Km3) was amplified by PCR with primers (SEQ ID NOs 301 and 302, Table 2) containing 50 bp sequences homologous to target sequences. Homology arms were designed based on the published sequence of rabbit germline kappa (b5; GenBank Accession No. K01363) and matched the intron-exon boundary of Cκ. The exchange of rabbit Cκ against the human Cκ in BAC 179L1 was verified by sequencing.

Human Vκ elements of the Vκ1 family (O2, L8, L4, A30, L11, L1, L5, L15, O8, L19, L12, A20, O4, L14, L23, L9, A4, L24, O6, L22, A9, A25, A15, O9) were amplified by PCR using primers (SEQ ID NOs 335-382, Table 2) and human genomic DNA as a template.

Amplification products were analysed by gel-electrophoresis, gel purified using GENECLEAN (Q-Biogen), subcloned into the Zero-Blunt TOPO™ vectors (Invitrogen) and sequenced. A rearranged human Vκ (O2) Jκ (J4) element was produced by PCR amplification, subcloned and sequenced. To combine human Vκ elements with rabbit spacers, human Vκ elements were amplified by PCR with

primers (SEQ ID Nos 383-430, Table 2) using plasmid DNA as a template. Primers contained AscI or MluI sites.

Rabbit spacer sequences are amplified by PCR using primers SEQ ID NOs 431-450 (Table 2). BACs 179L1 and 215M22 are digested with SpeI, NheI, AclI, SfoI, MluI, and SalI/XhoI. Fragments are gel purified and used as amplification templates.

The spacer sequence located at the 5-end is amplified by an upstream oligonucleotide containing a FRT and an attB site. PCR products are gel purified using the GENECLEAN kit and subcloned into XL-PCR-TOPO™ (Invitrogen) according to the manufacturer's instructions.

Human Vk elements and rabbit spacer sequences were cloned into pGem (Promega) modified as described in Example 4.

Human V kappa and the modified pGEM.genta vector are digested with AscI and MluI and ligated. Similarly, rabbit spacer sequences are cloned into pGEM.neo. Subsequently, pGem.neo. Vκ is cut with FseI and AscI and ligated with a purified insert of pGem.genta.spacer excised with FseI and MluI. Ligation of AscI and MluI complementary ends destroys the restriction enzyme site and allows repeated use of AscI and MluI for the construction of a Vκ locus comprising several Vκ and spacer elements. The final construct, consisting of fragments of a humanized BAC 179L1 and 215M22 and a humanized Vκ region is built in pBeloBAC. BAC 179L1 and 215M22 were modified and combined. Subsequently, BAC 179L1-215M22-huCk was further modified by ET cloning. Two cassettes containing restriction enzyme site, selection markers, and additional functional sites were inserted into the vector by ET-cloning as shown in FIG. **7**. Primers (SEQ ID NOs 451-454) used for the amplification of the cassettes are listed in Table 2.

To built the final construct, units consisting of human V elements, rabbit spacer elements and a resistance marker are excised out of pGEM with FseI and MluI and ligated with BAC 179L1-215M22 digested with FseI and AscI. Subsequently, the resistance marker is replaced with a new insert consisting of human V elements, rabbit spacer elements and another resistance marker. After several repeats the final construct will consist of many Vk segments (L8, L4, A30, L11, L1, L5, L15, O8, L19, L12, A20, O4, L14, L23, L9, A4, L24, O6, L22, A9, A25, A15, O9) separated by rabbit spacer sequences. The humanized light chain locus is used for the generation of transgenic animals.

EXAMPLE 9

Construction of a Humanized Heavy Chain Locus with Chicken Heavy Chain Locus Spacer Sequences

A synthetic humanized heavy chain locus containing a rabbit D region, a human J region, human Cμ, human Cγ, rabbit Cα4, the rabbit 3'α enhancer and human VH elements (including promotor and nonamer/heptamer sequences) separated by chicken spacer sequences is constructed.

Modified rabbit BAC 27N5 (see "Example 2) was further modified by ET cloning. The construct contained a humanized Cμ and Cγ and two unique restriction sites, BsiWI and AsiSI downstream of the α-4 membrane exon. DNA is amplified with oligonucleotides SEQ ID Nos 455 and 456 (Table 2).

Fosmid Fos15B is digested with NheI and the resulting 13 kb fragment containing the 3'α enhancer is subcloned into a cloning vector in such a way that the insert is flanked by BsiWI and AsiSI sites. Subsequently, the insert is excised with BsiWI and AsiSI and ligated with the modified BAC 27N5 to form BAC 27N5Fos.

The rabbit J region in BAC219D23 was exchanged with the corresponding human J region by ET cloning. The human J region was amplified by PCR using primers SEQ ID NOs 457 and 458 (Table 2).

Unique restriction enzyme sites are inserted in BAC219D23 upstream of the D region (A) and upstream of Cμ(B)□. In BAC 27N5Fos restriction site A is inserted upstream of the linker region and B is inserted in sequences homologous to BAC219D23. Following digestion with enzymes A and B, the fragment containing human J and rabbit D regions is isolated and ligated with BAC 27N5Fos to create BAC 219D23/27N5Fos.

Chicken heavy chain spacer sequences are amplified from chicken genomic DNA by PCR using primers (SEQ ID NOS 459 and 460, Table 2) specific for chicken heavy chain V pseudogenes (Mansikka et al., J Immunol 145(11), 3601-3609 (1990), Reynaud et al., Cell 59(1), 171-183 (1989)). Alternatively, spacer sequences are synthesized chemically.

The PCR products are gel purified, cloned into pTOPO (Invitrogen) and sequenced.

Human heavy chain variable elements are amplified by PCR using primers designed according to published sequences in GENBANK (eg Acc. No. NG_001019) or synthesized chemically. The human V elements contain the human promoter region, the human leader sequence, the human intron between leader and V-coding region, the human V-coding region and the human recombination signal sequence. The amplified or synthesized fragments are flanked by specific restriction endonuclease recognition. Chicken spacer sequences and human V elements are combined in one or several large DNA fragment comprising a humanized immunoglobulin locus. The construct is used to generate transgenic animals.

EXAMPLE 10

Construction of a Humanized Immunoglobulin Locus Containing Human V Elements and Non-Human Spacer Sequences (without Promoter Region and RSS)

A BAC library generated with non-human genomic DNA is screened with probes specific for immunoglobulin and BAC clones containing heavy and light chain immunoglobulin C, J and D regions are identified. The BAC clones are modified to contain restriction enzyme sites. Human heavy and light chain variable elements are amplified by PCR using primers designed according to published sequences in GenBank (eg., Acc. No. NG_001019). Sequences are amplified from genomic DNA or synthesized chemically. The human V elements contain the human promoter region, the human leader sequence, the human intron between leader and V-coding region, the human V-coding region and the human recombination signal sequence (RSS). The amplified or synthesized fragments have specific restriction endonuclease recognition sites at the ends. The non-human spacer sequences are amplified by PCR or synthesized chemically. Non-human spacer sequences and human V elements are combined in one or several large DNA fragment comprising a humanized immunoglobulin locus. The construct is used to generate transgenic animals. An example for the construction of a humanized V region using chicken spacer sequences is shown in FIG. **10**.

EXAMPLE 11

Construction of a Humanized Immunoglobulin Locus Containing Human V Elements and Non-Human Spacer Sequences

A BAC library generated with non-human genomic DNA is screened with probes specific for immunoglobulin and BAC clones containing heavy and light chain immunoglobulin C, J and D regions are identified. The BAC clones are modified to contain restriction enzyme sites. Human heavy and light chain variable elements are amplified by PCR using primers designed according to published sequences in GenBank (eg., Acc No. NG_001019). Sequences are amplified from genomic DNA or synthesized chemically. The human V elements contain the human V coding region. Non-human spacer sequences are amplified by PCR or synthesized chemically and contain a recombination signal sequence, a spacer sequence, a promoter region, a leader sequence and the intron between leader and V coding region. Such non-human spacer sequences are combined with human V elements in one or several large DNA fragments and used for the generation of transgenic animals. An example for the construction of a humanized V region using mouse or rabbit spacer sequences is shown in FIG. **11**.

EXAMPLE 12

Transgenic Rabbits Expressing Humanized Immunoglobulins

Transgenic rabbits were generated as described by Fan et al. (Pathol. Int. 49: 583-594, 1999). Briefly, female rabbits are superovulated using standard methods and mated with male rabbits. Pronuclear-stage zygotes are collected from oviduct and placed in an appropriate medium such as Dulbecco's phosphate buffered saline supplemented with 20% fetal bovine serum. BAC containing humanized immunoglobulin loci were microinjected into the male pronucleus with the aid of a pair of manipulators. Morphological surviving zygotes were transferred to the oviducts of pseudopregnant rabbits. Pseudopregnancy was induced by the injection of human chorionic gonadotrophin (hCG). Following injection of a humanized light chain construct into 4645 pronuclei of fertilized oocytes, 4043 oocytes were transferred into 132 recipients. In total, 253 live offspring were born, 11 of which were transgenic. Expression of human kappa light chain was detected by ELISA using human-kappa light chain specific reagents (for example, mouse anti-human Kappa, Southern Biotech, 9220-01; goat anti-human Kappa, Southern Biotech 2063-08).

A humanized heavy chain construct was injected into 4083 pronuclei of fertilized oocytes. 3485 oocytes were transferred into 119 recipients which delivered 433 offspring. Analysis by PCR and FISH revealed that 20 of these animals were transgenic. Humanized heavy chain in the blood of founder animals was detected by ELISA using antibodies specific for human IgM/IgG (for example, rabbit anti-human IgM, Rockland 609-4131; rabbit anti-human IgM, Rockland 609-4631; rabbit anti-human IgG, Pierce 31142, rabbit anti-human IgG, Southern Biotech 6145-08; rabbit anti-human IgG, Pierce 31784).

Sandwich-type ELISAs detecting humanized κ, μ and γ chains were performed using standard procedures. Briefly, microtiter plates were coated with capture antibody and incubated with diluted serum samples. Bound human immunoglobulin was detected using a secondary labeled antibody and peroxidase-streptavidin-conjugate (Sigma, S2438).

Double transgenic animals expressing both humanized heavy and light immunoglobulin chains were generated by breeding of founder animals.

EXAMPLE 13

Transgenic Mice Expressing Humanized Immunoglobulins

Transgenic mice were generated as described by Nagy et al. (Manipulating the Mouse Embryo: A Laboratory Manual; Cold Spring Harbor Laboratory Press, New York, 2003). Briefly, female mice were superovulated using standard methods and mated with male mice. Pronuclear-stage zygotes were collected from oviduct and placed in a suitable medium such as M2 medium. BAC containing humanized immunoglobulin loci were microinjected into the male pronucleus with the aid of a pair of manipulators. Morphologically surviving zygotes were transferred to the oviducts of pseudopregnant female mice. Pseudopregnancy was induced by mating with sterile males. Following injection of a humanized light chain construct into 1325 pronuclei of fertilized oocytes, 787 oocytes were transferred into 29 recipients. In total, 55 live offspring were born, 11 of which were transgenic.

A humanized heavy chain construct was injected into 1050 pronuclei of fertilized oocytes. 650 oocytes were transferred into 25 recipients which delivered 64 live offspring. Analysis by PCR revealed that 19 of these animals were transgenic.

Double transgenic animals expressing both humanized heavy and light immunoglobulin chains are generated by breeding of founder animals. Expression of humanized κ, μ and γ chains was detected by ELISAs using standard procedures. Briefly, microtiter plates were coated with capture antibody and incubated with diluted serum samples. Bound human immunoglobulin was detected using a secondary labeled antibody and peroxidase-streptavidin-conjugate (Sigma, S2438).

All references cited throughout the specification are hereby expressly incorporated by reference. While the invention is illustrated by reference to certain embodiments, it is not so limited. One skilled in the art will recognize that various modifications and variations are possible without diverting from the essence of the invention. All such modifications and variations are specifically included within the scope herein.

TABLE 1

ID	BAC	Accession#	Start	Finish	Description
1	38A2	AY386694	1	2137	Spacer 5' start-V34
2	38A2	AY386694	2433	9504	Spacer V34-V33
3	38A2	AY386694	9798	19384	Spacer V33-V32
4	38A2	AY386694	19690	35164	Spacer V32-V31
5	38A2	AY386694	35447	47669	Spacer V31-V30
6	38A2	AY386694	47973	52521	Spacer V30-V29
7	38A2	AY386694	52819	61798	Spacer V29-V28
8	38A2	AY386694	62100	74264	Spacer V28-V27
9	38A2	AY386694	74566	79145	Spacer V27-V26
10	38A2	AY386694	79449	84800	Spacer V26-V25
11	38A2	AY386694	85103	95717	Spacer V25-V24
12	38A2	AY386694	96009	102226	Spacer V24-V26
13	38A2	AY386694	102504	105307	Spacer V23-V22
14	38A2	AY386694	105603	107583	Spacer V22-V24
15	38A2	AY386694	107769	118033	Spacer V24-V20

TABLE 1-continued

ID	BAC	Accession#	Start	Finish	Description
16	38A2	AY386694	118334	125546	Spacer V20-V19
17	38A2	AY386694	125849	128059	Spacer V19-3' end
18	225P18	AY386697	1	4333	Spacer 5' start-V18
19	225P18	AY386697	4629	9255	Spacer V18-V17
20	225P18	AY386697	9561	15841	Spacer V17-V16
21	225P18	AY386697	16135	22502	Spacer V16-V15
22	225P18	AY386697	22794	32821	Spacer V15-V14
23	225P18	AY386697	33118	37738	Spacer V14-V13
24	225P18	AY386697	38044	44571	Spacer V13-V12
25	225P18	AY386697	44865	49447	Spacer V12-V11
26	225P18	AY386697	49745	56909	Spacer V11-V10
27	225P18	AY386697	57205	63678	Spacer V10-V9
28	225P18	AY386697	63977	71204	Spacer V9-V8
29	225P18	AY386697	71507	76261	Spacer V8-V7
30	225P18	AY386697	76560	79012	Spacer V7-V6
31	225P18	AY386697	79308	83467	Spacer V6-V5
32	225P18	AY386697	83768	88013	Spacer V5-V4
33	225P18	AY386697	88314	91233	Spacer V4-V3
34	225P18	AY386697	91531	95929	Spacer V3-V2
35	225P18	AY386697	96233	100963	Spacer V2-V1
36	225P18	AY386697	101262	133721	Spacer V1-D3
37	225P18	AY386697	133752	135212	Spacer D3-D1a
38	225P18	AY386697	135237	136922	Spacer D1a-D4
39	225P18	AY386697	136947	139446	Spacer D4-3' end
40	219D23	AY386695	8151	40612	Spacer V1-D3
41	219D23	AY386695	40643	42102	Spacer D3-D1a
42	219D23	AY386695	42127	43812	Spacer D4-D1b
43	219D23	AY386695	43837	48553	Spacer D1b-D6
44	219D23	AY386695	48577	48753	Spacer D6-D8
45	219D23	AY386695	48769	51181	Spacer D8-D2x
46	219D23	AY386695	51213	55826	Spacer D1c-Df
47	219D23	AY386695	55852	61112	Spacer Df-D1d
48	219D23	AY386695	61237	62445	Spacer D1d-D5
49	219D23	AY386695	62471	97024	Spacer D5-DQ52
50	219D23	AY386695	97036	97831	Spacer DQ52-J1
51	219D23	AY386695	97871	98090	Spacer J1-J2
52	219D23	AY386695	98140	98430	Spacer J2-J3
53	219D23	AY386695	98483	98642	Spacer J3-J4
54	219D23	AY386695	98690	98981	Spacer J4-J5
55	219D23	AY386695	99032	99550	Spacer J5-J6
56	219D23	AY386695	99604	106867	1501Spacer J6-IgM exon1
57	219D23	AY386695	107185	107290	Spacer IgM exon1-exon2
58	219D23	AY386695	107635	107863	Spacer IgM exon2-exon3
59	219D23	AY386695	108181	108268	Spacer IgM exon3-exon4
60	219D23	AY386695	108664	110499	Spacer IgM exon4-exonM1
61	219D23	AY386695	110615	110741	Spacer exonM1-exonM2
62	219D23	AY386695	108664	137302	Spacer IgM exon4-3' end
63	27N5	AY386696	5099	55582	Spacer IgM exon4-IgG exon1
64	27N5	AY386696	55867	56071	Spacer IgG exon1-exon2
65	27N5	AY386696	56104	56201	Spacer IgG exon2-exon3
66	27N5	AY386696	56531	56623	Spacer IgG exon3-exon4
67	27N5	AY386696	56946	58984	Spacer IgG exon4-exonM1
68	27N5	AY386696	56946	59205	Spacer IgG exon4-exonM2
69	27N5	AY386696	56946	69246	Spacer IgG exon4-IgE exon1
70	27N5	AY386696	69546	69719	Spacer IgE exon1-exon2
71	27N5	AY386696	70037	70132	Spacer IgE exon2-exon3
72	27N5	AY386696	70453	70532	Spacer IgE exon3-exon4
73	27N5	AY386696	70873	73061	Spacer IgE exon4-exonM1
74	27N5	AY386696	70873	73292	Spacer IgE exon4-exonM2

TABLE 1-continued

ID	BAC	Accession#	Start	Finish	Description
75	27N5	AY386696	70873	86059	Spacer IgE exon4-IgA4 exon1
76	27N5	AY386696	86362	86498	Spacer IgA4 exon1-exon2
77	27N5	AY386696	86876	87059	Spacer IgA4 exon2-exon3
78	27N5	AY386696	87450	89577	Spacer IgA4 exon3-exonM
79	27N5	AY386696	87450	103798	Spacer IgA4 exon3-IgA5b exon1
80	27N5	AY386696	104101	104231	Spacer IgA5b exon1-exon2
81	27N5	AY386696	104609	104787	Spacer IgA5b exon2-exon3
82	27N5	AY386696	105182	107227	Spacer IgA5b exon3-exonM
83	27N5	AY386696	105182	112077	Spacer IgA5b exon3-exonM
84	27N5	AY386696	105182	119223	Spacer IgA5b exon3-IgA1 exon1
85	27N5	AY386696	119511	119644	Spacer IgA1 exon1-exon2
86	27N5	AY386696	119984	120162	Spacer IgA1 exon2-exon3
87	27N5	AY386696	120557	122823	Spacer IgA1 exon3-exonM
88	27N5	AY386696	120557	127750	Spacer IgA1 exon3-exonM*
89	27N5	AY386696	120557	135838	Spacer IgA1 exon3-IgA2 exon1
90	27N5	AY386696	136138	136274	Spacer IgA2 exon1-exon2
91	27N5	AY386696	136652	136831	Spacer IgA2 exon2-exon3
92	27N5	AY386696	137229	139433	Spacer IgA2 exon3-exonM
93	27N5	AY386696	137229	146676	Spacer IgA2 exon3-3' end
94	Fos15B	AY386698	1	828	Spacer 5' start-IgA exonM
95	Fos15B	AY386698	1043	3596	Spacer IgA exonM-end contig1
96	Fos15B	AY386698	1	3596	Spacer 5' start-end contig1
97	Fos15B	AY386698	7404	7541	Spacer IgA exon1-exon2
98	Fos15B	AY386698	7908	8086	Spacer IgA exon2-exon3
99	Fos15B	AY386698	8481	10538	Spacer IgA exon3-exonM
100	Fos15B	AY386698	8481	13140	Spacer IgA exon3-end contig2
101	Fos15B	AY386698	8481	15871	Spacer IgA exon3-1,2 hs 3' enh
102	Fos15B	AY386698	8481	21447	Spacer IgA exon3-4hs enh
103	Fos15B	AY386698	21484	33297	Spacer 4hs enh-end contig4
104	Fos15B	AY386698	16633	33297	Spacer 1,2hs 3' enh-end contig4
105	179L1	AY495827	1	124285	Spacer 5' end-enh
106	179L1	AY495827	125411	131350	Enhancer-C□
107	179L1	AY495827	131664	134637	Spacer C□-J5
108	179L1	AY495827	134684	134915	Spacer J5-J4
109	179L1	AY495827	134952	135196	Spacer J4-J3
110	179L1	AY495827	135241	135485	Spacer J3-J2
111	179L1	AY495827	135525	135863	Spacer J2-J1
112	179L1	AY495827	135897	155257	Spacer J1-V1
113	179L1	AY495827	155572	170621	Spacer V1-V2
114	179L1	AY495827	170936	173443	Spacer V2-V3
115	179L1	AY495827	173752	177227	Spacer V3-V4
116	179L1	AY495827	177536	185356	Spacer V4-V5
117	179L1	AY495827	185664	200758	Spacer V5-V6
118	179L1	AY495827	201064	203580	Spacer V6-V7
119	179L1	AY495827	203886	205144	Spacer V7-3' end
120	215M22	AY495826	1	12829	Spacer 5' end-V6
121	215M22	AY495826	13136	15653	Spacer V6-V7
122	215M22	AY495826	15957	22241	Spacer V7-V8

TABLE 1-continued

ID	BAC	Accession#	Start	Finish	Description
123	215M22	AY495826	22551	32876	Spacer V8-V9
124	215M22	AY495826	33188	38276	Spacer V9-V10
125	215M22	AY495826	38582	41476	Spacer V10-V11
126	215M22	AY495826	41780	47827	Spacer V11-V12
127	215M22	AY495826	48133	48547	Spacer V12-V13
128	215M22	AY495826	48841	51408	Spacer V13-V14
129	215M22	AY495826	51638	55438	Spacer V14-V15
130	215M22	AY495826	55745	67437	Spacer V15-V16
131	215M22	AY495826	67743	77805	Spacer V16-V17
132	215M22	AY495826	78120	80628	Spacer V17-V18
133	215M22	AY495826	80937	84009	Spacer V18-V19
134	215M22	AY495826	84315	87339	Spacer V19-V20
135	215M22	AY495826	87648	89399	Spacer V20-V21
136	215M22	AY495826	89711	95414	Spacer V21-V22
137	215M22	AY495826	95720	106650	Spacer V22-V23
138	215M22	AY495826	106956	110940	Spacer V23-V24
139	215M22	AY495826	111246	117877	Spacer V24-V25
140	215M22	AY495826	118183	122396	Spacer V25-V26
141	215M22	AY495826	122706	126496	Spacer V26-V27
142	215M22	AY495826	126802	133358	Spacer V27-V28
143	196O2	AY495828	37134	48826	Spacer V15-V16
144	196O2	AY495828	49032	59195	Spacer V16-V17
145	196O2	AY495828	115057	125885	Spacer V28-V29
146	196O2	AY495828	126195	130012	Spacer V29-V30
147	196O2	AY495828	130318	136966	Spacer V30-V31
148	196O2	AY495828	137272	144512	Spacer V31-V32
149	196O2	AY495828	144819	148617	Spacer V32-V33
150	196O2	AY495828	148923	155402	Spacer V33-V34
151	196O2	AY495828	155714	171415	Spacer V34-V35
152	196O2	AY495828	171572	177676	Spacer V35-V36
153	196O2	AY495828	177979	178083	Spacer V36-3' end
154	CLC*	NA	1	443	Spacer 5' end-pV28**
155	CLC*	NA	486	1203	Spacer pV28-Pv27**
156	CLC*	NA	1528	1635	Spacer pV27-pV26**
157	CLC*	NA	1818	2242	Spacer pV26-pV25**
158	CLC*	NA	2585	2676	Spacer pV25-pV24**
159	CLC*	NA	2781	3327	Spacer pV24-pV23**
160	CLC*	NA	3464	3659	Spacer pV23-pV22**
161	CLC*	NA	3985	4241	Spacer pV22-pV21**
162	CLC*	NA	4578	4994	Spacer pV21-pV20**
163	CLC*	NA	5366	5425	Spacer pV20-pV19**
164	CLC*	NA	5749	5842	Spacer pV19-pV18**
165	CLC*	NA	6034	7043	Spacer pV18-pV17**
166	CLC*	NA	7266	7493	Spacer pV17-pV16**
167	CLC*	NA	7625	7625	Spacer pV16-pV15**
168	CLC*	NA	7988	8758	Spacer pV15-pV14**
169	CLC*	NA	9100	9410	Spacer pV14-pV13**
170	CLC*	NA	9787	10057	Spacer pV13-pV12**
171	CLC*	NA	10441	11022	Spacer pV12-pV11**
172	CLC*	NA	11380	11911	Spacer pV11-pV10**
173	CLC*	NA	12162	12349	Spacer pV10-pV9**
174	CLC*	NA	12691	13357	Spacer pV9-pV8**
175	CLC*	NA	13708	13882	Spacer pV8-pV7**
176	CLC*	NA	14229	14406	Spacer pV7-pV6**
177	CLC*	NA	14599	15338	Spacer pV6-pV5**
178	CLC*	NA	15613	16578	Spacer pV5-pV4**
179	CLC*	NA	16916	18219	Spacer pV4-pV3**
180	CLC*	NA	18439	18879	Spacer pV3-pV2**
181	CLC*	NA	19248	19343	Spacer pV2-pV1**
182	CLC*	NA	19609	22208	Spacer pV1-V**
183	CLC*	NA	22506	24313	Spacer V-J
184	CLC*	NA	24350	26088	Spacer J-C□
185	CLC*	NA	26402	36259	Spacer C-3' end

*CLC—Chicken light chain locus SEQ ID 184, FIG. 9

**pV—pseudo V gene (not functional)

Comments:

BAC sequences submitted to GenBank were modified by deletion of vector sequences at the 5' and 3' end as follows:

BAC	Accession#	Removed from 5' end	Removed from 3' end
38A2	AY386694	1-125	1281285-128225
219D23	AY386695	1-54	137357-137389
Fos15B*	AY3866968	1-97	33395-33427
179L1	AY495827	0	205145-205968
196O2	AY495828	1-32	178117-178171

*In addition contigs in GenBank are separated by 50 nt. In the Fos15B sequence submitted with the provisional application contigs were separated by 10 nt.

TABLE 2

ID	Region	Sequence
193	V_H1	5'CGCGGATCCGAGACTGGGCTGCGCTG3'
194	V_H1	5'CGCAAGCTTGAAATAGGTGGCCGTGTC3'
195	J_H	5'CGCGGATCCAGGCACCCTGGTCACCG3'
196	J_H	5'CGCAAGCTTGTGACCAGGGTGCCCTG3'
197	Cγ	5'CGCGGATCCCTGGAGCCGAAGGTCTAC3'
198	Cγ	5'CGCAAGCTTGAGATGGACTTCTGCGTG3'
199	3'Enh	5'CGCGGATCCCAGAGTGGGTCTGTGACA3'
200	3'Enh	5'CGCAAGCTTACAGGCGCATGCAAATGC3'
201	Vκ	5'CGCGGATCCGAGGCACAGTCACCATC3'
202	Vκ	5'CGCAAGCTTACAGTAGTAAGTGGCAGC3'
203	Jκ	5'CGCGGATCCGGAGGGACCGAGGTGGT3'

TABLE 2-continued

ID	Region	Sequence
204	Jκ	5'CGCAAGCTTACCATGGTCCCTGAGCC3'
205	C□	5'CGCGGATCCCCTCAGGTGATCCAGTTG3'
206	C□	5'CGCAAGCTTCTATTGAAGCTCTGGACG3'
207	K 3'Enh	5'CGCGGATCCGTGACTGGCCCAAGAAG3'
208	K 3'Enh	5'CGCAAGCTTATACAACCTTGGCCAGG3'
209	C□	5'AAACAGCTTTTCACACCTCCCCTTTCTCTCTTTGCTCCCC TGGGCCCTCAGGGAGTGCATCCGCCCCAACCCTTTTCC3'
210	C□	5'CAGGGTTAGTTTGCATGCACACACACACAGCGCCTGGTC ACCCAGAGGGGTCAGTAGCAGGTGCCAGCTGTGTCGGACATG3'
211	C□	5'GGTCAGGGGTCCTCCAGGGCAGGGGTCACATTGTGCCCC TTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTC3'
212	Cγ	5'CACAGCTGCGGCGTGGGGGGGAGGGAGAGGGCAGCTCG CCGGCACAGCGCTCATTTACCCGGAGACAGGGAGAGGCTCTTC3'
213	J_H	5'GTGTTATAAAGGGAGACTGAGGGGGCAGAGGCTGTGCTA CTGGTACCTGGCTGAATACTTCCAGCACTGGGGCCAGG3'
214	J_H	5'GGCCACAGAAAAGAGGAGAGAATGAAGGCCCCGGAGAG GCCGTTCCTACCTGAGGAGACGGTGACCGTGGTCCCT TG-3'
215	Genta	5'CCAGGCCGGCCTGGAGTTGTAGATCCTCTACG3'
216	Genta	5'CCAGGCGCGCCAAGATGCGTGATCTGATCC3'
217	Linker	5'GGCCGCGGCCGGCCATCGATGGCGCGCCTTCGAAACGCGTA3'
218	Linker	5'AGCTTACGCGTTTCGAAGGCGCGCCATCGATGGCCGGCCGC3'
219	pBB11.1	5'ATTCCCAAGCTTTTAATTAAGACGTCAGCTTCCTTAGCTCCTG3'
220	pBB11.1	5'ATTCGCGGATCCACGCGTTTCGTTCCCAAAGGCGCGCCTAGCG ATGAGCTCGGAC3'
221	Neo	5'GCAGGCATGCAAAGCTTATTACACCAGTGTCAGTAAGCG3'
222	Neo	5'GGTACCCGGGGATCCTCAGAAGAACTCGTCAAGAAGGCG3'
223	pBB11.2	5'AAATTCCCTTAATTAAGACGTCAGCTTCCTTAGCTCCTG3'
224	pBB11.2	5'GAAACCGGGGACGCGTTACCGTTCGTATAATGTATGCTATACGAA GTTATGCGGCCGCTAGCGATGAGCTCGGAC3'
225	CA	5'TTCTCTGTTTTTGTCCGTGGAATGAACAATGGAAGTCCGAGCTCA TCGCTAAGGGCACCAATAACTGC3'
226	CA	5'CACAGGAGAGAAACAGGACCTAGAGGATGAGGAAGTCCCTGTAG GCTTCCTACCGTTCGTATAATGTATGCTATACGAAGTTATTACCTGT GACGGAAGATC-3'
227	V_H3-9	5'ATAGAGAGATTGAGTGTG3'
228	V_H3-9	5'TCCTGTCTTCCTGCAG3'
229	V_H3-11	5'AGAGACATTGAGTGGAC3'
230	V_H3-11	5'AGGGAGGTTTGTGTC3'
231	V_H3-13	5'ACTAGAGATATTGAGTGTG3'
232	V_H3-13	5'AGGCATTCTGCAGGG3'
233	V_H3-15	5'ACTAGAGAGATTAAGTGTG3'
234	V_H3-15	5'TCACACTGACCTCCC3'
235	V_H3-20	5'TCATGGATCAATAGAGATG3'
236	V_H3-20	5'TGCAGGGACGTTTGTG3'
237	V_H3-23	5'AGAAAAATTGAGTGTGAA3'

TABLE 2-continued

ID	Region	Sequence
238	V_H3-23	5'GTGTCTGGGCTCACAA3'
239	V_H3-30	5'AGAGAGACTGAGTGTG3'
240	V_H3-30	5'TGCAGGGAGGTTTGTG3'
241	V_H3-43	5'TGAGTGTGAGTGAACATG3'
242	V_H3-43	5'ACCAGCTCTTAACCTTC3'
243	V_H3-64	5'TGAGTGTGAGTGGAC3'
244	V_H3-64	5'TGACGCTGATCAGTG3'
245	V_H3-66	5'TCTGACCAATGTCTCTG3'
246	V_H3-66	5'AGGTTTGTGTCTGGGC3'
247	V_H3-72	5'ACAAGGTGATTTATGGAG3'
248	V_H3-72	5'AGGTTTGTGTCCGGG3'
249	V_H3-9	5'TTGGCGCGCC TGTCGTCTGTGTTTGCAG GTGTCC3'
250	V_H3-9	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCATCTTTTGCAC3'
251	V_H3-11	5'TTGGCGCGCC TGTCGTCTGTGTTTGCAG GTGTCC3
252	V_H3-11	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTCG3'
253	V_H3-13	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAGGTGTCC3'
254	V_H3-13	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTTG3'
255	V_H3-15	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAGGTGTCC3'
256	V_H3-15	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTGTGG3'
257	V_H3-20	5'TTGGCGCGCC TGTCGTCTGTGTTTGCAGGTGTC3'
258	V_H3-20	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTC AGCCTGAGGGCCCCTCACTGTGTCTCTC3'
259	V_H3-23	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAG GTGTCCAGTGTG3'
260	V_H3-23	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCAGCCTGA GGGCCCCTCACTGTGTCTTTC3'
261	V_H3-30	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAGGTGTCCAGTGTC3'
262	V_H3-30	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCAGCCTG AGGGCCCCTCACTGTGTCTTTCG3'
263	V_H3-43	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAGGTGTCC3'
264	V_H3-43	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTTTTGCAC3'
265	V_H3-64	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAGGTGTCC3'
266	V_H3-64	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTCGCAC3'
267	V_H3-66	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAGGTGTCC3'
268	V_H3-66	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCCG3'
269	V_H3-72	5'TTGGCGCGCCTGTCGTCTGTGTTTGCAG GTTTCC3'
270	V_H3-72	5'TTGCACGCGTGCAGGGAGGTTTGTGTCTGGGCTCA GCCTGAGGGCCCCTCACTGTGTCTCTAGCAC3'

TABLE 2-continued

ID	Region	Sequence
271	V1-2	5'TTGGCGCGCCAGGGGAGTGCGGCTCCAC3'
272	V1-2	5'TTGCACGCGT TGGTCAGGACACTGTCACTCAC3'
273	V2-3	5'TTGGCGCGCCAGGGGCGCGCGGCTCCAC3'
274	V2-3	5'TTGCACGCGTTGATCACGAAACTGTCACTCACACTCTC3'
275	V3-4	5'TTGGCGCGCCAGGGGCGCGCGGCTCCAC3'
276	V3-4	5'TTGCACGCGTTCTGTTGGTCTCTTCTTCTCTTGCTATAAC3'
277	V4-5	5'TTGGCGCGCCAGGGGAGTGCGGCTCCAC3'
278	V4-5	5'TTGCACGCGTTGGTCAAGACACTGTCACTCAC3
279	V5-6	5'TTGGCGCGCCAGGGACGCACGGCTCCAC3'
280	V5-6	5'TTGCACGCGTTGGTCAGGAAGCTGTCACTCAC3'
281	V6-7	5'TTGGCGCGCCAGGGATGCGCGGCTCCAG3'
282	V6-7	5'TTGCACGCGTTGGTCAGGACACTGTCACTGACAC3'
283	V7-8	5'TT GGCGCGCCAGGGGAGTGCGGCTCCAC3'
284	V7-8	5'TTGCACGCGTTGGTCAGGAAGCTGTCACTCACTCTC3'
285	V21-22	5'TTGGCGCGCCGGGGCCCGCGGCTCCAC3'
286	V21-22	5'TTGCACGCGTTGGTCAGGAAGCTGTCAC3'
287	V22-23	5'TTGGCGCGCCAGGGACGTGAGGCTCTAC3'
288	V22-23	5'TTGCACGCGTTGGTCAGGGCACTGTCAC3'
289	Linker	5'GGCCGCGGCCGGCCATCGATGGCGCGCC TTCGAAACGCGTA3'
290	Linker	3'CGCCGGCCGGTAGCTACCGCGCGGAAGCTT TGCGCATTCGA5'
291	Linker	5'CGG CCG GCC ATC GAT GGC GCG CCT TCG AAA CGC GTG GTA C3'
292	Linker	3'TCG AGC CGG CCG GTA GCT ACC GCG CGG AAG CTT TGC GCA C5'
293	Genta	5'CCAGGCCGGCCTGGAGTTGTAGATCCTCTACG3'
294	Genta	5'CCAGGCGCGCCAAGATGCGTGATCTGATCC-3'
295	Neo	5'CCAGGCCGGCCATTACACCAGTGTCAGTAAGCG3'
296	Neo	5'CCAGGCGCGCCTCAGAAGAACTCGTCAAGAAGGCG3'
297	Linker	5'GAT CCG GCC GGC CAT CGA TGG CGC GCC TTC GAA ACG CGT TAG GGA TAA CAG GGT AAT A3'
298	Linker	3'GCC GGC CGG TAG CTA CCG CGC GGA AGC TTT GCG CAA TCC CTA TTG TC CCA TTA TCGA5'
299	Neo	5'ATCTGCACTCAGTGCGTCTTGAGCGCCCCCTGGTAGAGCCG CGCGACCCT GGCGCGCC ATTACACCAGTGTCAGTAAGCG3'
300	Neo	5'AAATGACCAGTCTGACAGCCGCGGACACGGCCACCTATTTC TGTGCGAGA GGCCGGCC TCAGAAGAACTCGTCAAGAAGGCG3'
301	Cκ Km3	5'GATGTCCACTGGTACCTAAGCCTCGCCCTCTGTGCTTCTTCCCTC CTCAGGAACTGTGGCTGCACCATCTGTCTTC3'
302	Cκ Km3	5'GAGGCTGGGCCTCAGGGTCGCTGGCGGTGCCCTGGCAGGCGTC TCGCTCTAACACTCTCCCCTGTTGAAGCTCTTTGTG3
303	Neo	5'CTTTCTCTGTCCTTCCTGTGCGACGGTTACGCCGCTCCATGAGCTT ATCGTAACTATAACGGTCCTAAGGTAGCGATGGACAGCAAGCGAA CCGGA3'
304	Neo	5'GGACCAGTTTACAATCCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGTCAGAAGAACTCGTCAAGAAG3'

TABLE 2-continued

ID	Region	Sequence
305	Zeo	5'CCCCCCCCGCCACTTCTCTTCTGTTTCGTTTAAGTTCTACACTGAC ATACTAGGGATAACAGGGTAATAACGTTTACAATTTCGCCTGATG3
306	Zeo	5'AGTGGGTAGGCCTGGCGGCCGCCTGGCCGTCGACATTTAGGTGA CACTATAGAAGGATCCTAGCACGTGTCAGTCCTGCT3'
307	Genta	5'TTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTTTG ATTGCTAACTATAACGGTCCTAAGGTAGCGATGAAGGCACGAACCC AGTTG3'
308	Genta	5'GCGGAATTCTATGTCTAGTGGAGGGTGAAGCTGGTGATTATAGA GTGAAAATTACCCTGTTATCCCTATCGGCTTGAACGAATTGTTAG3'
309	VJ	5'CATAAATATACTGTCTTCCAGGATCTTAGAGCTCACCTAAGGAAA CAAGAGTTCATTTGAAGTTTTTAAAGTG3'
310	VJ	5'ACTCCAGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAAT AGGAACTTCCTTTGATCTCCACCTTGGTC3'
311	Genta	5'GAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAAC TTCTGGAGTTGTAGATCCTCTACG3'
312	Genta	5'AAAACAAACCAATCAGGCAGAAACGGTGAGGAATCAGTGAAAC GGCCACTTACGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGG AATAGGAACTTCAAGATGCGTGATCTGATCC3'
313	FRT	5'TTATGCTGCATCCAGTTTGC3'
314	FRT	5'AAAACAAACCAATCAGGCAG3'
315	FRT	5'TGTGACATCCAGATGAC3'
316	FRT	5'AAAACAAACCAATCAGGCAG3'
317	Genta	5'GGACCAGTTTACAATCCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGGTGCCAGGGCGTGCCCTTGGGCTGGGGGCGCGATAACTTCG TATAGCATACATTATACGAAGTTATCGATCGTGGAGTTGTAGATCC TCTACG3'
318	Genta	5'TTACGCCAAGCTATTTAGGTGACACTATAGAATACTCAAGCTTTG ATTGCAAGATGCGTGATCTGATCCT3'
319	Linker	5'CGGGATCCGCGCGTACGGAAGTTCCTATACCTTTTGAAGAATAGG AACTTCGGAATAGGAACTTCATTACACCAGTGTCAGTAAGCG3'
320	Linker	5'GGGAAGCTTCGCGCGATCGCCGCTTTCGCAAAGGCGCGCCTCAG AAGAACTCGTCAAGAAGGCG3'
321	Genta	5'GGCGGCCGCCTGGCCGTCGACATTTAGGTGACACTATAGAAGGA TCCGCGTGGAGTTGTAGATCCTCTACG3'
322	Genta	5'AACTCAGTAAGGAAAAGGACTGGGAAAGTGCACTTACATTTGAT CTCCAGGCGCGCCAAGATGCGTGATCTGATCC3'
323	Neo	5'GGACCAGTTTACAATCCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGGTGCCAGGGCGTGCCCTTGGGCTGGGGGCGCGGAAGTTCCT ATTCCGAAGTTCCTATTCTTCAAAAGGTATAGGAACTTCCGTACGA TTACACCAGTGTCAGTAAGCG3'
324	Neo	5'GGACTGATGGGAAAATAGAGGAGAAAATTGACCAGAGGAAGTG CAGATGGTCAGAAGAACTCGTCAAGAAGGCG3'
325	RSS	5'AACCTGAAGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTA CCCCTTCCACAGTGATACAAGCCC3'
326	RSS	5'TGCCGGCCACGATGCGTCCGGCGTAGAGGATCTACAACTCCAGG CGCGCCTGGTCATGTCAGTGCTGCTGC3'
327	Genta	5'CTCCTTTCCTCCTCCTTGGTGGCAGCAGCACTGACATGACCAGGC GCGCC TGGAGTTGTAGATCCTCTACG3'
328	Genta	5'TGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGG GATCCCGTACGAAGATGCGTGATCTGATCC3'
329	Kana	5'GGCGGCCGCCTGGCCGTCGACATTTAGGTGACACTATAGAAGGA TCCGCGACCCTGTTATCCCTAGATTTAAATGATATCGG3'

TABLE 2-continued

ID	Region	Sequence
330	Kana	5'AACTTTCTCCTACAGATCCCAGATAACCATGAATTTATTACACCA TCTTGGGCGCGCCGAAGTTCCTATACTTTCTAGAGAATAGGAACTT CGGAATAGGAACTTCAGTTGGTGATTTTGAACTTTTGCTTTGCC3'
331	Amp	5'GGACCAGTTTACAATCCCACCTGCCATCTAAGAAAGCTGGTCTCA TCGTGGTGCCAGGGCGTGCCCTTGGGCTGGGGGCGCGGCGATCGCG AAGTTCCTATTCCGAAGTTCCTATTCTTCAAAAGGTATAGGAACTTC TACGGGGTCTGACGCTCAG3'
332	Amp	5'GAATTCAGAGCTCAATGAGTTGCCTTGTTCAGAGCTCTATTTTCA CTTGACGTACGACAGACAAGCTGTGACCGTC3'
333	J Region	5'GAGTTAGGCCTCAGAGCTGAGGCAGGGCTCGGTTCCCCTTGGGTG AGAAGGGTTTCTGTTCAGCAAGAC3'
334	J Region	5'TGGCCAATTAGAGCAAAATTTCAGACAGTAATAGGAAAAAGGTA CTTACGTTTAATCTCCAGTCGTGTC3'
335	O2	5'TCAGTACTGACTGGAAC3'
336	O2	5'CCAATGACTTTCAAAACC3'
337	L8	5'CCGTACAGCCTGGCTC3'
338	L8	5'AACACCATCAGAGTGTGC
339	L4	5'ATGATTAATTGTGTGGACC3'
340	L4	5'AGGTGATCTCATATCCTC3'
341	A30	5'CTCAGTACTGCTTTACTG3'
342	A30	5'TGACTTCATGTCCCCTTC3'
343	L11	5'ACATGATTAATTGTGTGGACC3'
344	L11	5'GGTGCAGAGGTGACTTCG3'
345	L1	5'CTCAGTACTGCTTTACTATTC3'
346	L1	5'GAGGAACACTCTCAGCTG3'
347	L5	5'CAGGGAACTTCTCTTACAG3'
348	L5	5'GAATTAGGGTGCAGAGGC3'
349	L15	5'TACTATTCAGGGAAATTC3'
350	L15	5'TGTCTGTGAAGTTGGTG3'
351	O8	5'TGGCTCTTGATGGAAGC3'
352	O8	5'ACTTCAAAGTGTGACTGC3'
353	L19	5'AGGGAACTTCTCTTACAGC3'
354	L19	5'AATTAGGGTGCAGAGGCG3'
355	L12	5'GAAGTCTTCCTATAATATGATC3'
356	L12	5'TGGCTGCATCTGAGGACC3'
357	A20	5'GCCACTAATGCCTGGCAC3'
358	A20	5'CTGCTGTCAGCAGAGGGC3'
359	O4	5'CTTCTTATAACATGATGG3'
360	O4	5'AAACGCTCTGAGCAGC3'
361	L14	5'CTCAGTACTGCTTTACTG3'
362	L14	5'GAGGAACAATCTCAGCCG3'
363	L23	5'AGCCAGGCTGTACGGAAC3'
364	L23	5CCCAGCCTCACACATCTC3'

TABLE 2-continued

ID	Region	Sequence
365	L9	5'TGGCCCTTCAGGGAAG3'
366	L9	5'ACCATCAGAGTGTGGTTG3'
367	A4	5'CCAGTGTAGCCATTAATG3'
368	A4	5'TACCAAAACTTCCCAGGG3'
369	L24	5'GGGAAATTCTCTTACTAC3
370	L24	5'CCCCCTCTACCAATAC3'
371	O6	5'CCATTCAGGGAAGTCTTC3'
372	O6	5'TGAGTCTGAGAAGTGTTG3'
373	L22	5'GGAATTTTCTTAGCCCAC3'
374	L22	5'ATGTTCAGGCTTGTAACC3'
375	A9	5'TCATCTTACAAATAGTTG3'
376	A9	5'TCTGACCATTCCTGC3'
377	A25	5'GGGAAATCATCTTATAAATAG3'
378	A25	5'TGCAGATGAGACTTCTGG3'
379	A15	5'ATTCAGGAAAGTCCTCTC3'
380	A15	5'CAGTGACCTTCAGAGTG3'
381	O9	5'ATTCAGGAAAGTCCTCTC3'
382	O9	5'CAGTGACCTTCAGAGTG3'
383	O2	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC
384	O2	5'CGCACGCGTGTTTGATCTCCACCTTGGTCCCTCCGCCGAAAGTGA GAGGGGTACTGTAACTCTGTTG3'
385	L8	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC
386	L8	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTAAG3'
387	L4	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGCCATC
388	L4	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTAAAC3'
389 7	A30	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGGTGTGACATC3'
390	A30	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATGC3'
391	L11	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGCCATC3'
392	L11	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAATTGTAATC3'
393	L1	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC3'
394	L1	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATAC3'
395	L5	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTTCCAGATGCGACATC3'
396	L5	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAAACTGTTAG3'
397	L15	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC
398	L15	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATAC3'
399	O8	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC

TABLE 2-continued

ID	Region	Sequence
400	O8	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAGATTATCATAC3'
401	L19	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTTCCAGATGCGACATC3'
402	L19	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAAACTGTTAG3'
403	L12	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAAATGTGACATC3'
404	L12	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGAATAACTATTATAC3'
405	A20	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGATACCAGATGTGACATCC3'
406	A20	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGGCACTGTTATAC3'
407	O4	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGACATC3'
408	O4	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGGCATTGTAAG3'
409	L14	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTAACATCC3'
410	L14	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATTATGC3'
411	L23	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGCCATC3'
412	L23	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGGTACTATAATAC3'
413	L9	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGCCATC3'
414	L9	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTATAATAC3'
415	A4	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGATACCAGATGTGACATCC3'
416	A4	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGGCACTGTTATAC3'
417	L24	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATGTGTCATC3'
418	L24	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGAAACTATAATAC3'
419	O6	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGGACCAGAAGTGACATC3'
420	O6	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAATTTTTATAC3'
421	L22	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGTCAGATTTGACATCC3'
422	L22	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTAACTGAAGTC3'
423	A9	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGAGTCAGATGTGATTTCC3'
424	A9	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GATGGCTGCTGTAAG3'
425	A25	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGAGTCAGATGTGATTTC C3'
426	A25	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GATGGCTGCTGTAAG3'
427	A15	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATATGACATGC3'
428	A15	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG GAGGGTCACTTTTATAC3'
429	O9	5'TTGGCGCGCCTTCTGTTTCCCTTCTCAGGTGCCAGATATGACATGC3'
430	O9	5'CGCACGCGTCCTGGGGGGTTTTTGTTAGGGCTTGTATCACTGTGG

TABLE 2-continued

ID	Region	Sequence
		GAGGGTCACTTTTATAC3'
431	V7-8	5'TTGGCGCGCC GGAGGAAACAGAAACACAG3'
432	V7-8	5'CGCACGCGT CAGCTGCTCGTCCTGGG3'
433	V11-10	5'TTGGCGCGCC GGAGGGAAACAGAAACAC3'
434	V11-10	5'CGCACGCGTAGCTGCTCCTCCTGGG3'
435	V15-14	5'TTGGCGCGCC GAAGGGAAACAGAAACACAG3'
436	V15-14	5'CGCACGCGTAGCTGCTCCTCCTGGG3'
437	V18-17	5'TTGGCGCGCC GAGGGAAACAGAAACAC3'
438	V18-17	5'CGCACGCGTCAGCTGCTGCTCCTGGG3'
439	V19-18	5'TTGGCGCGCC GAAGGGAAACAGAAACACAG3'
440	V19-18	5'CGCACGCGT AGCTGCTCCTCCTGGG3'
441	V20-19	5'TTGGCGCGCC GAGGAGGGAAACAGAAACAC3'
442	V20-19	5'CGCACGCGTCAGCTGCCCCTCCTGGG3'
443	V21-20	5'TTGGCGCGCC GGAGGAAACAGAAACACAG3'
444	V21-20	5'CGCACGCGTCCCTAGCTGCTCCTGGG3'
445	V24-23	5'TTGGCGCGCC GGAGGGAAACAGACACAC3'
446	V24-23	5'CGCACGCGTCAGCTGCTCCTCCTGGC3'
447	V26-25	5'TTGGCGCGCC GAAGGGAAAGAGAAACACAG3'
448	V26-25	5'CGCACGCGTAGCTGCTCCTCCTGGG3'
449	V27-26	5'TTGGCGCGCC GGAGGGAAACAGAAACAC3'
450	V27-26	5'CGCACGCGTCCCAGCTGCTCCTGGG3'
451	Genta	5'AGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGC ATGCAAGCTTGGCCGGCCTGGAGTTGTAGATCCTCTACG3'
452	Genta	5'AAAACAAACCAATCAGGCAGAAACGGTGAGGAATCAGT GAAACGGCCACTTACGGCGCGCCAAGATGCGTGATCTGATCC3'
453	Hygro	5'CGTTGGACCAGTTTACAATCCCACCTGCCATCTAAGAAAGC TGGTCTCATATAACTTCGTATAATGTATGCTATACGAACGGTA ACGCGTGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGT ATAGGAACTTCTCAGAGCAGATTGTACTG3'
454	Hygro	5'GGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCT GGTAAGGTTAAGATGCGTGATCTGATCC3'
455		5'ACTGCACCTCAGCGTCCCCCTGCCCATGTCAGGGCCGATGAA GGGCACAGCGTACGATTACACCAGTGTCAGTAAGCG3'
456		5'TGTAATACGACTCACTATAGGGCGAATTGAGCTCGGTACCCG GGGATCCTGCGATCGCTCAGAAGAACTCGTCAAGAAGGCG3'
457	J_H	5'GTGTTATAAAGGGAGACTGAGGGAGGCAGAGGCTGTGCTA CTGGTACCTGGCTGAATACTTCCAGCACTGGGGCCAGG3'
458	J_H	5'GGCCACAGAAAAGAGGAGAGAATGAAGGCCCCGGAGAGG CCGTTCCTACCTGAGGAGACGGTGACCGTGGTCCCTTG3'
459	Spacer	5'AACAACCTCAGGGCTGAGGACACC3'
460	Spacer	5'CTGCCCGTTGTCCCTCGAGATGGTGGCACGGCC3'

SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=[US08652842B2]*USRE047131E1*). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. A B cell from a transgenic rabbit, wherein said transgenic rabbit comprises a humanized immunoglobulin (Ig) locus comprising multiple Ig gene segments, [present in a transgenic vector] wherein:

(a) at least one of said gene segments is a human Ig gene segment comprising two or more identical or different units consisting of, from 5' to 3' direction, a 5' nucleotide sequence, a human immunoglobulin heavy or light chain V gene segment, and a 3' nucleotide sequence, wherein said 5' nucleotide sequence and said 3' nucleotide sequence have the nucleotide sequence of SEQ ID NO: 35;

(b) said gene segments are juxtaposed in an unrearranged, partially rearranged or fully rearranged configuration, and

(c) said humanized Ig locus is capable of undergoing gene rearrangement, if necessary, and gene conversion and/or hypermutation, and producing a repertoire of humanized immunoglobulins in said transgenic rabbit.

2. The B cell of claim **1**, wherein said transgenic rabbit preserves an essentially intact endogenous regulatory and antibody production machinery.

3. The B cell of claim **1** or claim **2**, wherein said human Ig heavy chain V gene segment is a member of the VH3, VH1, VH5, or VH4 family.

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