

(43) International Publication Date
2 September 2010 (02.09.2010)

PCT

(10) International Publication Number
WO 2010/099384 A1

(51) International Patent Classification:

C12N 15/85 (2006.01) G01N 33/50 (2006.01)
A01K 67/027 (2006.01)

(21) International Application Number:

PCT/US2010/025507

(22) International Filing Date:

26 February 2010 (26.02.2010)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/156,299 27 February 2009 (27.02.2009) US

(71) Applicant (for all designated States except US):
GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, California 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MISAGHI, Shahram** [US/US]; 311 Windmill Canyon Place, Clayton, California 94517 (US). **ZARRIN, Ali** [CA/US]; 70 Golden Aster Court, Brisbane, California 94005 (US).(74) Agents: **BOYD, Victoria L.** et al.; Genentech, Inc., 1 DNA Way, MS-49, South San Francisco, California 94080 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

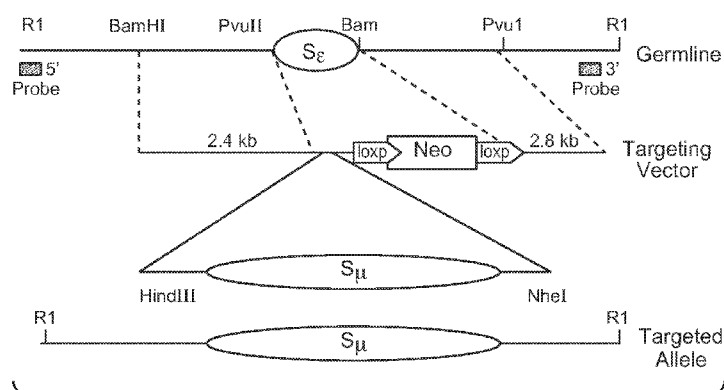
(54) Title: HYPER IgE ANIMAL MODEL WITH ENHANCED IMMUNOGLOBULIN HEAVY CHAIN CLASS SWITCHING TO C ϵ 

FIG. 2A

(57) Abstract: Animal model in which the immunoglobulin heavy chain gene has an enhanced probability of switching to c-ep-silon

Hyper IgE Animal Model with Enhanced
Immunoglobulin Heavy Chain Class Switching to C ϵ

CROSS-REFERENCE TO RELATED APPLICATIONS

[01] The present application claims priority to U.S. Provisional Patent Application Serial No. 61/156,299, entitled "Hyper IgE Animal Model with Enhanced Immunoglobulin Heavy Chain Class Switching to C ϵ ", filed February 27, 2009.

SEQUENCE LISTING

[02] A sequence listing comprising SEQ ID NOS: 1-20 is attached hereto as Table 1. Each sequence provided in the sequence listing is incorporated herein by reference, in its entirety, for all purposes.

TECHNICAL FIELD

[03] This disclosure relates to a recombinant mouse and methods for testing allergy treatments.

BACKGROUND

[04] Asthma is a debilitating disease affecting one fifth of the population of the developed world. Severe asthma is a major cause of hospitalization and health care costs. In clinical practice, asthma is classified as atopic or nonatopic, according to the presence or absence of circulating IgE directed against local aeroallergens detected by skin prick test (SPT) or *in vitro* techniques (RAST or ELISA). These IgE antibodies interact with the high-affinity IgE receptor (Fc ϵ RI) on mast cells, which may result in immediate hypersensitivity on allergen provocation and acute exacerbation of disease.

[05] About one third of adult patients with asthma are classified as nonatopic. They tend to have more severe disease, often associated with chronic rhinosinusitis, but apart from their lack of acute reactivity to allergens, their disease is clinically similar.

[06] An allergy is an immunological reaction, generally of the immediate hypersensitivity type, to a particular type of antigen termed an allergen. Such reactions underlie attacks of anaphylaxis, allergic rhinitis (hay fever), hives, and allergic asthma, and may be triggered by common allergens such as ragweed, pollen, bee or wasp venom, animal dander, mold, or a component of house dust (such as mites).

[07] There is a close concordance between asthma, allergic rhinitis and atopic dermatitis; the presence of one of these entities increases the relative risk of the other two by 3- to 30-

fold over the lifetime of the subject. All three of these diseases are associated with high levels of nonspecific and antigen-specific serum immunoglobulin E (IgE).

[08] In humans, immediate hypersensitivity (IH) is mediated by antibodies of the IgE isotype anchored to the surfaces of mast cells and basophils in the skin and elsewhere. Binding of antigen to these cell-bound IgE molecules triggers release of mediators such as histamine from the cells, which mediators induce the clinical phenomena such as tissue swelling, itching, or bronchial smooth muscle contraction that typify an allergic reaction.

[09] IgE antibodies specific for a given allergen are produced and secreted by B lymphocytes upon contact with that allergen. Initially, B lymphocytes (or B cells) express antibodies of the IgM isotype, with each B cell committed to producing antibody specific for a particular antigenic determinant. Contact with both an allergen bearing that antigenic determinant, and certain factors produced by T lymphocytes, will induce the B cell to undergo what is termed an antibody heavy chain class switch, in which the antigen-specific portion of the antibody produced by the B cell remains the same, but it is attached to the ϵ -heavy chain (to yield IgE antibody) rather than the μ -heavy chain of the IgM isotype. Such a class switch is apparently permanent for a given B cell, which thereafter secretes IgE antibody specific for the allergen whenever stimulated to do so.

[10] Ovalbumin (OVA)-induced asthma in mice is one of the most commonly used models of human asthma. Th2 type cells are believed to be critical in pathogenesis of OVA-induced asthma. While we know that Th2 lymphocytes play an important role in the initiation, progression and persistence of allergic asthma, there is a lot to be understood about the immunoregulatory mechanisms. This model fails to mimic human disease associated with hyper-IgE.

[11] Allergic asthma models have also been described in large animal models, e.g., cats, dogs, pigs, sheep, and monkeys. Among these species, the feline one is of particular interest because cats spontaneously develop idiopathic asthma. However, large animal models are expensive and time consuming and have limited availability of immunological and/or molecular tools.

[12] US 6118044 (2000) provides transgenic mice which constitutively express an antibody-type molecule encoded by a transgene and which has an IgE heavy chain constant region and is specific for a pre-defined antigen (i.e., TNP). It does not provide a polyclonal response to an unknown or non-specific antigen.

[13] Currently there is no good model of chronic airway disease as they lack many key pathological features of human asthma such as mast cell infiltration of smooth muscle. In addition, almost all of them resolve spontaneously over time because mice don't get asthma.

Thus, it would be desirable to have a non-human animal model that will allow the generation of an elevated IgE response relative to a native non-human animal wherein the antibody repertoire is polyclonal.

[14] Currently, common treatments for allergy include avoidance of the suspected allergen; injections of the allergen as immunotherapy to stimulate certain protective mechanisms and thereby eventually desensitize the host to the allergen; drugs such as corticosteroids, which interfere with the release of the mediators of allergy from mast cells; and drugs such as antihistamines, which block the biological action of the released mediators. However, there is no good animal model for testing allergy therapies, especially therapeutic agents that will block IgE isotype switching in B cells without adverse effects.

[15] Thus, it is desirable to have cells and/or animals that have the ability to generate an elevated IgE response. In other words, selectively enhancing the rate of chain switch recombination for IgE production would be desirable.

BRIEF SUMMARY OF THE INVENTION

[16] Despite ongoing research, there still remains a need for an *in vivo* model for IgE involvement in asthma, allergies and other immunologic pathologies that provides a polyclonal response to a non-specific antigen, i.e., an antigen that is not predefined. Also lacking is an *in vivo* animal model for hyper-IgE generation wherein there is an elevated serum IgE response.

[17] Provided herein is a recombinant non-human animal, and a method for using it, that is useful as a reliable model for the search for, and/or evaluation of, anti-allergic drugs.

[18] An animal model that has a genomic structure within the immunoglobulin locus that is substantially similar to the wild-type (i.e., native or unmodified) immunoglobulin locus and retains the potential to provide a full repertoire of immunoglobulins in response to antigen challenge would allow the search for, and/or evaluation of, anti-allergic drugs that inhibit IgE isotype switching in B cells.

[19] The present disclosure provides an animal model for testing allergy therapies. The animal model provides a wide diversity of antibody production in response to antigenic challenge, producing the full diversity of antibody isotypes and full complement of specificities to epitopes on the antigen. The animal model further provides a means to further understand the physiological importance of IgE to allergy and asthma.

[20] In a first embodiment there is provided a targeting vector comprising:

- a) a fragment of DNA homologous to the 5' end of the switch region to be altered (the 5' arm/acceptor) is selected from the group consisting of at least 1500

nucleotides, at least 1800 nucleotides, at least 2000 nucleotides, at least 2200 nucleotides and at least 2400 nucleotides corresponding to Nucleotides 25470628 to 25468161 of NCBI accession number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J) (SEQ ID NO:5);

- b) a selectable gene marker;
- c) a desired/donor DNA sequence encoding a donor switch region; and
- d) a second fragment of DNA homologous to the 3' end of the switch region to be altered (the 3' arm/acceptor) is selected from the group consisting of at least 1500 nucleotides, at least 1800 nucleotides, at least 2000 nucleotides, at least 2200 nucleotides, at least 2400 nucleotides and at least 2800 nucleotides corresponding to Nucleotides 25470628 to 2546816 of NCBI accession number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J 1) (SEQ ID NO:8).

[21] In one aspect the targeting vector has a 5' arm comprising SEQ ID NO:4 or 5. In an embodiment the 5' arm comprises residues 25-2471, inclusive, of SEQ ID NO:4. In a further aspect, the 5' arm is homologous to a region 3' of the endogenous I ϵ and 5' of the endogenous S ϵ . In a second aspect, the targeting vector has a 3' arm comprising SEQ ID NO:7 or 8. In an embodiment the 3' arm comprises residues 2-2495, inclusive, of SEQ ID NO:7. In a third aspect, the targeting vector has a selectable gene marker that is selected from the group consisting of Neomycin and thymidine kinase. In a further aspect, the selectable gene marker is Neomycin. In a fourth aspect, the targeting vector has the selectable gene marker flanked by loxp sites. In a fifth aspect, the targeting vector has a desired switch region that is selected from the group consisting of human and mouse. In a sixth aspect, the desired switch region is selected from S μ , Sy1, Sy2a, Sy2b and Sy3. In a seventh aspect, the desired switch region is the HindIII/NheI fragment containing most of mouse S μ region. In an eighth aspect, the desired switch region comprises Nucleotides 25617172 to 25615761 of NCBI accession number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J) (SEQ ID NO:6). In a ninth aspect the S μ region comprises a 4.9 kb NheI-HindIII fragment was subcloned from a plasmid containing a genomic fragment isolated from BAC clone RP23-354L16.

[22] In second embodiment there is provided a method for producing an altered embryonic stem cell *in vitro*, comprising the steps of:

- a) Altering the genomic DNA in said cell to enhance the probability of class switch recombination (CSR) to express the C ϵ selected from

- b) increasing the S ϵ length by adding at least one additional S ϵ copy in tandem with the endogenous S ϵ region;
- c) S ϵ region substitution; and
- d) Selecting the cell for correctly altered genomic DNA.

[23] In one aspect, the alteration is a substitution of a switch region selected from S μ , S γ 1, S γ 2a, S γ 2b and S γ 3 for the S ϵ region. In a further aspect, the alteration is a substitution of a S μ region for the S ϵ region. In a further aspect, the alteration is a substitution of any acceptor S region (S γ 1, S γ 2a, S γ 2b and S γ 3, S α) with S m or vice versa.

[24] In a third embodiment there is provided a method for producing an altered embryonic stem cell (ESC) *in vitro*, comprising the steps of:

- a) Using the vector according to Claim 1 to exchange the S μ for the S ϵ region
- b) Selecting the cell for correctly altered genomic DNA.

[25] In one aspect, the method provides an alteration that is a substitution of a switch region selected from S μ , S γ 1, S γ 2a, S γ 2b and S γ 3 for the S ϵ region. In a further aspect, the method provides an alteration that is a substitution of a S μ region for the S ϵ region. In another aspect, the method provides that the ESC are from a mouse strain selected from BALB/c or C57BL/6.

[26] In a fourth embodiment there is provided a non-human animal wherein:

- a) At least one allele of the IgH locus has been altered to enhance the rate of IgE expression/production/secretion/ relative to a non-altered allele; and
- b) Has an IgE profile selected from the group consisting of:
 - i. The IgE fraction of all serum antibodies is greater than 0.04%;
 - ii. The IgE serum concentration is above 4,000 ng/ml;
 - iii. The IgG/IgE ratio is less than 10.

[27] In a first aspect, the non-human mammal has an IgE serum level greater than 4,000 ng/ml, greater than 10,000 ng/ml, greater than 15,000 ng/ml, greater than 30,000 ng/ml, greater than 90,000 ng/ml, greater than 10 μ g/ml, greater than 20 μ g/ml, greater than 30 μ g/ml, greater than 40 μ g/ml, greater than 50 μ g/ml, greater than 60 μ g/ml, greater than 70 μ g/ml, greater than 80 μ g/ml, greater than 90 μ g/ml or greater than 100 μ g/ml. In a second aspect, the non-human mammal has an IgG/IgE ratio that is between 0.1 and 10. In a third aspect, the non-human mammal having an unchallenged (i.e., resting) IgE serum concentration of between 100 ng/mL and 10000 ng/mL. In a fourth aspect, the non-human mammal has a challenged (i.e., activated or stimulated) IgE serum concentration of between 1000 ng/mL and 1000000 ng/mL. In a fifth aspect, the animal model is a nonhuman vertebrate. In a sixth aspect, the animal model is a mouse, rat, guinea pig, rabbit, or

primate. In a seventh aspect, the genome of the non-animal described herein has had the S ϵ region of the IgH locus altered to express/produce more IgE. In an eighth aspect, the non-human animal/mammal model has an alteration that is achieved by gene targeting. In a ninth aspect, the non-human mammal has an IgE fraction of at least 0.04%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% or 1.0%.

[28] In a fifth embodiment there is provided a method of testing an allergy therapy using the animal model comprising exposing said animal to an allergen prior to, simultaneous with or after the administration of said method of treatment for allergic disorders and evaluating the IgE response. In a first aspect of the method the IgE levels in response to antigen challenge is less than without the allergy therapy. In a second aspect, the test animal and the control animal are littermates.

[29] In a sixth embodiment there is provided a use of a compound identified by the method of testing an allergy therapy as a medicament for the treatment of an allergy.

[30] In a seventh embodiment there is provided a cell line obtainable from the animal model described herein.

[31] In a eighth embodiment there is provided a cell isolated from an animal model described herein.

[32] In a ninth embodiment there is provided a process for making a non-human animal model, said process comprising:

- a) microinjecting linearized fragments of plasmids encoding SEQ ID NO:6 (S μ) into a fertilized egg of a mouse such that the fragment is incorporated in the genomic DNA upstream from and operably linked to the C ϵ -encoding region,
- b) transferring said fertilized egg to the oviduct of a female mouse which has previously been treated to induce pseudopregnancy, and
- c) allowing said egg to develop in the uterus of the female mouse.

[33] In a tenth embodiment there is provided a recombinant mouse comprising, in its germline, a modified genome wherein said modification comprises at least one allele of the IgH locus altered to enhance the rate of IgE production. In a first aspect, the recombinant mouse has an alteration that comprises replacing the S ϵ with the S μ region or a functional portion thereof. In a second aspect, the S μ functional portion is between at least 1kb and 10kb in length.

[34] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications

within the scope and spirit of the invention will become apparent to one skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[35] Figure 1 is a schematic of the genetic alterations of the mouse IgH locus. (A) Genomic organization of the variable region up to Cm in germ line configuration. (B) V(D)J recombination assembles the functional coding variable region generating a large pool of low affinity IgM producing B cells. (C) Activation of B cells accompanied by induction of AID and germline transcription results in SHM, where point mutations are introduced into assembled V region (asterisks). AID-mediated DSBs (lightning symbol) in Sm and a downstream S region (e.g., Sg1) are joined to generate new isotypes (e.g., IgG1) transcript. In addition, an excised circular fragment is generated by joining the intervening sequence.

[36] Figure 2A illustrates a schematic of the gene targeting strategy and recombination sites for modification of the mouse S ϵ region. The structure of the targeted allele after Cre_{loxP} recombination is illustrated at the bottom. Restriction enzyme cleavage sites are designated. R1 indicates the splice site for *EcoRI*. All other restriction enzymes have their full name.

[37] Figure 2B is a schematic of the targeting vector, pSW312. See Example 3.

[38] Figure 3 is a schematic of the overall mouse IgH locus before and after replacement of the S ϵ region with a donor switch region. In this diagram the donor switch region is S μ . In the upper panel is the unmodified IgH locus; the lower panel illustrates a modified IgE locus as described herein.

[39] Figure 4A is a schematic of the unmodified (i.e., wild-type) genomic locus and illustrates the relative locations of the restriction sites, probe and switch region. The 5' homology arm is represented by the black box. The 3' homology arm is represented by the gray box.

[40] Figure 4B is a schematic of the modified genomic locus and illustrates the relative locations of the restriction sites, probe and switch region with S ϵ replaced with S μ . The 5' homology arm is represented by the black box. The 3' homology arm is represented by the gray box.

[41] Figure 4C is a Southern blot confirming the replacement of the S ϵ with S μ . While wild-type B6 samples show only one band of relevant size indicating existence of a single genomic I ϵ region, targeted embryonic stem cell samples show the wild-type and the targeted S ϵ sites (where S ϵ is replaced with S μ) manifested as two bands with distinct size differences. This shows successful targeting and replacement of the intended switch region.

[42] Figure 4D is a Southern blot confirming the replacement of the S_{ϵ} with S_{μ} . While wild-type B6 samples show only one band of relevant size indicating existence of a single genomic I_{ϵ} region, targeted embryonic stem cell samples show the wild-type and the targeted S_{ϵ} sites (where S_{ϵ} is replaced with S_{μ}) manifested as two bands with distinct size differences. This shows successful targeting and replacement of the intended switch region.

[43] Figures 5-11 show nucleotides described herein. The nucleotide base codes are: A or a is adenine; C or c is cytosine; G or g is guanine; T or t is thymine; M or m is adenine or cytosine; S or s is cytosine or guanine; and N or n is adenine or cytosine or guanine or thymine.

[44] Figure 5 shows two nucleotide sequences from the Mouse. The motif GGGCTGGGCTG (SEQ ID NO:1 shown in Fig. 5A) is found in S_m and S_{ϵ} and a second motif GAGCTGACT is slightly modified in the S_{ϵ} region as GAGCTGAGCT (has an added G relative to the S_m motif) (SEQ ID NO:2 shown in Fig. 5B).

[45] Figure 6 shows a 2055 bp (SEQ ID NO:3) deleted from the the BamHI/PvuI fragment of the IgH locus.

[46] Figure 7 shows A) SEQ ID NO:4, the 2471 bp 5' arm (for 129 mice) and B) SEQ ID NO:5, the 2467 bp 5' arm (for C57BL/6J strain) corresponding to Nucleotides 25470628 to 25468161 of NCBI NT_166318.

[47] Figure 8 shows SEQ ID NO:6 corresponding to nucleotides 25617172 to 25615761 of NCBI Accession Number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J)(in caps) (1141 bp).

[48] Figure 9 shows A) SEQ ID NO:7, the 3' arm (129 mouse sequence) and B) SEQ ID NO:8, the 3' arm (C57BL/6J sequence) corresponding to 25466106 to 25463273 of NCBI NT_166318.

[49] Figure 10 shows A) the 3.7 kb upstream of BamHI (used to design 5' probe) (SEQ ID NO:9): and B) the PVUI to ECORI fragment (used for 3' probe design) (SEQ ID NO:10).

[50] Figure 11 shows probes used in Example 2. A) SEQ ID NO:11, I-mu Forward-1 (21 bp); and B) SEQ ID NO:12, C-epsilon Reverse-1 (30 bp); C) SEQ ID NO:13, E-mu Forward-2 (20 bp); D) SEQ ID NO:14, C-epsilon Reverse-2 (30 bp); E) SEQ ID NO:15, Forward: SM5' (20 bp); F) SEQ ID NO:16, 9225F (19 bp); G) SEQ ID NO:17, 9518F (26 bp); and H) SEQ ID NO:18, Reverse (30 bp).

[51] Figure 12 is the retrieved IgE C57BL/6 genomic sequence (SE region to be deleted shown in bold, underlined font) from BAC RP23-135L12 (Invitrogen) (SEQ ID NO:19).

[52] Figure 13 A-D summarizes the FACS data for intracellular levels of various immunoglobulins in wild-type (WT) and heterozygotes (HET) splenocytes following immune

stimulation. Figure 13A is a bar graph showing the IgM levels after lipopolysaccharide (LPS) stimulation is the same for both WT and HET animals. Figure 13B is a bar graph showing the IgG3 levels after lipopolysaccharide (LPS) stimulation is the same for both WT and HET animals. Figure 13C is a bar graph showing the IgG1 levels after IL-4 in combination with anti-CD40 (4/40) stimulation is decreased in HET animals compared to WT. Figure 13D is a bar graph showing the IgE levels after IL-4 in combination with anti-CD40 (4/40) stimulation is increased in HET animals compared to WT.

[53] Figure 14 A-D summarizes the ELISA data for the same splenocytes as used to generate the data presented in Figure 13. At day 6 post stimulation, supernatants from the same stimulated splenocytes (three Het and three WT mice) that were used for FACS analysis were used in ELISA assay. In agreement to what we observed in FACS analysis, we also observed increase in levels of IgE expression and decrease in levels of IgG1 expression in Het compared to WT when stimulated with IL4/anti-CD40. This suggests that there are more frequent breaks occurring in SmK1 site that competes with switching to IgG1 and increases levels of IgE switching. LPS stimulation serves as control and shows that both WT and Het have similar levels of IgM and IgG3, suggesting that the locus is intact and functions normally when other switch sequences are accessible for class switching.

DETAILED DESCRIPTION

[54] The invention will now be described in detail by way of reference only using the following definitions and examples. All patents and publications, including all sequences disclosed within such patents and publications, referred to herein are expressly incorporated by reference.

[55] Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton, *et al.*, DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2D ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide one of skill with a general dictionary of many of the terms used in this invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Practitioners are particularly directed to Sambrook *et al.*, 1989, and Ausubel FM *et al.*, 1993, for definitions

and terms of the art. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary.

[56] Numeric ranges are inclusive of the numbers defining the range.

[57] Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

[58] The headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

Definitions

[59] Novel recombinant non-human hosts, particularly mammalian hosts, usually murine, are provided, wherein the host is capable of mounting an immune response to an immunogen (also called an antigen). The immune response produced is a full repertoire of antibodies albeit with an elevated IgE component or fraction of the total serum Ig concentration.

[60] By "recombinant" is meant that the DNA of an animal or cell contains a genetically engineered modification. Thus, for example, a "recombinant animal" would be one in which at least a portion of its cells contain a genetic modification as described herein. Similarly, a "recombinant cell" would be one in which its genome has a genetic modification as described herein.

[61] "Non-specific antigen" means any substance (as an immunogen or a hapten) foreign to the body that evokes an immune response either alone or after forming a complex with a larger molecule (as a protein) and that is capable of binding with a product (as an antibody or T cell) of the immune response.

[62] As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG₁) that is encoded by heavy chain constant region genes.

[63] As used herein, "isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

[64] As used herein, "nonswitched isotype" refers to the isotypic class of heavy chain that is produced when no isotype switching has taken place; the C_H gene encoding the nonswitched isotype is typically the first C_H gene immediately downstream from the functionally rearranged VDJ gene.

[65] As used herein, the term "switch sequence" refers to those DNA sequences responsible for switch recombination. During class switch recombination (CSR) a "switch donor" sequence, typically a μ switch region, will be 5' (i.e., upstream) of the region to be

deleted during the switch recombination. The "switch acceptor" region will be between the region to be deleted and the replacement constant region (e.g., γ , ϵ , etc.). As there is no specific site where recombination always occurs, the final gene sequence will typically not be predictable. Switch sequence may be used interchangeably with switch region herein.

[66] In the genetically modified (i.e., recombinant) animal described herein the switch acceptor region is modified to enhance CSR so that the serum IgE levels are elevated.

[67] S regions are large, repetitive intronic sequences that vary greatly in length (repetitive regions range from 2.0 to 6.5 kb in mice). Mammalian S regions are unusually G-rich on the nontemplate strand and are composed primarily of tandem repetitive units within which certain motifs—such as TGGGG, GGGGT, GGGCT, GAGCT, and AGCT predominate.

[68] The term "rearranged" as used herein refers to a configuration of a heavy chain or light chain immunoglobulin locus wherein a V segment is positioned immediately adjacent to a D-J or J segment in a conformation encoding essentially a complete V_H or V_L domain, respectively. A rearranged immunoglobulin gene locus can be identified by comparison to germline DNA; a rearranged locus will have at least one recombined heptamer/nonamer homology element.

[69] The term "unrearranged" or "germline configuration" as used herein in reference to a V segment refers to the configuration wherein the V segment is not recombined so as to be immediately adjacent to a D or J segment. Reference is made to Figure 1.

[70] For nucleic acids, the term "substantial homology" indicates that two nucleic acids, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least about 80% of the nucleotides, usually at least about 90% to 95%, and more preferably at least about 98 to 99.5% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is "isolated" or "rendered substantially pure" when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. See, F. Ausubel, *et al.*, ed. *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience, New York (1987).

[71] The nucleic acid compositions of the present invention, while often in a native sequence (except for modified restriction sites and the like), from either cDNA, genomic or

mixtures may be mutated, thereof in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid sequence as desired. In particular, DNA sequences substantially homologous to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where "derived" indicates that a sequence is identical or modified from another sequence).

[72] A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. For switch sequences, operably linked indicates that the sequences are capable of effecting switch recombination.

[73] The design of a non-human animal that responds to foreign antigen stimulation with an antibody repertoire requires that the immunoglobulin genes contained within the animal function correctly throughout the pathway of B-cell development. Correct function of a heavy chain gene includes isotype switching. Accordingly, the genes of the invention are constructed so as to produce isotype switching and one or more of the following: (1) high level and cell-type specific expression, (2) functional gene rearrangement, (3) activation of and response to allelic exclusion, (4) expression of a sufficient primary repertoire, (5) signal transduction, (6) somatic hypermutation, and (7) domination of the IgE antibody locus during the immune response.

[74] In the mouse, CH genes are arranged in the order 5'-V(D)J-C μ -C δ -C γ 3-C γ 1-C γ 2b-C γ 2a-C ϵ -C α -3'. CSR occurs in switch (S) regions, which are 1- to 10-kilobase (kb) repetitive DNA elements 5' of individual CH genes. CSR results from recombination between the S region upstream of C μ (S μ) and a downstream S region, accompanied by deletion of intervening sequences.

Immunoglobulins

[75] The immune system responds to foreign invaders (antigens) by producing antibodies. Antibodies are protein molecules that attach themselves to invading microorganisms and mark them for destruction or prevent them from infecting cells. Antibodies are antigen specific. That is antibodies produced in response to antigen exposure are specific to that antigen.

[76] Mammals produce four isotypes (or classes) of Ig: IgM, IgG, IgE, and IgA, encoded by the μ , γ , ϵ , and α constant regions, respectively.

[77] Related IgG subclasses are encoded by distinct Cy regions. Each Ig isotype is specialized for particular modes of antigen removal. IgM, the first isotype synthesized by a B cell, activates complement. IgG, the most abundant isotype in serum, binds receptors on phagocytic cells. IgG antibodies cross the placenta to provide maternal protection to the fetus. IgA antibodies are abundant in secretions, such as tears and saliva; they coat invading pathogens to prevent proliferation. IgE antibodies can provide protection against parasitic nematodes, but in developed countries they are the bad guys: They bind basophils and mast cells, activating histamine release and resulting in an allergic response.

[78] Immunogens (or antigens) can trigger an antibody response. Successful recognition and eradication of many different types of antigens requires diversity among antibodies; their amino acid composition varies allowing them to interact with many different antigens. It has been estimated that humans generate about 10 billion different antibodies, each capable of binding a distinct epitope of an antigen. Although a huge repertoire of different antibodies is generated in a single individual, the number of genes available to make these proteins is limited. Several complex genetic mechanisms have evolved that allow vertebrate B cells to generate a diverse pool of antibodies from a relatively small number of antibody genes.

B-cell development

[79] B cells undergo a series of differentiation checkpoints in the bone marrow and spleen before they become mature functional cells. Decisions as whether to continue differentiation or to undergo cell death occur at these checkpoints and revolve principally around the immunoglobulin B-cell receptor (BCR) and its ability to function as an antigen-binding and signal-transduction molecule. The first two such checkpoints are in the bone marrow at the pro-B to pre-B transition, where the newly synthesized heavy (H) chains associate with surrogate light (L) chains to form a pre-BCR, and at the pre-B to immature B-cell stage, where the H chains associate with conventional L chains to form a BCR. Cells that are unable to form a pre-BCR or BCR undergo apoptosis (programmed cell death), whereas those that can form a BCR continue differentiating. The mature B cell that moves into the periphery can be activated by antigen and become an antibody-secreting plasma cell or memory B cell, which will respond more quickly to a second exposure to antigen. When antigen-activated B cells stop proliferating they can differentiate into mature plasma cells. Plasma cells are essentially 'antibody factories'. (See Hardy & Hayakawa, *B Cell Development Pathways*, Annu Rev Immunol. (2001) 19:595-621.)

[80] Initially, all B cells produce IgM antibodies. The V, D and J elements encoding the variable-region domains of the m heavy chain are located adjacent to the Cm exons that encode the IgM C-regions at the 5' end of the immunoglobulin heavy-chain (IgH) locus.

Following appropriate stimulation, B cells can alter the isotype of the antibodies they produce via class switching while retaining their antigenic specificity. Class switching occurs in the heavy chain gene locus by a mechanism called class switch recombination (CSR). This mechanism relies on conserved nucleotide motifs, called switch (S) regions, found in DNA upstream of each constant region gene (except in the δ -chain). In this process, genomic DNA is spliced and rejoined to juxtapose the VDJ elements to the C-region exons that encode the γ , ϵ and α chains of IgG, IgE and IgA isotypes, respectively; these C-region exons are located further downstream in the IgH region. This process results in an immunoglobulin gene that encodes an antibody of a different isotype.

S Regions

[81] The molecular basis of antibody class switching to the expression of C_γ , C_ϵ , and C_α genes in activated B cells is a recombination which positions the new C_H gene 3' next to the VDJ gene. The apparent sites of Ig class switch recombination are located within the S regions, highly repetitive DNA sequences which are present 5' of each C_H gene, except C_δ .

[82] All murine and most humans S regions are sequenced at least partially. They are 1–10 kb in length, highly repetitive and GC-rich. Murine and human S_μ are almost homogeneously composed of the two pentamer sequences GAGCT and GGGGT and the heptamer sequence (C/T)AGGTTG. All other S regions also contain multiple copies of the pentameric sequences. All murine S regions except S_μ are composed of tandem repeats that vary both in sequence and in length, with 49 bp repeats for $S_\gamma 1$, $S_\gamma 3$ and $S_\gamma 2b$, 52 bp repeats for $S_\gamma 2a$, 80 bp repeats for S_α and 40 bp repeats for S_ϵ . Both human and murine S_μ are more homologous to S_ϵ and S_α than to the S_γ regions, which have considerable homology among each other. The S regions are sufficiently conserved between human and mouse to allow human S regions to be used as substrate for switch recombination in murine cells. The S_μ , S_ϵ and S_α regions are more homologous between the two species than the S_γ regions. Indeed, the Mouse Sm motif GGGCTGGGCTG (SEQ ID NO:1) is found in S_ϵ and a second motif GAGCTGACT is slightly modified in the S_ϵ region as GAGCTGAGCT (has an added G relative to the Sm motif) (SEQ ID NO:2). The length of the S regions is subject to considerable allelic variation (length polymorphism) indicating that there is no functional requirement for a particular size of a given S region.

IgE and Serum IgE Levels

[83] Immunoglobulin E (IgE) is a class of antibody (or immunoglobulin "isotype") that has only been found in mammals. It plays an important role in allergy, and is especially associated with type 1 hypersensitivity. IgE has also been implicated in immune system responses to most parasitic worms like *Schistosoma mansoni*, *Trichinella spiralis*, and

Fasciola hepatica, and may be important during immune defense against certain protozoan parasites such as *Plasmodium falciparum*.

[84] Although IgE is typically the least abundant isotype - blood serum IgE levels in a normal ("non-atopic") individual are only 0.05% of the IgG concentration, compared to 10 mg/ml for the IgGs (the isotypes responsible for most of the classical adaptive immune response) - it is capable of triggering the most powerful immune reactions.

[85] Atopic individuals can have up to 10 times the normal level of IgE in their blood (as do sufferers of hyper-IgE syndrome). IgE that can specifically recognise an "allergen" (typically this is a protein, such as dust mite DerP1, cat FelD1, grass or ragweed pollen, etc.) has a unique long-lived interaction with its high affinity receptor, FcεRI, so that basophils and mast cells, capable of mediating inflammatory reactions, become "primed", ready to release chemicals like histamine, leukotrienes and certain interleukins, which cause many of the symptoms we associate with allergy, such as airway constriction in asthma, local inflammation in eczema, increased mucus secretion in allergic rhinitis and increased vascular permeability, ostensibly to allow other immune cells to gain access to tissues, but which can lead to a potentially fatal drop in blood pressure as in anaphylaxis. Although the mechanisms of each response are fairly well understood, why some allergics develop such drastic sensitivities when others merely get a runny nose is still not well understood.

[86] Total serum IgE concentration tests allows for measurement of the total IgE level in a serum sample. Elevated levels of IgE are associated with the presence of allergy. One method of testing for total serum IgE is the PRIST (paper radioimmunosorbent test). This test involves causing serum samples to react with IgE that has been tagged with radioactive iodine. Bound radioactive iodine, calculated upon completion of the test procedure, is proportional to the amount of total IgE in the serum sample. In clinical immunology, levels of individual classes of immunoglobulins are measured by nephelometry (or turbidimetry) to characterize the antibody profile of patient. Other methods of measuring IgE levels are ELISA, immunofluorescence, Western blot, immunodiffusion and immunoelectrophoresis.

[87] Measurement of a total serum IgE concentration using a UniCAP 250® system (Pharmacia, Uppsala, Sweden) above 100 kU/L is considered elevated. In one study, using a sensitive double antibody radioimmunoassay to measure IgE, serum IgE from normal subjects free from evident allergic symptoms varied over a 130-fold range from 6 to 1000 ng/ml. In patients with allergic respiratory diseases the range of IgE concentrations overlapped that of normal subjects to a considerable extent, but approximately 35% of untreated allergic individuals had IgE concentrations above the 97th percentile for normals and 51% are above the 95th percentile. (See G.J. Gleich, A.K. Averbach and H.A.

Swedlund, Measurement of IgE in normal and allergic serum by radioimmunoassay. *J. Lab. Clin. Med.* **77** (1971), p. 690.)

[88] In another study, the geometric mean IgE level of normal adults was 105 ng/ml with a 95% interval of 5 to 2045. The normal level of IgE in adults has been reported to be approximately 100 to 400 ng/ml, 1/400,000 of that of IgG. (see Waldmann *et al.*, *The Journal of Immunology*, 1972, 109: 304-310; see also Medical Immunology - 10th Ed. (2001) TG Parslow, DP Stites, AI Terr and JB Imboden, eds., Table 7-2).

[89] For a comparison of serum Ig levels in young (24-43 y.o.), old (66-96) and centenarians (99-108) see Listi *et al.*, A Study of Serum Immunoglobulin Levels in Elderly Persons That Provides New Insights into B Cell Immunosenescence. *Ann. N.Y. Acad. Sci* (2006) 1089:487-495. In particular see Table 2 of Listi *et al.* (*supra*) for the age- and gender-related serum concentration of immunoglobulins for normal individuals

[90] Although it normally represents only a minute fraction (0.004%) of all serum antibodies, immunoglobulin E (IgE) is extremely important from the clinical standpoint because of its central involvement in allergic disorders. Two specialized types of inflammatory cells involved in allergic responses, the mast cell and the basophil, carry a unique, high-affinity Fc receptor that is specific for IgE antibodies. Thus, despite the very low concentration of IgE (roughly 10^{-7} M) in blood and tissue fluids, the surfaces of these cells are constantly decorated with IgE antibodies, adsorbed from the blood, that serve as antigen receptors. When its passively bound IgE molecules contact an antigen, the mast cell or basophil releases inflammatory mediator substances that produce many of the acute manifestations of allergic disease. Elevated levels of serum IgE may also signify infection by helminths or certain other types of multicellular parasites. Like IgG and IgD, IgE exists only in monomeric form. Fc receptors appear to recognize primarily the C_H3 domain of the ϵ chain. See Medical Immunology - 10th Ed. (2001; *supra*)

[91] Despite the variability in the serum concentrations of immunoglobulin E in humans it is clear that serum IgE levels of greater than about 2500 ng/ml are associated with a variety of diseases. Similar low levels of IgE is reported in mice (see Pinaud *et al.*, *Localization of the 3' IgH locus Elements that Effect Long-Distance Regulation of Class Switch Recombination*, *Immunity* (2001) 15(2):187-199).

Gene Targeting and Plasmid

[92] Gene targeting is a technique utilizing homologous recombination between an engineered exogenous DNA fragment and the genome of the mouse embryonic stem (ES) cells. Recombination between identical regions contained within the introduced DNA fragment and the native chromosome will lead to the replacement of a portion of the

chromosome with the engineered DNA. These modified ES cells can then be injected into blastocysts where they can incorporate and contribute to the fetal development along with the blastomeres from the ICM (inner cell mass).

[93] In brief, gene targeting vectors are designed which, through homologous recombination, replace the wild-type allele of a given gene with a mutated form. The targeted ES cells are then implanted into 2-4 day blastocysts and transferred to pseudopregnant mothers (see below).

[94] The targeting vectors used herein have four components:

- a. a 5' arm (also referred to as a 5' flanking region);
- b. a selection marker;
- c. a DNA sequence encoding a donor switch region; and
- d. a 3' arm (also referred to as a 5' flanking region).

The 5' arm is a fragment of DNA homologous to the 5' end of the switch region to be replaced. The selection marker confers a selectable phenotype upon homologous recombination. The selection marker may be flanked by loxp sites. The donor switch region may be either before or after the selection marker. The 3' arm is a fragment of DNA homologous to the 3' end of the switch region to be replaced.

[95] The 5' and 3' flanking regions may be any length but is dependent on the degree of the homology. As used herein "substantial homology" between two DNA sequence portions means that the sequence portions are sufficiently homologous to facilitate detectable recombination when DNA fragments are co-introduced into a recombination competent cell. Two sequence portions are substantially homologous if their nucleotide sequences are at least 40%, preferably at least 60%, more preferably at least 80% and most preferably, 100% identical with one another. This is because a decrease in the amount of homology results in a corresponding decrease in the frequency of successful homologous recombination. A practical lower limit to sequence homology can be defined functionally as that amount of homology which if further reduced does not mediate detectable homologous recombination of the DNA fragments in a recombination competent mammalian cell. The 5' and 3' flanking regions are preferably at least 500 bp, more preferably, 1000 bp, next most preferably about 1800 bp, and most preferably, greater than 1800 bp for each homologous sequence portion.

[96] Desirably, a marker gene is used in the targeting construct to replace the deleted sequences. Various markers may be employed, particularly those which allow for positive selection. Of particular interest is the use of G418 resistance, resulting from expression of the gene for neomycin phosphotransferase ("neo"). The presence of the marker gene in the genome will indicate that integration has occurred.

[97] The donor switch region may be the S μ , S γ 1, S γ 2a, S γ 2b or S γ 3 region when the S ϵ region is the region to be replaced. The donor region should be one that under stimulated, non-recombinant conditions (i.e., the switch regions have not been altered) results in its associated heavy chain is expressed at a higher level than C ϵ .

[98] For the most part, DNA analysis by Southern blot hybridization will be employed to establish the location of the integration. By employing probes for the insert and the sequences at the 5' and 3' regions flanking the region where homologous integration would occur, one can demonstrate that homologous targeting has occurred.

[99] PCR may also be used with advantage in detecting the presence of homologous recombination. PCR primers may be used which are complementary to a sequence within the targeting construct and complementary to a sequence outside the construct and at the target locus. In this way, one can only obtain DNA molecules having both the primers present in the complementary strands if homologous recombination has occurred. By demonstrating the expected size fragments, e.g. using Southern blot analysis, the occurrence of homologous recombination is supported.

[100] Once a targeting construct has been prepared and any undesirable sequences removed, e.g., procaryotic sequences, the construct may now be introduced into the target cell, for example an ES cell (see below). Any convenient technique for introducing the DNA into the target cells may be employed. Techniques include protoplast fusion, e.g. yeast spheroplast:cell fusion, lipofection, electroporation, calcium phosphate-mediated DNA transfer or direct microinjection.

[101] After transformation or transfection of the target cells, target cells may be selected by means of positive and/or negative markers, as previously indicated, neomycin resistance and acyclovir or gancyclovir resistance. Those cells which show the desired phenotype may then be further analyzed by restriction analysis, electrophoresis, Southern analysis, PCR, or the like. By identifying fragments which show the presence of the desired alteration at the target locus, one can identify cells in which homologous recombination has occurred to alter the IgH in a manner that enhances switching to C ϵ .

EMBRYONIC STEM (ES) CELL METHODS

A. Introduction of cDNA into ES cells

[102] Methods for the culturing of ES cells and the subsequent production of recombinant animals, the introduction of DNA into ES cells by a variety of methods such as electroporation, calcium phosphate/DNA precipitation, and direct injection are described in detail in Teratocarcinomas and embryonic stem cells, a practical approach, ed. E.J. Robertson, (IRL Press 1987), the teachings of which are incorporated herein. Selection of

the desired clone of recombinant ES cells is accomplished through one of several means. In cases involving sequence specific gene integration, a nucleic acid sequence for recombination with the gene of interest or sequences for controlling expression thereof is co-precipitated with a gene encoding a marker such as neomycin resistance. Transfection is carried out by one of several methods described in detail in Lovell-Badge, in Teratocarcinomas and embryonic stem cells, a practical approach, ed. E.J. Robertson, (IRL Press 1987) or in Potter *et al.*, Proc. Natl. Acad. Sci. USA 81, 7161 (1984). Calcium phosphate/DNA precipitation, direct injection, and electroporation are the preferred methods. In these procedures, a number of ES cells, for example, 0.5×10^6 , are plated into tissue culture dishes and transfected with a mixture of the linearized nucleic acid sequence and 1 mg of pSV2neo DNA (Southern and Berg, J. Mol. Appl. Gen. 1:327-341 (1982)) precipitated in the presence of 50 mg lipofectin in a final volume of 100 μ l. The cells are fed with selection medium containing 10% fetal bovine serum in DMEM supplemented with an antibiotic such as G418 (between 200 and 500 μ g/ml). Colonies of cells resistant to G418 are isolated using cloning rings and expanded. DNA is extracted from drug resistant clones and Southern blotting experiments using the nucleic acid sequence as a probe are used to identify those clones carrying the desired nucleic acid sequences. In some experiments, PCR methods are used to identify the clones of interest.

[103] DNA molecules introduced into ES cells can also be integrated into the chromosome through the process of homologous recombination, described by Capecchi, (1989) Science 244:1288-1292. Direct injection results in a high efficiency of integration. Desired clones are identified through PCR of DNA prepared from pools of injected ES cells. Positive cells within the pools are identified by PCR subsequent to cell cloning (Zimmer and Bruss, Nature 338, 150-153 (1989)). DNA introduction by electroporation is less efficient and requires a selection step. Methods for positive selection of the recombination event (i.e., neo resistance) and dual positive-negative selection (i.e., neo resistance and ganciclovir resistance) and the subsequent identification of the desired clones by PCR have been described by Joyner *et al.*, Nature 338, 153-156 (1989) and Capecchi, (1989), the teachings of which are incorporated herein.

B. Embryo Recovery and ES cell Injection

[104] Female animals are induced to superovulate using methodology adapted from the standard techniques used with mice, that is, with an injection of pregnant mare serum gonadotrophin (PMSG; Sigma) followed 48 hours later by an injection of human chorionic gonadotrophin (hCG; Sigma). Females are placed with males immediately after hCG injection. Approximately one day after hCG, the mated females are sacrificed and embryos

are recovered from excised oviducts and placed in Dulbecco's phosphate buffered saline with 0.5% bovine serum albumin (BSA; Sigma). Surrounding cumulus cells are removed with hyaluronidase (1 mg/ml). Pronuclear embryos are then washed and placed in Earle's balanced salt solution containing 0.5% BSA (EBSS) in a 37.5°C incubator with a humidified atmosphere at 5% CO₂, 95% air until the time of injection.

[105] Naturally cycling or superovulated females mated with males are used to harvest embryos for the injection of ES cells. Embryos of the appropriate age are recovered after successful mating. Embryos are flushed from the uterine horns of mated females and placed in Dulbecco's modified essential medium plus 10% calf serum for injection with ES cells. Approximately 10-20 ES cells are injected into blastocysts using a glass microneedle with an internal diameter of approximately 20 µm.

C. Transfer of Embryos to Pseudopregnant Females

[106] Randomly cycling adult females are paired with vasectomized males. Recipient females are mated such that they will be at 2.5 to 3.5 days post-mating (for mice, or later for larger animals) when required for implantation with blastocysts containing ES cells. At the time of embryo transfer, the recipient females are anesthetized. The ovaries are exposed by making an incision in the body wall directly over the oviduct and the ovary and uterus are externalized. A hole is made in the uterine horn with a needle through which the blastocysts are transferred. After the transfer, the ovary and uterus are pushed back into the body and the incision is closed by suturing. This procedure is repeated on the opposite side if additional transfers are to be made.

[107] The procedures for manipulation of the embryo and for microinjection of DNA are described in detail in Hogan *et al.*, Manipulating the mouse embryo, Cold Spring Harbor laboratory, Cold Spring Harbor, NY (1986), the teachings of which are incorporated herein. These techniques are readily applicable to embryos of other animal species, and, although the success rate is lower, it is considered to be a routine practice to those skilled in this art.

D. Identification of Recombinant Animals

[108] Samples (1-2 cm of mouse tails) are removed from young animals. For larger animals, blood or other tissue can be used. To test for chimeras in the homologous recombination experiments, i.e., to look for contribution of the targeted ES cells to the animals, coat color has been used in mice, although blood could be examined in larger animals. DNA is prepared and analyzed by both Southern blot and PCR to detect recombinant founder (F₀) animals and their progeny (F₁ and F₂).

[109] Once the recombinant animals are identified, lines are established by conventional breeding.

SOUTHERN ANALYSIS

[110] DNA was obtained from cell lines by standard phenol extraction procedure or by cesium gradient centrifugation.

A. Phenol extraction

[111] Flasks of cells are washed with HBSS buffer, then 2.5 ml/100cm² of lysing solution (1% sodium dodecyl sulfate/150 mM NaCl/10mM EDTA/10 mM Tris, pH 7.4) is added. After all cells are solubilized, they are transferred to a 50 ml conical tube and proteinase K to a final concentration of 0.4 mg/ml is added. The lysate is incubated at 65°C for 10 minutes to inactivate DNase enzymes, then incubated overnight at 37°C. To this lysate an equal volume of fresh phenol that has been equilibrated in 50 mM Tris, pH 8.0, is added, and the tube is gently inverted for 5 minutes at room temperature (about 22°-24°C) then centrifuged at 2000g for 5 minutes, and the top (aqueous) layer is transferred to a second tube. An equal volume of 50% phenol/50% chloroform (v/v) is then added, and the inversion centrifugation process repeated. The supernatant is then transferred to a third tube, and an equal volume of chloroform is added. After a third inversion, centrifugation cycle, the supernatant is transferred to a fourth tube and the DNA precipitated by the addition of 1/10 volume of 3M NaAcetate, 2.5 vol of cold ethanol. After washing the resulting precipitate with 70% ethanol and air-drying the pellet, it is resuspended in TE buffer (10 mM Tris pH 7.4/1 mM EDTA) and RNase to a final concentration of 50% µg/ml is added (The RNase is prepared to be DNase-free by heating the freshly suspended enzyme at 70°C for 30 minutes. The solution is then extracted with an equal volume of 1:1 SS-phenol:chloroform. The phases are separated by centrifugation, as above, and the supernatant extracted with an equal volume of chloroform. Following centrifugation, as above, and the supernatant extracted with an equal volume of chloroform. Following centrifugation, the DNA in the supernatant is precipitated with 12.5 ml of ethanol, then washed with 70% ethanol and air-dried. The pellet is then suspended in TE buffer, and the DNA yield determined by O.D. reading at 260 nM and the purity determined by 260/280 ratio. The DNA preparation is stored in TE at 4°C.

B. Cesium chloride preparation of RNA and DNA from cultured cells

[112] Flasks of cells were washed with HBS then 2.5 ml/100cm² of guanidine isothiocyanate (GIT) buffer was added. The guanidine isothiocyanate buffer was 4M GIT/25 mM sodium acetate, pH6/0.8% beta-mercaptoethanol (v/v). After 3-5 minutes, with gentle rocking, the cell lysates were layered on top of 4 ml of cesium chloride buffer in Beckman SW41 10 ml ultracentrifuge tubes. The tubes were filled to nearly the top with GIT buffer, then they were spun overnight at 32,000 rpm (174,000 x g) at 200C. The GIT

solution in the upper two-thirds of the tube was then removed and discarded, the CsCl solution in the lower one third of the tube that contains the DNA was transferred to a second tube. The RNA pellet in the bottom of the tube was resuspended in 200 μ l of 0.3 M sodium acetate, pH 6 and transferred to a 1.5 ml microfuge tube. To this tube was added 750 μ l of ethanol, and the tube was placed on dry ice for 10 minutes. After microcentrifugation for 10 min., the supernatant was discarded, 300 μ l of 70% ethanol was added, and the tube was microfuged again. The supernatant was discarded, and the pellet was dried in a vacuum centrifuge. The pellet was resuspended in 200 μ l of dH₂O. The RNA preparation was stored as an ethanol precipitate of -70°C. The 4 ml of CsCl containing the DNA was diluted with dH₂O. To this was added 30 ml of cold ethanol. The DNA precipitate was recovered, transferred to a new 50 ml tube, and rinsed with 70% ethanol, then air-dried. The pellet was then resuspended in PK buffer, and 10mg of proteinase K was added. After incubation at 65°C for 15 minutes, the solution was incubated overnight at 37°C. The hydrolysate was then extracted with 1:1 SS-phenol:chloroform, followed by chloroform, ethanol precipitation, and quantitated as described above.

C. Restriction digestion, electrophoresis, and Southern transfer

[113] Restriction endonuclease digest conditions were according to the recommendations of the suppliers. For genomic DNA, the restriction digestion was for 4-6 hrs. at 37°C. For simple DNA preparations (cloned or PCR amplified) the incubation was for 1-2 hours at 37°C. Generally, 10 μ g of DNA was digested in a volume of 150 μ l. The digest was precipitated by addition of 3 μ l 5M NaCl and 375 μ l (2.5 vol) of cold ethanol, microfuged for 10 minutes at 4°C, washed with 500 μ l cold 70% ethanol and microfuged.

[114] The pellet was air-dried in a vacuum microfuge and resuspended in 17 μ l of electrophoresis running buffer (routinely TAE buffer) and 3 μ l of gel loading buffer (TAE buffer containing 50% glycerol/1% saturated bromphenol blue), heated to 68°C for 10 minutes, and loaded into wells of an agarose gel, along with a lambda-HindIII digest in a separate well to serve as a size marker. The concentration of agarose in the gel was 1.0%. Following electrophoresis for 8-16 hours, the gel was stained with ethidium bromide, the migration distance of the marker bands measured and recorded, and the gel photographed.

[115] The digested DNA was vacuum-transferred to a Nytran membrane. The gel was laid on top of the Nytran membrane on the vacuum apparatus, covered with 500 ml of 0.4 M NaOH/0.8 M NaCl and a vacuum pressure of 50 cm of water applied for four minutes. The NaCl-NaOH solution was removed, 500 ml of 10 X SSC added, and a pressure of 50 cm water applied for 30-60 minutes. The Southern blot was then baked at 80°C for 2 hours and stored in a vegetable freezing bag.

D. Southern hybridization

[116] The Southern blot was placed in a heat sealable plastic bag and incubated with 10 ml of pre-hybridization buffer containing 1 M NaCl, 1% SDS, 10% dextran sulphate, and 200 µg/ml herring sperm DNA, and incubated for 15 minutes at 65°C. A corner of the bag was then cut off, and the radiolabelled oligonucleotide probe was added (approximately 10^7 dpm). The bag was resealed and placed at 65°C in an oven or water bath and gently rocked or shaken for 12-16 hours. The membrane was then removed from the bag and washed in a series of increasingly dilute and higher temperature (increasing stringency) SSC buffers until the background radioactivity was low relative to the specifically bound probe. In a darkroom, the membrane was then placed in a plastic bag which was positioned in an X-ray film cassette equipped with intensifier screens, a sheet of Kodak XAR-5 film was added, and the sealed cassette was placed at -70°C for variable time depending on the intensity of signal. Usually, exposures after varying time periods are useful. The film was developed in a Kodak X-OMAT automatic developer. Membranes may be re-hybridized several times. Nytran membranes may be stripped of labeled probe by heating in boiling 0.1 X SSC for 2 minutes.

B-Cell culture

[117] B cells may be purified from spleens by negative selection. Briefly, T cells in single cell suspensions are coated with antibodies and depleted by complement lysis. The remaining spleen cells were layered over a discontinuous Percoll (GE Healthcare) gradient. Resting B cells may be selected from the 66% to 70% interface and total B cells (50–70% Percoll interface) were used. B cells may be cultured in B cell media consisting of RPMI 1640 media (Sigma Aldrich), supplemented with 10% heat inactivated FBS, 100 U/ml penicillin and streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM Hepes, 100 mM non-essential amino acids and 5×10^{-5} M 2-mercaptoethanol.

Confirming *in vivo* hyper-IgE production

[118] Recombinant animals will be tested for elevated IgE serum levels using techniques known in the art. For example, the ImmunoCAP Specific IgE blood test (which the literature may also describe as: CAP RAST, CAP FEIA (fluorezymeimmunoassay), and Pharmacia CAP) may be used.

[119] Other methods are known in the art. Such methods include for example Enzyme-linked immunosorbent assay and potentially FACS using an anti-IgE antibody. Enzyme-Linked ImmunoSorbent Assay, also called ELISA, Enzyme ImmunoAssay or EIA, is a biochemical technique used mainly in immunology to detect the presence of an antibody or

an antigen in a sample. Fluorescence-activated cell sorting (FACS) is a powerful technique for analyzing large mixed populations of single cells. A higher proportion of IgE positive cells would indicate an elevated serum IgE level.

Hybridoma production

[120] Standard techniques known in the art are used to prepare hybridomas that produce IgE. See, for example, Köhler & Milstein (1975) Continuous cultures of fused cells secreting antibody of predefined specificity, *Nature* 256:495, and Köhler & Milstein (1976) Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* 6:511.

[121] Briefly, immunized splenocytes are washed and fused to myeloma cells under appropriate conditions. The hybridomas are exposed to HAT or other selection agent 24 hours later, and the non-fused myeloma cells will die. The non-fused splenocytes also have a finite lifetime, and the hybridomas are then the only proliferating cells left in the culture.

Assay for Class Switching

[122] Assays are known in the art and are described in, for example, Shinkura, R. *et al. Nat. Immunol.* (2003) **4**, 435–441 and Zarrin, *et al., Nat. Immunol.* (2004) **5**, 1275–1281. Briefly, splenocytes were stimulated with anti-CD40 and IL-4 for 4 days to generate hybridomas or for 6 days to perform ELISA. A monoclonal anti-IgE antibody may be used to detect IgE (mutated alleles). Total IgE may be measured by polyclonal anti-IgE antibodies (Southern Biotechnology Associates).

[123] See also, for example, Southern and Berg (Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Appl Genet* (1982) **1**: 327-341) describes Southern blot analysis to assess DNA rearrangement and CSR in IgH locus.

[124] ϵ Germline transcription marks the first step in the commitment of B cells to the synthesis of IgE. Therefore, RT-PCR may be used to examine ϵ immunoglobulin heavy-chain germline gene transcripts (GLTs; ϵ GLTs), ϵ circle transcripts (CTs; I ϵ -C μ CT or I ϵ Cy CT), and mRNA encoding the heavy chain of IgE (ϵ mRNA) and activation-induced cytidine deaminase (AID) (see Takhar *et al.*, *J Allergy Clin Immunol* (2007) **119**(1):213-218).

[125] In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μ M (micromolar); N (Normal); mol (moles); mmol (millimoles); μ mol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); kg (kilograms); μ g (micrograms); L (liters); ml (milliliters); μ l (microliters); cm (centimeters); mm (millimeters); μ m (micrometers); nm (nanometers); °C. (degrees Centigrade); h (hours); min (minutes); sec

(seconds); msec (milliseconds); Ci (Curies) mCi (milliCuries); μ Ci (microCuries); TLC (thin layer chromatography).

EXAMPLES

[126] The present invention is described in further detail in the following examples which are not in any way intended to limit the scope of the invention as claimed. The attached Figures are meant to be considered as integral parts of the specification and description of the invention. All references cited are herein specifically incorporated by reference for all that is described therein. The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1 Gene Targeting/Generation of mutant mice

[127] This example illustrates the construction of the targeting vector, the transformation of embryonic stem cells (ES) and generation of mutant mice.

I. Generalized Procedure

A. Transformation of embryonic stem cells (ES)

[128] Targeting constructs were designed based on sequence information available in the NCBI for NT_166318. (See also, Waterston *et al.*, *Initial sequencing and comparative analysis of the mouse genome*, Nature. (2002) 420(6915):520-62.) A BamHI/PVul fragment (7022 bp with Se region (129 mice); 7355 bp with Se region (B6 mice)) was isolated from 129 or C57B6 BAC clones and amplified.

[129] The following sequence (5'→3'; SEQ ID NO:3) was deleted from the BamHI/PVul fragment and was replaced with a Neo cassette plus the HindIII/NheI containing most of mouse Sm region (see Figure 2 and SEQ ID NO:6).

tgggttaagc	agagctgtgc	tgggctggta	tgagctggtc	caagttgggc	50
taaacagagc	tgggccaggc	tagtatgagc	tggctctgaac	tacactaagc	100
aggactaggc	tgggctgagc	tgagctggac	tggctggact	tggctgagat	150
gtgttgagct	gggttaagta	tggctgggct	gggctggcct	gggctgggct	200
ggactggatt	ggtatgagct	ggtccaagtt	gggctaagca	gagctgggcc	250
aggctggtat	gagctggtct	aaactgaact	aagtagggct	gggctaagct	300
gagctggtct	acactagcct	gacctgagct	agggtaggct	ggactgggct	350
gagctaagtt	gcactgggca	ggggtgggct	ggaccgagct	gatttgagct	400
gggatgggct	gagatggggt	cagcaggcct	aagcaggcct	agctggggtt	450
agctagattt	agctaggcaa	ggctgagcta	ggctgggcgg	ggcggggcta	500
ggctgggcag	ggctggactg	agctagcttt	tgtatatctg	gttgaaatgg	550
gttggctctg	tctggactga	actgactgag	ctgggctagc	ctgagctcga	600
tgggggggtat	actcagctga	gatgggctgg	tctggctaga	ctgaactgga	650
ttgggctagg	ctgagctagg	ctgacctgaa	ctggcctggt	ctgggctgga	700
ctgggcaggg	ctggctctcag	ctagactaca	ctgagttaac	ctgggctgga	750

ccatactggg	ttaaactag	ttgcactggc	tgggttagac	ttggctgagc	800
tgggcttggc	tgagctgagt	caagatggtc	tgagttgatt	tgagttggct	850
aagctaagct	gagctacact	gaactaggca	aggctgggct	ggaaaggtct	900
gggttaagtt	aggagggact	tggcttggct	tagctgggcc	aagctaggct	950
gaactgggct	gaactgagct	gagctgggct	gagctgggct	gagctgggct	1000
gagctgggca	aggctaaact	ggaatggact	gaattggcct	aagatgggcc	1050
cggctaagct	aagtaaggct	gccctgaact	gagcaggact	ggcctggcct	1100
ggattgacct	ggcatgagct	taacttgact	agactagtct	atcttgggtg	1150
aactgggcta	agcaggacta	atctggcctg	atctgagcta	gactgaacta	1200
ggctaagctg	agctgagttt	agcttggctg	aactgggctg	ggctgcactg	1250
aactgtattg	agctatgtag	aactgagctg	gtcttgtctg	aggtgggttg	1300
ggctggctctg	ggctgaacca	gattgcacta	gactgagctt	agctggacct	1350
ggctgagctg	gactgcattg	tgctaaactg	gctctcttta	gaccgagctt	1400
agctggactg	gactgagcta	ggttgggtgg	gctgatctaa	gctgagctag	1450
gctggtctca	cctgaggaat	gctgtgctgt	gctgagctga	actaaactga	1500
gctcagctaa	ggaagtgtga	gctagactga	gctgagctag	gctgggttgg	1550
gctgaactga	gctaccttgg	gtggactagg	ctgagctgag	ctgggttgag	1600
ctgagctata	gatttggttg	gactggactg	gattgggcta	aactgaactg	1650
gtttggggta	ggctgggatg	agctggactg	agctaggctg	tactggtctg	1700
agctaaacta	agttgagtgg	ggctaagagg	agctgagtga	ggctgggctg	1750
gaatgagcta	ggctaggggt	gtgagctagg	gttggtactgg	tctaagctga	1800
gttttagctga	gagaggctgg	gctagacttc	cataagggtg	ctgagtcata	1850
ctacagtgca	ctgagctgtg	ttgagcttaa	cttggattaa	gtggaatggg	1900
ttgagctggc	tgaactgggc	tgaactgaga	taaactagac	tgagctggga	1950
cacgctggga	cgagctggaa	cgagctagaa	ttactgttct	aatctgatct	2000
gggctgaggt	aaactgggcc	tggttgagct	ctactaggct	aagtagagtt	2050
gagct					2055

[130] A similar construct may be made to target 129SvEv ES cells to account for strain allelic differences that exist in the IgH locus.

B. Transformation of embryonic stem cells (ES)

[131] The plasmid from above was linearized using *PvuI* restriction enzyme. DNA was washed in 70% Ethanol and subsequently pelleted and resuspended in 50 µl TE. Using techniques known in the art (see, for example, Templeton *et al.*, *Efficient gene targeting in mouse embryonic stem cells*, Gene Therapy (1997) 4:700-709), 10⁶ ES cells were transfected with linearized vector by electroporation and selected using G418 (400 µg/ml). Subsequently the Neo gene was deleted using cre/loxP recombination system. Correctly targeted clones were identified by Southern analyses with two probes of at least 300 base pairs and designed from SEQ ID NO:9 (for the 5' probe) and SEQ ID NO:10 (for the 3' probe) of the construct (shown in Figure 2) identified recombinants.

C. Generation of mutant mice

[132] Germline mice were generated to create a mouse with a modified IgH locus. ES cell clones showing homologous recombination of *Sμ/Sε* were injected into C57B6 blastocysts,

and the resulting male chimeras were mated with C57B6 females. Germline transmission in heterozygous and homozygous mutant mice was assessed by coat color.

II. Specified Procedure

A. Target Vector Construction

[133] The construct for targeting the C57BL/6 IgE locus in ES cells was made using recombineering and standard molecular cloning techniques. See, for example, Liu *et al.*, A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Research* (2003) vol. 13 (3) pp. 476-84.

[134] Targeting constructs were designed based on sequence information available in the NCBI for NT_166318. (See also, Waterston *et al.*, *Initial sequencing and comparative analysis of the mouse genome*, *Nature*. (2002) 420(6915):520-62.).

[135] First, a 6988 bp genomic fragment (SEQ ID NO:19; Figure 13) from a mouse BAC (RP23-135L12; Invitrogen, Carlsbad, CA) containing the C57BL/6 Switch epsilon (designated as SE or S ϵ or Sepsilon, herein) region/sequence was isolated and introduced into a plasmid containing the negative selection marker Diphtheria toxin A (DTA) called "pBlight-DTA" (see Warming *et al.*, *Mol. Cell. Biol.* (2006) 26 (18):6913-22) for subsequent use in embryonic stem (ES) cell targeting, resulting in "pBlight-DTA-IgE".

[136] Second, a loxP-PGK-em7-Neo-BGHpA-loxP-HindIII-Sall-Ascl-NheI cassette was inserted into the IgE fragment using homologous recombination, replacing the endogenous S ϵ region with a floxed Neo and a polylinker for subsequent insertion of switch mu region (designated as SMu or S μ or Sm, herein) ("pBlight-DTA-IgE-lox-Neo-lox-MCS").

[137] Finally, a 4.9 kb HindIII-NheI fragment containing C57BL/6 SMu was isolated from the BAC RP23-135L12 (Invitrogen) and this fragment was cloned into pBlight-DTA-IgE-lox-Neo-lox-MCS using three-way ligation (ligation of a 6.2 kb XhoI-HindIII fragment and a 4.1 kb XhoI-NheI fragment, both from pBlight-DTA-IgE-Neo-MCS, to the 4.9 kb HindIII-NheI Smu fragment). The resulting construct is called "pSW312" (pBlight-DTA-IgE-lox-neo-lox-Smu). See Figure 2B.

B. Transformation of embryonic stem cells (ES)

[138] C57BL/6 ES cells were targeted using standard methods (G418 positive and DTA negative selection), and positive clones were identified using PCR and taqman analysis. Correctly targeted clones were confirmed by Southern blotting analysis using HindIII digested genomic DNA and an external 3' probe (the sequence between the 3' end of the construct and an endogenous IgE HindIII site) (**SEQ ID NO:20**):

```

taatggacga tcgggagata actgatacac ttgcacaaac tgttctaato    50
aaggaggaag gcaaactagc ctctacctgc agtaaactca acatcactga    100

```

```

gcagcaatgg atgtctgaaa gcaccttcac ctgcaaggctc acctcccaag      150
gcgtagacta tttggccac actcggagat gccaggtag gtctacactc      200
gcctgatgtc cagacctcag agtcctgagg gaaaggcagg ctctcacaca      250
gcccttcctc cccgacagat catgagccac ggggtgtgat tacctacctg      300
atcccacca gccccctgga cctgtatcaa aacggtgctc ccaagctt      348

```

C. Generation of mutant mice

[139] Germline mice were generated to create a mouse with a modified IgH locus. ES cell clones showing homologous recombination of $S\mu/S\epsilon$ were injected into C57B6 blastocysts, and the resulting male chimeras were mated with C57B6 females. Germline transmission in heterozygous and homozygous mutant mice was assessed by coat color.

Example 2

In vitro stimulation (anti-CD40 /IL4; LPS) to induce isotype switching

[140] The following example details how B-cells were collected and analyzed for class switch recombination (CSR).

[141] Spleen cells from 6-8 week old wild-type or heterozygotes animals were stimulated *in vitro* with anti-CD40 (1 μ g/mL; HM40-3, Pharmingen) plus IL-4 (25 ng/mL), or Lipopolysaccharide (LPS, 20 μ g/mL). 1.5×10^6 cells were seeded in one well of 6 well plates (0.5×10^6 /mL) in RPMI media supplemented with 10% Fetal Bovine Serum, 2mM glutamine, 100 units/mL of penicillin-streptomycin and 100 μ M β -mercaptoethanol. After stimulation, activated B cell cultures were used to generate hybridomas (using standard methods; see, for example, *Monoclonal Antibodies: Methods and Protocols* in Methods in Molecular Biology (2007) vol. 378:1-13) at day 4-5 or to measure Ig levels by ELISA (day 6). Monoclonal anti-mouse antibodies (Pharmingen) were used to detect Ig levels followed with alkaline phosphatase-conjugated goat anti-mouse IgG1 (Southern Biotechnology) as the detection antibody. Purified mouse Ig (Pharmingen) was used as the standard.

[142] Surface Ig staining was performed using PE anti-mouse Ig (Pharmingen) antibodies on splenocytes. FACS analyses was done at day four of stimulation. Samples were collected on a FACS Scan (Becton Dickinson) and analyzed using Flojo analyses software.

[143] CSR was evaluated by Southern blot analysis. Briefly, genomic DNA from hybridoma clones was used to assess DNA rearrangement and CSR in IgH locus. Hybridoma genomic DNA (about 150 μ l) was digested overnight with EcoRI (NEB) restriction enzyme and the digest was resolved by applying the samples to a 0.7% agarose gel. The resolved samples were then transferred to Zeta-probe blotting membrane (Bio-Rad), fixed by UV cross-linking and/or baking in 80° Celsius vacuum oven (20 min), and probed with ^{32} P labeled I-mu, C-mu, I-epsilon, or C-epsilon probes following standard southern blotting protocols (Molecular

Cloning, 3rd Edition Vol.1, pages 6.33-6.64). The labeled DNA was visualized by putting the membranes on X-ray films (Kodak).

[144] In order to sequence the junctions of S-mu and S-epsilon CSR in IgE positive hybridoma clones, nested PCR was used to amplify and sequence this region. First we used I-mu Forward-1: 5'-CTCTGGCCCTGCTTATTGTTG-3' (SEQ ID NO:11) and C-epsilon Reverse-1: 5'-CCTGATAGAGGCTGTGAGAAAGGAAGGACC-3' (SEQ ID NO:12) primers to amplify this region from genomic hybridoma DNA with PCR. The PCR cycles were 94°C for 2 min; (94°C for 10 sec, 60°C for 30 sec, and 68 °C for 150 sec) X 35 cycles, 68°C for 7 min. The product from this PCR step was used as template (2 ul) for a second PCR cycle, using the following primers: E-mu Forward-2: 5'-AGACCTGGGAATGTATGGTT-3' (SEQ ID NO:13) and C-epsilon Reverse-2: 5'-TAGGTTAGACTTATTTATATCACTGCATGC-3' (SEQ ID NO:14). The PCR program was the same as above except the annealing temperature was lowered to 55°C. The PCR products were then gel purified (Quigen) and directly sequenced using the following primers: Forward: SM5': 5'- GTTGAGAGCCCTAGTAAGCG-3' (SEQ ID NO:15); 9225F: 5'-TTGAGAGCCCTAGTAAGCG-3' (SEQ ID NO:16); 9518F: TGAGCTCAGCTATGCTACGCGTGTTG-3' (SEQ ID NO:17); Reverse: 5'-GCCCGATTGGCTCTACCTACCCAGTCTGGC-3' (SEQ ID NO:18).

Example 3 Intracellular IgE Staining & FACS Analysis

[145] This example demonstrates the intracellular staining of IgE and FACS analysis from tissue obtained from heterozygotic mice and wild-type mice after exposure to different stimuli.

[146] 0.5×10^6 splenocytes were spun down and resuspended in FACS buffer (PBS+0.5% Fetal Bovine Serum). Anti-IgE antibody (e-Bioscience, San Diego, CA; Cat. No. 14-5992-85) was added at 1 µg/sample to block surface IgE molecules. The cells were incubated for 15 min at 4°C then washed twice with FACS buffer and pelleted by centrifugation for 3 minutes at 1700 rpm. The pelleted cells were resuspended in 1% Fetal Bovine Serum in PBS.

[147] Cells were vortexed and BD Cytofix/Cytoperm (BD Biosciences, San Jose, CA; Cat. No. 554722) was added at 200 µl/sample to fix the cells. Incubated at 4°C for 20 min.

[148] Cells were washed twice with 1X BD Perm/Wash buffer (BD Biosciences, San Diego, CA; Cat. No. 554723) and were resuspended in 250 µl of 1X BD Perm/Wash buffer with Fc - blocker. Cells aliquots were then used for staining with Biotin-Isotype Control (e-Bioscience,

13-4301-82) or anti-IgE-biotin (e-Bioscience, 13-5992-82) along with B220-FITC at 1:200 final antibody dilution. Incubated for 30 min at 4°C.

[149] Cells were washed twice with 1X BD Perm/Wash buffer.

[150] Streptavidin-PE (Pharmingen) in 1X BD Perm/Wash was added at 1:200 dilution. Incubated 10 min at 4°C.

[151] Washed twice with BD Perm/Wash buffer and resuspended cells in 200 µl of FACS buffer and performed FACS analysis using a BD FACS Calibur equipment and CellQuest Pro program to analyze the cells.

[152] Results are shown in Figure 13. In Figure 13, it can be seen that the number of cells that stain positive for IgE is increased relative to the WT. FACS results show that the percentage of IgE expressing B-cells increase (approximately twice) in the Het animal compared to WT animal while the levels of IgG1 drops approximately by half in Het compared to WT. This demonstrates that having SmKI (in place of Se) increases switching to IgE and at the same time reducing the chance of switching to IgG1 by competing with this locus.

Example 4 IgE Assays/Measurements

[153] This example demonstrates the use of ELISA (luminex or conventional ELISA) to measure the IgE in an unchallenged or challenged recombinant animal or in *in vitro* cell culture. For *in vitro* cell cultures, the cells may be stimulated with LPS or antiCD40/IL4.

Luminex Mouse 7-plex Immunoglobulin Isotyping Assay

[154] This assay uses a multiplex assay kit (Millipore Beadlyte Mouse Immunoglobulin Isotyping Kit, Cat#48-300,) for isotyping (heavy chain: IgG1, IgG2a, IgG2b, IgG3, IgA, IgE, IgM; and light chain: kappa or lambda) mouse monoclonal cell culture supernatants or serum samples (using the Millipore Mouse Isotyping Serum Diluent) in a single well with the Luminex® Instrument system.

[155] Before use, the cell culture supernatants should be centrifuged at 14,000×g to remove any particulates. Similarly, serum and plasma samples should be spun down (8000×g) prior to assay to remove particulate and lipid layers. This will prevent the blocking of wash plate as well as sample needle.

Materials used for Bead-Based Multiplex Assays

[156] Millipore Beadlyte Mouse Immunoglobulin Isotyping Kit Cat#48-300 (Contains: Beadlyte Cytokine Assay Buffer, Cat#43-002, Beadlyte mouse multi-Immunoglobulin Beads, Cat#42-045, Beadlyte mouse Immunoglobulin positive control, Cat#43-008, Beadlyte anti-

mouse k light chain, PE (100X), Cat#44-029, Beadlyte anti-mouse lambda light chain, PE (100X), Cat#44-029).

[157] Millipore Beadlyte mouse Isotyping Serum Diluent (5X), Cat#43-033, Phosphate Buffered Saline with 1% Bovine Serum Albumin.

[158] Ig Standard Curve Reagents: Millipore: Beadlyte Mouse Multi-Immunoglobulin Standard (IgG1, IgG2a, IgG2b, IgG3, IgA, IgE, IgM) Lyophilized, from balb/c mouse Cat#47-300.

[159] Filter Plate: Millipore multiscreen-HA 0.45um surfactant-free.

[160] Millipore Filtration System (NOTE: Any system that provides ddH₂O will work.)

[161] The assay may be performed according to the manufacturer's instructions.

General Protocol for Processing Bead-Based Multiplex Assays

[162] Centrifuge the sample (as appropriate) to precipitate any particulates before diluting into appropriate diluent. Resuspend the standard into appropriate diluent and prepare an eight-point standard curve using twofold serial dilutions. Wet filter plate with 50-100 µl assay diluent per well.

[163] Plate fitting: Add 50 µl of the standard or sample to each well. Sonicate the coupled beads for 15–20 s to yield a homogeneous suspension. Thoroughly vortex the beads for at least 10 s. Dilute the beads to 1500 beads per well, and add 25 µl of diluted bead suspension to each well. Incubate for 15 min in the dark at room temperature (Incubation time can be varied, typically between 15 min and 2 h. The primary incubation of the bead and sample can be performed overnight at 4°C for greater low-end sensitivity.).

[164] Washing step: Apply vacuum manifold to the bottom of filter plate to remove liquid and blot. Wash by adding 75 µl of assay diluent, vacuum and blot. Repeat washing twice. Resuspend the beads in 75 µl of assay diluent. Add 25 µl of the detection antibody solution to each well. Incubate for 15 min in the dark at room temperature. Apply vacuum manifold to the bottom of filter plate to remove liquid. Wash by adding 125 µl of assay diluent, vacuum and blot. Repeat washing twice. Resuspend the beads in 125 µl of assay buffer. Incubate on a plate shaker for 1 min. Read the results on Luminex® 100 instrument. Data evaluation: extrapolate the sample concentrations from a 4-PL or 5-PL curve.

[165] At day 6 post stimulation, supernatants from the same stimulated splenocytes (three Het and three WT mice) that were used for FACS analysis were used in an ELISA assay as described above. In agreement to what we observed in FACS analysis, we also observed an increase in levels of IgE expression and decrease in levels of IgG1 expression in Het compared to WT when stimulated with IL4/anti-CD40. This suggests that there are more frequent breaks occurring in SmK1 site that competes with switching to IgG1 and

increases levels of IgE switching. LPS stimulation serves as control and shows that both WT and Het have similar levels of IgM and IgG3, suggesting that the locus is intact and functions normally when other switch regions are accessible for class switching. See Figure 14.

Total Mouse IgE Binding ELISA

[166] This assay is run to quantitate mouse IgE serum levels in both naïve and immunized animals.

I. Coat with Capture Antibody:

[167] Dilute the purified anti-mouse IgE capture mAb (Rat Anti-mu IgE, Clone R35-92 BD Pharmingen, San Diego, CA, Stored at 4°C. Cat. #553416 (0.5 mg/ml)) to 2 µg/ml^a in coating buffer (0.05 M Carbonate/bicarbonate, pH 9.6. Add 100 µl per well to an enhanced protein-binding ELISA plate (e.g., Nunc immunoplate Cat #464718, 384-Well). Shake plate to ensure all wells are covered by capture antibody solution. Cover the plate and incubate for overnight at 4°C. [Note: may be done at 1 hour at 37°C. Wash the plate 3X with PBS/Tween® (PBS + 0.05% Tween 20). For each wash, wells are filled with 200 µl PBS/Tween ® and allowed to stand at least 1 min prior to aspirating or dumping. As a final step, tap plate on paper towels to remove excess buffer.

II. Blocking:

[168] Block the plate with 50 µl blocking buffer (PBS + 0.5% BSA + 10 ppm Proclin pH 7.4) per well. Cover the plate and incubate at RT for 1 hour with gentle agitation. Wash the plate 3X with PBS/Tween ® , as above.

III. Apply Standards and Samples:

[169] Leave column 1 as blank wells (i.e., no antigen added, 25 µl per well blocking buffer only). Use columns 2 and 3 for duplicates of the standard, 25 µl per well: Prepare standard curve from 500 ug/ml Stock standard antibodies to starting standard concentration of 10ng/ml (1:50,000). Make Serial 1:2 dilutions (PBS + 0.5% BSA + 0.05% Tween 20 + 15ppm Proclin + 0.2% BgG + 0.25% CHAPS + 5 mM EDTA , pH 7.4) The mouse IgE standard curve: 10.0, 5.0, 2.50, 1.25, 0.625, 0.313, 0.156, 0 ng/ml. Assay Controls are mouse IgE kIsotype control, BD Bioscience, Catalog #557079, Main Stock: 0.5 mg/ml, keep in 4°C) at the following dilutions: 8ng/ml, 4ng/ml, 0.5ng/ml. Use the remaining columns to add samples at various dilutions in blocking buffer, 25 µl per well. Dilute the serum samples with Assay Diluent (PBS + 0.5% BSA + 0.05% Tween 20 + 15ppm Proclin) 1:25 minimum initial dilution, serial 1:3, using *Hamilton* Diluter. Cover the plate and incubate for 2 hours

with gentle agitation. [Note: May be done for at least 1 hour at RT or overnight at 4°C.] Wash the plate 6X with PBS/Tween® , as above, and incubate with agitation for one hour.

IV. Incubation with Detection Antibody:

[170] Add 25 µl biotinylated anti-mouse IgE (Rat Anti-mu IgE-Biotin, Clone R35-118, BD Pharmingen, San Diego, CA, 0.5ug/ml, 4°C, Cat #553419) per well. Cover the plate and incubate at RT for 30 minutes with gentle agitation. Wash the plate 6X with PBS/Tween® , as above.

V. Add Streptavidin-Horseradish Peroxidase (SAv-HRP):

[171] Dilute Streptavidin-HRP (GE Healthcare, formerly Amersham Biosciences, Piscataway, NJ, 1mg/ml, Stored at 4°C, Cat.#RPN4401) 1:20,000 in blocking buffer to a final concentration of 50 ng/ml. Add 25 µl per well and incubate with agitation for 30 minutes. [Note: Avidin-HRP may be used instead of Streptavidin-HRP with appropriate modification as noted in the art.] Cover the plate and incubate at RT for 30 min. Wash the plate 6X with PBS/Tween® , as above, of this protocol.

VI. Add Substrate and Develop:

[172] Mix 1 Part of TMB A to 1 Part of TMB B (TMB peroxidase solutions A & B (KPL, Gaithersburg, MD, cat # 50-76-02 and 50-65-02, respectively), store at 4°C). Add 25 µl TMB substrate to each well and shake. Incubate 15 min. at room temperature for color to develop and add 25 µl of 1 M H₃PO₄ to quench the development. Read the plate at 450-650 nm.

[173] Interpolate the serum sample IgE levels from the standard curve.

Example 5 **Hybridoma to quantitate antibody isotypes**

[174] Hybridomas are constructed using techniques known in the art. Using the assays of Example 4 the immunoglobulin isotypes are characterized.

[175] The antibodies are then further characterized for binding affinity, epitope characterization and mode of action on a relevant pathway.

Example 6 **Immunization: TNP-OVA; OVA; Ficol to induce isotype switching *in vivo***

[176] This example illustrates the *in vivo* immunization of recombinant animals as described herein.

[177] Eight-week-old sex-matched Balb/C mice aged 8 weeks, weighing approximately 25-30 g are immunized i.p. with TNP-OVA 50ug/alum 2mg or TNP-Ficoll 50ug injected i.p. in

100 ul sterile PBS, boosted at day 28. 60ul samples are collected on day -3, 7, 14, 21, 28, 35 and 42 via tail vein for antibody isotype measurements using the assays described in Example 4.

II. Antigen-induced peritonitis model with measurement of histamine in the peritoneal fluid.

Animals and sensitization procedure

[178] Balb/C mice aged 8 weeks, weighing approximately 25-30 g raised at the Pasteur Institute (Paris, France), are actively sensitized by a subcutaneous (s.c.) injection of 0.4 ml 0.9% w/v NaCl (saline) containing 100 gLg ovalbumin adsorbed in 1.6 mg aluminium hydroxide (Andersson & Brattsand, 1982). Seven days later, the animals receive the same dose of ovalbumin in the presence of Al(OH)₃ and are used 7 days thereafter.

Antigen-induced peritonitis

[179] Peritonitis is induced by the intraperitoneal (i.p.) injection of 0.4 ml of a solution containing 2.5 or 25 gm/ml ovalbumin diluted in sterile saline (1 or 10 µg of ovalbumin, as final doses injected per cavity). Control animals receive the same volume of sterile saline. At various time intervals after antigen challenge (30 min- 164 h), animals are euthanized by an overdose of ether and the peritoneal cavity is opened and washed with 3 ml of heparinised saline (10 U per ml). Approximately 90% of the initial volume is recovered. In rare cases, when hemorrhages are noted in the peritoneal cavity, the animals are used.

[180] Histamine levels are measured using methods known in the art.

Example 7

Infection with *Nippostrongylus brasiliensis* to induce IgE

[181] This example illustrates the IgE response to infection with parasitic worms, *Nippostrongylus brasiliensis*.

[182] The course of development of *N. brasiliensis* has been well characterized in the mouse (Love, *Nippostrongylus brasiliensis* infections in mice: the immunological basis of worm expulsion, (1975) Parasitology 70:11). In the mouse once infective larvae (L3) have penetrated the skin they are transported to the lungs via the lymph and blood vascular systems. After a tracheal-esophageal migration, fourth-stage larvae (approx. 15-35% of the original L3 dose) are carried to the lumen of the small intestine and mature. The infection is patent by the seventh day after larval inoculation. Worm expulsion that is preceded by a sharp fall in fecal egg output occurs soon after patency (approx. day 9 and is virtually complete by the twelfth day).

[183] The maintenance of *N. brasiliensis* under laboratory conditions, methods of infection, worm transfer and collection of worms for counting have been described previously (Love & Ogilvie, *Nippostrongylus brasiliensis* in young rats. Lymphocytes expel larval infections but not adult worms. (1975) *Clin. Exp. Immunol.* 21:155). Live worms are purified from a worm mount. The purified worms are counted and resuspended at 2500 worms/ml in phosphate buffered saline (PBS). Mice are infected with 500 worms/200ul via a subcutaneous injection as described by Ogilvie (Reagin-like antibodies in animals immune to helminth parasites *Nature* (1964) 204:91). The infected mice are kept on a normal diet and provided ad lib antibiotic water (0.5g polymyxin B and 10g neomycin sulfate in 5000 ml ddH₂O) for 5 days. The mice are checked for lung inflammation at day 9 and serum IgE levels checked at days 9 and 15 using the methods provided in Example 4.

Example 8

Sensitization with panel of allergens to induce IgE (airways, skin)

[184] This example illustrates the IgE response to various allergens.

[185] A panel of allergens (used in clinic) such as Dust mite *D. farinae*, *D. pteronyssinus*, American Cockroach, *Alternaria tenuis*, *Aspergillus mix*, *Cladosporidium herbarum*, *Cladosporidium herbarum*, Cat, Dog, Plantain-Sorrel mix, Short Ragweed, West Oak mix, Grass mix/Bermuda/Johnsonand fungus and other allergens are injected with varying doses and the serum immunoglobulin levels are assessed as described above.

Example 9

Evaluation of serum IgE or memory IgE positive B cells following administration of desired therapeutics

[186] This example illustrates how various therapeutics influence the IgE response under various conditions. Both preventative and therapeutic interventions are evaluated in a similar manner. Reference to proposed therapeutic agents is intended to cover both preventative and therapeutic interventions.

[187] The serum IgE concentration of naïve animals is measured. The animals are randomly assigned to one of seven group. The first group will receive no therapeutic intervention or antigen challenge. The second group receives vehicle only (i.e., no antigen) then the proposed therapeutic agent. The third group receives antigen challenge then the proposed therapeutic agent. The fourth group receives the proposed therapeutic agent then vehicle only. The fifth group receives the proposed therapeutic agent then antigen challenge. The sixth group group receives vehicle only (i.e., no proposed therapeutic agent). The seventh group receives antigen challenge only (i.e., no proposed therapeutic agent).

[188] The time between antigen challenge and the administration of the proposed therapeutic agent (or vice versa) may be varied to determine optimal administration times.

[189] The animals IgE levels are measured over time to evaluate the proposed therapeutics ability to modulate IgE serum levels. 60ul samples are collected on days 3, 7, 14, 21, 28, 35 and 42 (post antigenic challenge) via tail vein for antibody isotype measurements using the assays described in Example 4.

[190] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

INDUSTRIAL APPLICABILITY

[191] The embryonic stem cells provided herein allow the generation of an *in vivo* model IgE response to non-specific allergens.

[192] The *in vivo* animal model described herein provides a full repertoire IgE response to a non-specific allergen.

Table 1: Summary of Sequences

SEQ ID NO	Sequence	Figure
1	GGGCTGGGCTG	N/A
2	GAGCTGAGCT	N/A
3	tgggttaagc agagctgtgc tgggctggta tgagctggtc caagttgggc taaacagagc tgggccaggc tagtatgagc tggctctgaac tacactaagc aggactaggc tgggctgagc tgagctggac tggctggact tggctgagat gtgttgagct ggggttaagta tggctgggct gggctggcct gggctgggct ggactggatt ggtatgagct ggtccaagtt gggctaagca gagctgggcc aggctggtat gagctggtct aaactgaact aagtagggct gggctaagct gagctggtct acactagcct gacctgagct agggtaggct ggactgggct gagctaagtt gcactgggca ggggtgggct ggaccgagct gatttgagct gggatgggct gagatggggt cagcaggcct aagcaggcct agctgggttt agctagattt agctaggcaa ggctgagcta ggctgggcgg ggcgggggcta ggctgggcag ggctggactg	3

SEQ ID NO	Sequence				Figure
	agctagcttt tgtatatattcg gttgaaatgg gttggtctcg tctggactga actgactgag ctgggctagc ctgagctcga tgggggggtat actcagctga gatgggctgg tctggctaga ctgaactgga ttgggctagg ctgagctagg ctgacctgaa ctggcctgggt ctgggctgga ctgggcaggg ctggtctcag ctagactaca ctgagttaac ctgggctgga ccatactggg ttaaactagg ttgcactggc tgggttagac ttggctgagc tgggcttggc tgagctgagt caagatggtc tgagttgatt tgagttggct aagctaagct gagctacact gaactaggca aggctgggct ggaaaggctct gggttaagtt aggagggact tggcttggct tagctgggcc aagctaggct gaactgggct gaactgagct gagctgggct gagctgggct gagctgggct gagctgggca aggctaaact ggaatggact gaattggcct aagatgggcc cggctaagct aagtaaggct gccctgaact gagcaggact ggcctggcct ggattgacct ggcatgagct taacttgact agactagtct atcttgggtg aactgggcta agcaggacta atctggcctg atctgagcta gactgaacta ggctaagctg agctgagttt agcttggctg aactgggctg ggctgcactg aactgtattg agctatgtag aactgagctg gtcttgtctg aggtgggttg ggctggtctg ggctgaacca gattgcacta gactgagctt agctggacct ggctgagctg gactgcattg tgctaaactg gctctcttta gaccgagctt agctggactg gactgagcta ggttgggtgg gctgatctaa gctgagctag gctggtctca cctgaggaat gctgtgctgt gctgagctga actaaactga gctcagctaa ggaagtgtga gctagactga gctgagctag gctgggttgg gctgaactga gctaccttgg gtggactagg ctgagctgag ctgggttgag ctgagctata gatttgggtg gactggactg gattgggcta aactgaactg gtttggggta ggctgggatg agctggactg agctaggctg tactggtctg agctaaacta agttgagtgg ggctaagagg agctgagtga ggctgggctg gaatgagcta ggctaggggt gtgagctagg gttgtactgg tctaagctga gttttagctga gagaggctgg gctagacttc cataagggtg ctgagtcata ctacagtgca ctgagctgtg ttgagcttaa cttggattaa gtggaatggg ttgagctggc tgaactgggc tgaactgaga taaactagac tgagctggga cacgctggga cgagctggaa cgagctagaa ttactgttct aatctgatct gggctgaggt aaactgggcc tggttgagct ctactaggct aagtagagtt gagct				
4	GATCCCTGTG AAGCCCTGGG CCATGGGAAG AGATAGAAGG AAGGCTAGGT GGGGCAAGAC GAGGGA ACTA AAGCCACTGT GCTGCTGGGG AACTGTGGA CACTgatgga cagaaagga gtgatcagtc tgtggacagg agggggaggg gCAAGGATGA TGCTGACAGA GAGTCACAGT GGAGTCCGTA GCAGGAAAGA GAGAGAGCGC CCAGTGTAGT CCTAAGGCTT AGGAAGTTGC AACTGCCTCC TCTCCTTCCA GAGGATCACT CACTGCCACC TAGCATAGAA CTCAGAGGAC CCAGAACCAG CAGCTCAGCC				7A

SEQ ID NO	Sequence	Figure
	CAACCTGTGT GTCACAGAAG AATCAGGCCC GGTGAGGCTA GACACAAAGG CTCTTGGCCC TCATGCTGTG AGGGAGGTAC ACACTGGAGG CACACCACAA ACAGTTGGAG CAGAGGCTTC TCGCCCCCTAT TTTTCCCTCT GAACAATAGT TGCTTCCAGG GAACTCTGCA TTTACCCCTC AGGCTCCAC CCATGTCTAT TAGGCTGAAG GCCAAGCCTG TCACCTCAGA CAGACAGTGT ATCTGAAAGA CAGAAGGCCG TGCAAGACCA CAATTCCCTT GAATCTCACA CTCTGTCTTC CCAAAGTTCC TAACTGCATC TGACCTTTCT GGGCCAGCCT CTCAGCCTGC CTGGCTCTGC CACTATCAGG AAGATCTCTA ATATCTTCCA AATGCAATTA AACACGCTCC TGTGAAAGTC AGACTTGGCA TAGCCTAAGT CCCTTCGGTC CCTTTCCTG GACCAACGA CCCTGAGCAG CCAGGGTCCA AGGGATGGGG CTCTCATTTT CTCCCCAAA TCTCTGTGTG CCTCTCTCAA GACTCAAGAC TCACAAGCAA AATTAGTGGC TCCTATAGTT TGTATGTATG TTTTCTTAGA ACTCCTAGGA ACCATGGGCC TACAGAGACA TCAGAGTGTA GAGGGAATCC CTGAACCCAG AAGATGACCT TGCTCTACAA AGCTGCAGCT GAGACAGACA CTACTAGTAC CCCATGAAAG CTGCTGAGCC AAAGCCCAGC CCTCACACCA TCTTTACCCT CATCCCTCCC CTCAGTGCAG ACATAGACCA CAGGCCTGGA AGAGACGTTA GCTGTTTCTA CACAGCTCCG TGAAACCCAG TCACAACCCA GATGTGCTCT GTCCTTCTGG ACTCCTTGCC AGAGTAGCAG GTAGAGGACC TCAAGCTGAA AGATAATCAC TTGTGAGTGG GCACCAGGGA AGGCCACTGT CCCTCGCATG CCAGCTCCAA AGCTGATACA GGAAGTAGGG TGCCTCTATC AGAGGCCCTG CAATGTCATA TCTGGCCAC AGGCTGTTCC TCTTTGTGCA CCATTAATAA CTTACAAAGT GACAGCCACA CTCCCCTGAA GGGCTGCCAA AGGAACAGAA AAAGCAATGG CGAGGGTCTA GTCCTGCCTC AGGGCAGTGA CACTCCAAAG GGGCAGGCAT GGTGACTGCA CGCASNNCAC ACATGCAAGG CTTTAATACG AGAGCTATGC AAGGAGACCT GGGATCAGAC GATGGAGAAT AGAGAGCCTT GACCAGAGTG TGCAGGTGTG TCTCCTAGAA AGAGGCCTCA CCTGAGACCC CACTGTGCCT TAGTCAACTT CCAAGAACA GAATCAAAAG GGAAGTTCCA AGGCTGCTAA GGCCGGGGGT TCCCACCCCA CTTTTAGCTG AGGGCACTGA GGCAGAGCGG CCCCTAGGTA CTACCATCTG GGCATGAATT AATGGTTACT AGAGATTCAC AACGCCTGGG AGCCTGCACA GGGGGCAGAA GATGGCTTCG AATAAGAACA GTCTGGCCAG CCACTCACTT ATCAGAGGAC CTCAGGTATT ACAACCCATG GGACCCTGAG CAAAAGGGTT TGCCTAAGGA GAAGGGACAA ACAGGTTACA GGGTCCTGGG TGGGGAAGGG GACACCTGGG CTGCCTTCTA ATGTGGACAG TCTCTTGACC ACCGAATGTC CTTAGCTAT CACTTCCCTG CACTAAGGCA CACAGGTATT AGAACTGCT ATAGCTATTC ATGAAGACGG GGGACTGTGG ATCTCAACCA GAGAGGGCTG AACCAAGATA AACTGAATAT GTTGTGAGAA ACTCAAAAC TGCAGGAGAG GCTGGAGAGG AATCGGCCAG CAAGCCATCA GACAAGAATG CAATGACAAA TGTCAGATCC AGCAAATGAC AGCAAGGAAT	

SEQ ID NO	Sequence	Figure
	TGCCCTGTGA TGAAC TAACA ACCAAGAGGA CTGTCCACAG CTGGGCTGAC CCAGGCAGCA CTGGGCTAAA TTGGGTGGGA TCTGTGCTGC CCTGGGCTGG TATGAGCCAG GATGAGCCAA GTGAAGTGGG CTGGACTAGT TTGGGCTGGA CTGGCCTGGA GTAGGCTAAA CCAGTTTAAA CTAGAGTAGG CTGGGCTGAG GTGAATCAGA CTAGGCTAGA CTAGTCTGAG C	
5	GATCCCTGTG AAGCCCTGGG CCATGGGAAG AGATAGAAGG AAGGCTAGGT GGGGCAAGAC GAGGGAAC TA AAGCCACTGT GCTGCTGGGG AACTGTGGA CACTGATGGA CAGAAAGGGA GTGATCAGTC TGTGGACAGG AGGGGAGGG GCAAGGATGA TGCTGACAGA GAGTCACAGT GGAGTCCGTA GCAGGAAAGA GAGAGAGCGC CCAGTGTAGT CCTAAGGCTT AGGAAGTTGC AACTGCCTCC TCTCCTTCCA GAGGATCACT CACTGCCATC TAGCATAGAA CTCAGATGAC CCAGAACCAG CAGCTCAGCC CAACCTGTGT GTCACAGAAG AATCAGGCCC AGTCAGGCTA GACACAAAGG CTCTTGGCCC TCATGCTGTG AGGGAGGTAC AACTGGGGG CACACCACAA ACAGTTGGAG CAGAGGCTTC TCACCCCTAT TTTTCCCTCT GAACAATAGT TGCTTCCAGG GAACTCTGCA TTTACCCCTC AGGCTCCAC CCATGTCTGT TAGGCTGAAG GCCAAGCCTG TCACCTCAGA CAGACAGTGG ATCTGAAAGA CAGAAGGCCG TGCAAGACCA CAATTCCCTT GAATCTCACA CTCTGTCTTC CCAAAGTTCC TAACTGCATC TGACCTTTCT GGGCCAGCCT CTCAGCCTGC CTGGCTCTGC CACTATCAGG AAGATCTCTA ATATCTTCCA AATGCAATTA AACACGCTCC TGTGAAAGTC AGACTTGGCA AAGCCTAAGT CCCTTCGGTC CCTTTCAGTG GGACCAACGA CTCTGAGCAG CCAGGGTCCA AGGGATGGGG CTCTCATTTT CTCCCCAAA TCTCTGTGTG CCTCTCTCAA GACTCAAGAC TCACAAGCAA AATTAGTGGC TCCTATAGCT TGTATGTATG TTTTCTTGGA ACTCCTAGGA ACCATGGGCC TACAGAGACA TCAGAGTGTA GAGGGAATCC CTGAACCCAG AAGATGACCT TGCTCTACAA AGCTGCAGCT GAGACAGACA CTACTAGTAC CCCATGAAAG CTGCTGAGCC AAAGCCCAGC CCTCACACCA TCTTTACCCT CATCCCTCCC CTCAGTGCAG ACATAGACCA CAGGCCTGGA AGAGACGTTA GCTGTTTCTA CACAGCTCCG TGAAACCCAG TCACAACCCA GATGCGCTCT GTCCTTCTGG ACTCCTTGCC AGAGTAGCAG GTAGAGGACC TCAAGCTGAA AGATAATCAC TTGTGAGTGG GCACCAGGGA AGGCCACTGT CCCTCGCATG CCAGCTCCAA AGCTGATACA GGAAGTAGGG TGCCTCTATC AGAGGCCCTG CAATGTCATA TCTGGCCAC AGGCTGTTCC TCTTTGTGCA CCATTAATAA CTTACAAAGT GACAGCCACA CTCCCTGAA GGGCTGCCAA AGGAACAGAA AAAGCAATGG CTAGGGTCTA GTCCTGCTTC AGGGCAGTGA CACTCCAAAG GGGCAGGCAT GGTGACTGCA CACA---CGC ACATGCAAGG CTTTAATACG AGAGCTATGC AAGGAGACCT GGGATCAGAC GATGGAGAAT AGAGAGCCTT GACCAGAGTG TGCAGGTGTG TCTCCTAGAA AGAGGCCTCA CCTGAGACCC CACTGTGCCT	7B

SEQ ID NO	Sequence				Figure
	TAGTCAACTT CCCAAGAACA GAATCAAAAG GGAAGTTCCA AGGCTGCTAA GGCCGGGGGT TCCCACCCCA CTTTTAGCTG AGGGCACTGA GGCAGAGCGG CCCCTAGGTA CTACCATCTG GGCATGAATT AATGGTTACT AGAGATTCAC AACGCCTGGG AGCCTGCACA GGGGGCAGAA GATGGCTTCG AATAAGAACA GTCTGGCCAG CCACTCACTT ATCAGAGGAC CTCAGGTATT ACAACCCATG GGACCCTGAG CAAAAGGGTT TGCCTAAGGA GAAGGGACAA ACAGGTTACA GGGTCCTGGG TGGGGAAGGG GACACCTGGG CTGCCTTCTA ATGTGGACAG TCTCTTGGTC ACCGAATGTC CTTTCAGCTAT CACTTCCCTG CACTAAGGCA CACAGGTATT AGAAACTGCT ATAGCTATCC ATGAAGACAG GGGACTGTGG ATCTCAACCA GAGAGGGCTG AACCAAGATA AACTGAATAT GTTGTGAGAA ACTCAAAAC TGCAGGAGAG GCTGGAGAGG AATCGGCCAG CAAGCCATCA GACAAAAATG CAATGACAAA TGTCAGATCC AGCAAATGAC AGCAAGGAAT TGCCCTGTGA TGAAC TAACA ACCAAGAGGA CTGTCCACAG CTGGGCTGAC CCAGGCAGCA CTGGGCTAAA TTGGGTGGGA TCTGTGCTGC CCTGGGCTGG TATGAGCCAG GATGAGCCAA GTGAAGTGGG CTGGACTAGT TTGGGCTGGA CTGGCCTGGA GTAGGCTAAA CCAGTTTAAA CTAGAGTAGG CTGGGCTGAG GTGAATCAGA CTAGGCTAGA CTAGTCTGAG C				
6	agctcacccc agctcagctc AGCTCACCCC AGCTCAGCCC AGCTCAGCCC AGCTCAGCCC AGCTCAGCCC AGCTCAGCCC AGCTCAGCTC AGCTCAGCCC AGCTCAGCCC AGCTCACCCC AGCTCAGCTC ACCCCAGCTC AGCTCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCAGCCC AGCTCAGCTC AGCTCACCCC AGCTCAGCTC ACCCCAGCTC AGCCCACTC AGCCCACTC AGCCCACTC ACCCCAGCTC AGCCCACTC AGCTCAGCTC ACCCCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCCCACTC ACCCCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCAGCTC AGCTCACCCC AGCTCAGCCC AGCTCACCCC AGCTCAGCCC AGCTCAGCTC ACTCCAGCTC AGCTCAGCTC ACCCCAGCTC ACCCCAGCTC AGCTCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC ACCCCAGCTC AGCTCAGCTC ACCCCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCACCCC AGCTCAGCCC AGCTCAGCTC ACTCCAGCTC AGCTCAGCTC ACCCCAGCTC ATCCCACTT ACCCCAGCTC AGCTCAGCTC ACCCCAGCTC AGCCCACTC ACCCTAGCTC AGCCAAGCTC AGCTCAGCTC ACCCCAGCTT AGCTCAGCTC ACCCAGCTCA GCTCACCCCTA GCTCAGCTCA GTCTAGCTCA GCCCAGCTCA GCTCACCCCA TCTCACCCCA				8

SEQ ID NO	Sequence	Figure
	TCTCAGCTAC TCCAGAGTAT CTCATTTTCAG ATCAGCTCAC CCCAACACAG CGTAGCATAG CTGAGCTCAC CCCAGCTCAT CTCAGCTCAG AACAGTCCAG TGTAGGCAGT AGAGTTTAGC TCTATTCAAC CTAGATTAAT GAAGTTCATT CCAGTTTGGC TCATCTCGGT TAAGCCAGCC TAGTTTAGCT TAGCGGCCCA GCTCATTCCA GTTCATTACA GTCTACTTCA TTTTGGCTCA AGCCAGCTT TGCTTACCTC AGAGTAATCA CCTCAGTTTA GCGCATTTTA GAAGCACTCA GAGAAGCCCA CCCATCTCAG CTCAGCTGTG CTTTTTAGAG CCTCGCTTAC TAGGGCTCTC AACCTTGTTT CCTTAATTTT GCTCAGCAAG CTTTATGAGT	
7	TATGAGATGA AAGTAAGCTG AGTTGGGCAG TTCTAGACTA TTCTAGGCTG TTCTGGGCTG ACCTGAACTG GGTGGGGTTG AGCTGAATGA AGTAGGCTGT GCTGAGCTCT GCCAGGCTGG ATGAGTTTAT TGATCTGAGT TGGACTGGCC TGGGCTGCAC TAAATGGGAC TGAGATGAGA TTGGCCAGGC TAGGAGGGAT TGAGCAAGGC TAAGCTAAGT TGTTCCTGGAC TAGGCCAAAC TATTAGAGGT CTTTTGGTTT AGTTGAACTT TCTGGTACTG AACTAACTG TCTTTAAGAT AGAATGCTCA AAATTATTTG TGGGTGTTTT AACTGTCCTC AAAGAAGATT GTCCTGTTGT AGGATACAAC AACAGCTACT AGCCAGACTG GGTAGGTAGA GCCAATCGGG CCTAGCAGGA ATATCCTGTG CTTTCTGAGG ACCTGGCACA GAGCTGAGCT GAGCCCCTCT CTCAGGAGAA TGTCTGGGC ATGTGGACAC TCTAGAGCAT CAAGGTGGCT TCTGAAGTGG TTGTATTCTC TATGTGCTTT CTGGGATCCA CGGAGGTCAT CTTGGAGGCA GAACACTGTG CAGGTTAGCC TATGGTAAAG CAGAGAGCCT CATGTATCTG AAACCCAAAG ATCCGATTAA TTGCCATTGT AAGTTTGCCT CTTCATCCAA ACTCGTGCCC AGCTCTCCTG GAAGCCCCTG TGCTAAGCCA GCTAGGGGCA GTGAGTGAGC AAAGCCTGAT GGGGTGTAAG GAATCAGGGG GATCCCTAGG TCTGTGTTTG GGTTTAGTGA ATAAAGACAA GACCCAGAAG GAATCTATGA CCAACAGCCC TAGGAAACAA GAATCTCACC ATTCTGTCCT CAATGTGTCC CAAAACAGAT TTAATGTGTC TCACCAAGAA ACTGGTGGTC CTGGGAAAGC TCTGAATCCC CAGGCCCCAA GAGTGGGGAC AGAAGAGACA ATGGCAATTC ATCGGATCTC TGGGCCACCA AGCCCTGTGG GGTACCTATG TCCTGGACAT AAAGGACAAC CTAGTCCCTC TGTCAACATT ACATAGCCTA CCTTAAAGCT ACTCCATTCA TCCTGAGACC ATAATGGCTT CCAGTCTGCC ACCCAGCTCT CATGCTTCAT TTCTGGACAT TCCCTAGATG GCGTCACTGT CACCTGGTCT AAAGGACAGA CAGGAGATAC CTCACACATA TCCACAAAAT TTCCAATCA AGAAAGAGGG CAAGTTTGCC TCTTTATCCA AACTTGTGCC CAGCTCTCCT GGAAGCCCCT GTGCTAAGCC AGCGAGGGGC AGTGAGTGAG CAAAGCCTGG CGGGGTGTAA GGAATCAGGG AGCTCCCTAG GTCTGTGTTT GGGTTTAGAG AATAAAGACA AGACCCAGAA GGAATCTAAC CATCTGTCTC CTAGACTGGA ATGGGGTCCC CAGAGCCCTG CTCCTGTCAC AGCTGCCCTT AATCAGTTCC	9A

SEQ ID NO	Sequence	Figure
	CCATGCTGCA GSNNGCATGC AGTGATATAA ATAAGTCTAA CCTAGGTCCT TCCTTTCTCA CAGCCTCTAT CAGGAACCCCT CAGCTCTACC CCTTGAAGCC CTGTAAAGGC ACTGCTTCCA TGACCCCTGGG CTGCCTGGTA AAGGACTACT TCCCTGGTCC TGTGACTGTG ACCTGGTATT CAGACTCCCT GAACATGAGC ACTGTGAACT TCCCTGCCCT CGGTTCTGAA CTCAAGGTCA CCACCAGCCA AGTGACCAGC TGGGGCAAGT CAGCCAAGAA CTTCACATGC CACGTGACAC ATCCTCCATC ATTCAACGAA AGTAGGACTA TCCTAGGTAA GTAGGGATGG GCTGacagtt acactgtgta ttctcccttg gagatggaac agtttctgtc taatcaggaa cttgtcacaa tttcctttca tagaggactt cataagagat tttttttt-ct acttctatca tgtttagtga tccaaataga ttttaaaaac tggttgagtg catattactt ttagcctcag aagacatcat gtatatTTAA gaggcattta actattgtaa attattctga tgacttttaa aaatgttcat gctgagttgt atattttttaa ataaatttta ttagtttagt ttaaaaaaag aaaagaaaat tattaatttt attaaaaaat ctcctatatt taaaaaaaaa agagaaaaaG GCAGAGCTGG GCTGGCTACA GTTACCACAA GAACATGGTC AGAGGAGGAA GGGACTCTTA TACATACCTA TGACAGGAGA ACGGGAGACC CAACATACTC GGGGGCCTAC CTTCAGAGAA CACAAGGCCA GGGCAATACT CACAGSNNCT CATTGTTCGA CCCTGCCCTA GTTTCGACCTG TCAACATCAC TGAGCCCACC TTGGAGCTAC TCCATTTCATC CTGCGACCCC AATGCATTCC ACTCCACCAT CCAGCTGTAC TGCTTCATTT ATGGCCACAT CCTAAATGAT GTCTCTGTCA GCTGGCTAAT GGACGATCGG GAGATAACTG ATACACTTGC ACAAACGTGT CTAATCAAGG AGGAAGGCAA ACTAGCCTCT ACCTGCAGTA AACTCAACAT CACTGAGCAG CAATGGATGT CTGAAAGCAC CTTACCTGC AAGGTCACCT CCCAAGGCGT AGACTATTTG GCCCACACTC GGAGATGCCC AGGTAGGTCT ACACTCGCCT GATGCCCAGA CCTCAGAGTC CTGAGGGAAA GGCAGGCTCT CACACAGCCC TTCTCCCSN NCGACAGATC ATGAGCCACG GGGTGTGATT ACCTACCTGA TCCCACCCAG CCCCCTGGAC CTGTATCAAA ACGGTGCTCC CAA	
8	TATGAGATGA AAGTAAGCTG AGTTGGGCAG TTCTAGACTA TTCTAGGCTG TTCTGGGCTG ACCTGAACTG GGTGGGGTTG AGCTGAATGA AGTAGGCTGT GCTGAGCTCT GCCAGGCTGG ATGAGTTTAT TGATCTGAGT TGGACTGGCC TGGGCTGCAC TAAATGGGAC TGAGATGAGA TTGGCCAGGC TAGGAGGGGT TGAGCAAGGC TAAGCTAAGT TGTCTGGAC TAGGCCAAAC TATTAGAGGT CTTTTGGTTT AGTTGAACTT TCTGGTACTG AACTAAACTG TCTTTAAGAT AGAATGCTCA AAATTATTTG TGGGTGTTTT AACTGTCCTC AAAGAAGATT GTCCTGTTGT AGGATACAAC AACAGCTACT AGCCAGACTG GGTAGGTAGA GCCAATCGGG CCTAGCAGGA ATATCCTGTG CTTTCTGAGG ACCTGGCACA GAGCTGAGCT GAGCCCCTCT CTCAGGAGAA TGTCTGGGC ATGTGGACAC TCTAGAGCAT CAAGGTGGCT	9B

SEQ ID NO	Sequence	Figure
	TCTGAAGTGG TTGTATTCTC TATGTGCTTT CTGGGATCCA CGGAGGTCAT CTTGGAGGCA GAACACTGTG CAGGTTAGCC TATGGTAAAG CAGAGAGCCT CATGTATCTG AAACCCAAAG ATCCGATGAA TTGCCATTGT AAGTTTGCCT CTTTCATCCAA ACTCGTGCCC AGCTCTCCTG GAAGCCCCTG TGCTAAGCCA GCTAGGGGCA GTGAGTGAGC AAAGCCTGAT GGGGTGTAAG GAATCAGGGG GATCCCTAGG TCTGTGTTTG GGTTTAGTGA ATAAAGACAA GACCCAGAAG GAATCTATGA CCAACAGCCC TAGGAAACAA GAATCTCACC ATTCTGTCCT CAATGTGTCC CAAAACAGAT TTAATGTGTC TCACCAAGAA ACTGGTGGTC CTGGGAAAGC TCTGAATCCC CAGGCCCCAA GAGTGGGGAC AGAAGAGACA ATGGCAATTC ATCGGATCTC TGGGCCACCA AGCCCTGTGG GGTACCTATG TCCTGGACAT AAAGGACAAC CTAGTCCCTC TGTC AACATT ACATAGCCTA CCTTAAAGCT ACTCCATTCA TCCTGAGACC ATAATGGCTT CCAGTCTGCC ACCCAGCTCT CATGCTTCAT TTCTGGACAT TCCCTAGATG GTGTCACTGT CACCTGGTCT AAAGGACAGA CAGGAGATAC CTCACACATA TCCACAAAAT TTCCAATCA AGAAAGAGGG CAAGTTTGCC TCTTTATCCA AACTTGTGCC CAGCTCTCCT GGAAGCCCCT GTGCTAAGCC AGCGAGGGGC AGTGAGTGAG CAAAGCCTGG CGGGGTGTAA GGAATCAGGG AGCTCCCTAG GTCTGTGTTT GGGTTTAGAG AATAAAGACA AGACCCAGAA TGAATCTAAC CATCTGTCTC CTAGACTGGA ATGGGGTCCC CAGAGCCCTG CTCTGTCTAC AGCTGCCCTT AATCAGTTCC CCATGCTGCA G---GCATGC AGTGATATAA ATAAGTCTAA CCTAGGTCCT TCCTTTCTCA CAGCCTCTAT CAGGAACCCCT CAGCTCTACC CCTTGAAGCC CTGTAAAGGC ACTGCTTCCA TGACCCCTGGG CTGCCTGGTA AAGGACTACT TCCCTGGTCC TGTGACTGTG ACCTGGTATT CAGACTCCCT GAACATGAGC ACTGTGAACT TCCCTGCCCT TGGTTCTGAA CTCAAGGTCA CCACCAGCCA AGTGACCAGC TGGGGCAAGT CAGCCAAGAA CTTCACATGC CACGTGACAC ATCCTCCATC ATTCAACGAA AGTAGGACTA TCCTAGGTAA GTAGGGATGG GCTGACAGTT ACACTGTGTA TTCTCCCTTG GAGATGGAAC AGTTTCTGTC TAATCAGGAA CTTGTCACAA TTTCTTTTCA TAGAGGACTT CATAAGAGAT TTTTTTTTCT ACTTCTATCA TGTTTAGTGC TCCAAATAGA TTTTAAAC TGTTGAGTG CATATTACTT TTAGCCTCAG AAGACATCAT GTATATTTAA GAGGCATTTA ACTATTGTAA ATTATTCTGA TGACTTTTAA AAAAGTTAAT GCTGAGTTGT ATATTTTAA ATAAATTTTA TTAGTTTAGT TTAAAAAAG AAAAGAAAAT TATTAATTTT ATTTAAAAAT CTCCTATATT TAAAAAAGAA AGAGAAAAAA GCAGAGCTGG GCTGGCTACA GTTACCACAA GAACATGGTC AGAGGAGGAA GGGACTCTTA TACATACCTA TGACAGGAGA ACGGGAGACC CAACATACTC GGGGGCCTAC CTTCAGAGAA CACAAGGCCA GGGCAATACT CACAG---CT CATTGTTCTGA CCCTGCCCTA GTTTCGACCTG TCAACATCAC TGAGCCCACC TTGGAGCTAC TCCATTTCATC CTGCGACCCC AATGCTTTCC ACTCCACCAT	

SEQ ID NO	Sequence	Figure
	CCAGCTGTAC TGCTTCATTT ATGGCCACAT CCTAAATGAT GTCTCTGTCA GCTGGCTAAT GGACGATCGG GAGATAACTG ATACACTTGC ACAAACCTGTT CTAATCAAGG AGGAAGGCAA ACTAGCCTCT ACCTGCAGTA AACTCAACAT CACTGAGCAG CAATGGATGT CTGAAAGCAC CTTACCTGC AAGGTCACCT CCCAAGGCGT AGACTATTTG GCCCACACTC GGAGATGCCC AGGTAGGTCT AACTCGCCT GATGTCCAGA CCTCAGAGTC CTGAGGGAAA GGCAGGCTCT CACACAGCCC TTCCTCCC-- -CGACAGATC ATGAGCCACG GGGTGTGATT ACCTACCTGA TCCCACCCAG CCCCCTGGAC CTGTATCAAA ACGGTGCTCC CAA	
9	CTCTCCCTGT GGACCACAAA AGTTTATATT CTTCTACAT ACAGTGCCCC TCCCCCATG CCAACATCCC AAAGTCTCAT CCTTCCAGCA TTCAGCTCTG CCCCAAGTCT CTCCTAAGG TCTGCTAGTC AAGTAAGGGT CAGATTGTGT GAGGTTTGTT TTGAGATAGG ATTCAGTCCA CCTAGGCCAT AGCTGTCAGG AGGAAGGGGA AGGAGAGAGG CACAGAAGGG AGAGGTATAC CGTGATGAAC TGGGCAGACT GATAACATGC TGTAGAGCCA AAAGCTGAGG GCAAGTGGGG TCCCCTCCCT CTCATGCTAA GGTGACAGTT TCTAAGGGAG AGCAAGGGAT TTGGAGAAAG AAGTGAAGGT TTGGTTCAGC ACTGGCCTTC CTGGTCCAGC AACTGCCCC TGCTCAAAC TTTGCACATA CAGATCCCCC ACTGTACTCC CCTCCTGCAT TTTCCCCACT ACCTTCAGCA CACCACCAAC CTCTCTTCCA TGACTGCCTC CATCCCACCT AGCATGGAGC CCCACTCCTG TGTGAGCCTA GTTCACTCAA TGACCATGGG TGTCCATCTT CCAATGAAAC ATGAGCTCCA TGGACAGGAA TATCCCTCCA GACCCATGTT CCTGCAGTTC TATCTAACTG TTGGGCATTT ATGATGAAGT CACACCAGGT CCCTCATTC TAACTAACCT TCTAATCTGG CACAGTTATC TGCTGGGAAC TAAGAGTGTG GTCAAAGTAA GATATGATGC TGGCCGACCA GTAGGTCCAT GTGCTGGCTT TCAACTCTAA ATCTTCCCCA CGCCTACCAT GTAGCCACAG GATCTTTCCC CAAGGCAGGG TACAGATAGC ACATAGAGGA AGGAGGCCAA GTGGATGGGG CTCTCCCGTA GCAGGTGTGG GAGAGGCAGG TTGCACACAT GTGTGAATCC ATATGGTTCT GAGGTTTGGG GGGTTTTTTT GTCCCAAAT ATCATGTTCC AGAAGTACTT GCTCCTTTGA CCTATACACC AGAGAAGAGA CCAAAAACCTG TGGTAGACAC AGGAGCAAGA ACAAAACCAT GCTGTTGTTT TTCTACAGGC AAAACTTGTT GTCACCCTTA TTCCTGGCAA AGCTATGTCG TCAAATTGCT AGTCCTGGTT GTAATTACAA ATTTAATAAT ATATATAAAT ATATGTGCTA GACAACAGTT AAAGAAATAA GGACAATGTT GGAGGGAGGA AATGAAAGTG GGAAATGATA CAATAATTAT ATTTTCATCC CAAAGATAAA GATATATGTT TTATTTCTGG TCCTGGGCTA AGGATATAGT TCAATTGTTA AAGTACTTAT ATAGCATGCA GGAAGCCCTG GATCTGATCT CCATAAATAA ACCAACTGTG GTCATTCTA CCTGTAATTC CAGTACTCCA AAGGCAGACA CAGGAAGATG GGGGCTTTGA AGTCATTCTT GGCTACATAG CAAATTAGAG	10A

SEQ ID NO	Sequence				Figure
	ACCAGCCTTA	TCTTTAATAA	TAATAATAAT	CCTGACATTC	
	CACTTGAAGT	AAATGTATCC	AGTAATTGTA	CCTGATACCA	
	TGCCATGTTA	GATTTTATTT	CAGGGTTTCA	GAGAAAAGTA	
	CCCAGGGTTT	TCCAGAGACG	CACAAGTAGT	GGAAGACAGC	
	AGACTGAGGA	CTGGTAGATG	AACGCATGAA	CAACTTAGGA	
	CAGAGCTGGA	GGACCATAAG	GCTGATAGGT	AGGTAGAAAC	
	AAGGATAACA	CATAATAGGC	TTACAGGTTT	TATGGTTCAC	
	CTTGACTGGA	CTAAGGGATG	GAAGGCCTGT	GGAGGTGTCT	
	GCAAAGGATT	TTCCAGAAGT	GTTAGGTCTT	GGGGACTCTG	
	ATTTAACC GC	ATGGTGCTGA	ACAGTAGGAA	GAAGGCCAGG	
	TGAAAGAAGC	AGGTTACTGT	GGGTAAGCCC	GGGTAATTCT	
	ATCAGATCCT	GGCACTGTAT	TCCTGTTATC	TCTACTTCCC	
	ATCAGTTGAG	AAACAAACAG	CCTCTTCCAC	ACAACCCCGC	
	TGCCAAGATG	GCCCTAAGCA	CATAGGGCCA	AACAAAAGTA	
	AATCTTTTCC	CCCTTATGTT	TCTTTGAGGT	ATCAGGTCAT	
	CCTAGCTCCC	GGCTCCAAGT	CACACAGAGG	GCTCCTATGA	
	GCAAGCATTT	CCAACCTCTAC	CCTTTTTTTC	TGTCACAAAC	
	CGCTGGCTAT	TTGAGACATT	TCAGACAGCT	AAAAGATGGC	
	TCCCTTAGCA	CTGATGGCTG	AGTTCAAGAG	GCTCACCTGC	
	TGGCTCGCAA	ACCCAGAGGT	AACCTGGGTT	TGTGTTGCTT	
	TAGGCATAGA	GAATGCTGAC	ATCCAGCGGC	TACGTGACTG	
	CTTCTGGGTT	TTGGAACCTG	TTCTTCAGTA	CCATCCCCTG	
	CATCCAATC	TTCTCAGCTT	GTAATCTTCT	CAATATTTCC	
	ACCGTTCTTC	ATCACCCCTAG	ATTCTTGCAG	ATGCTGCCCT	
	CGATGGCTCA	CTGGCTCCTC	TACTCCCTGC	CACTATCACC	
	CTCGGGGCCA	GAAACCTGGA	GCCATGTCTG	CTCTTTGCAC	
	AGTAACACAG	CCTACTCTAC	CCATGACAAA	GACCATAAGA	
	AGACTTGAG	ACCATTTAGG	AAAAGCCTTT	ATCATGGCCT	
	AATGCTGCAC	ACGTGGATCA	GGAGAAGCCT	CAAAATATAG	
	TAGGGGGCAC	ACTGTAGAGA	CAGAATAGAG	TCCATGATAC	
	GCTCATACAT	GGATTATACT	TCCAACAAGC	ACTGCCCTGT	
	TTGTGCTCAT	CTCCTGGTTC	GACCAAGCAC	AGTCTTCCCA	
	TATGAACCCA	TCACAAGCCC	TGCAGAATCA	CAGATCACAG	
	GTCTTAGATA	GGACCAGCTT	TCTTTCTGAC	AATAACCAGG	
	ATTTATTTGT	TATTTCTTTT	TATTGTATTT	ATTTTTATTC	
	ATAATTTTAC	ATCCCTCTCA	CTGCCCCCTC	CCAGTCATTC	
	CCTTCTGTTT	TCCTCTCCCC	ACTCCCCCTC	CCTTTCTCCT	
	CTAAGCCAGT	TAAGCCCCCT	CTGTGTACCC	CCCACCCTAA	
	TAATCAGGAG	TTTTGAGCCA	CCAGAGATGT	TCTTCCTCCT	
	CTCTGACCTT	GCTGAGAGCC	TCTATGCCAA	GGTCCTCTCG	
	AGCTGCATGT	GAAGTCACTT	GGAAGTCGTA	GGTGAAGTGG	
	AGTTTTCCAG	CTACAGTGCA	GGCTGGAGCC	CTGGTAACTA	
	GAACAAGGCT	GTAGTTTCAG	CAGCAGCCAT	GATTGCAGGA	
	TACCTTGACG	CTCAAATATG	GCCTCCTTGG	GGCTCTGTGA	
	GGTATTCAAA	GCATCTAGAA	TCCCATGATG	ACAGTTCTAC	
	CAGTCCCTAA	AAGAAACCTA	AGACGACTAG	ATATAAGGAA	
	AGACCCACCT	GAGTGCATCA	AAAGGTCAAA	TCAGCCTGGC	
	GCTCAACAGC	TCATTTTACA	TGAAGAAAAG	GTGAACACTA	

SEQ ID NO	Sequence	Figure
	CCCTATTCCC AATAAAGACA TGTGTGTTACA CTTACACTAA CATCCTTGGC AGCCCTTAGC AGATGATCCT AGGGAGAGCT GAGCAGTCTC ATCTACCTCA CCTCCACCCA GGCATCAAGT TAACACTGTT CTAAGGTGCA CTTCTGAAAC TTACAGAGTT GGGGTAGCAG TCAGACCTTT CCCTGACCCC CAAGATATGA TCACACCCAC AACCACATAC ATGAGTTCGC AGACACTAAC CGACACAGTG GATCTTAGAC CTGGCCCATT CCGGAATAGA TCACTGTCAC AGTCACTTGA GTGAAGGAGC CACCCAAGGG AATGGCTAAA GGA CTG	
10	CGGGAGATAA CTGATACACT TGCACAACT GTTCTAATCA AGGAGGAAGG CAAACTAGCC TCTACCTGCA GTAAACTCAA CATCACTGAG CAGCAATGGA TGTCTGAAAG CACCTTCACC TGCAAGGTCA CCTCCCAAGG CGTAGACTAT TTGGCCCACA CTCGGAGATG CCCAGGTAGG TCTACACTCG CCTGATGCCC AGACCTCAGA GTCCTGAGGG AAAGGCAGGC TCTCACACAG CCCTTCCTCC CSNNGACAG ATCATGAGCC ACGGGGTGTG ATTACCTACC TGATCCCACC CAGCCCCCTG GACCTGTATC AAAACGGTGC TCCCAAGCTT ACCTGTCTGG TGGTGGACCT GGAAAGCGAG AAGAATGTCA ATGTGACGTG GAACCAAGAG AAGAAGACTT CAGTCTCAGC ATCCCAGTGG TACACTAAGC ACCACAATAA CGCCACAACCT AGTATCACCT CCATCCTGCC TGTAAGTTGCC AAGGACTGGA TTGAAGGCTA CGGCTATCAG TGCATAGTGG ACCACCCTGA TTTTCCCAAG CCCATTGTGC GTTCCATCAC CAAGACCCCA GGTGAGTACA GGAGGTGGAG AGTGGGCCAG CCCTSNNSMT CTTTCATGTTT AGAGAACATG GTTAACTGGT TAAGTCATGT CTGCCCACAG GCCAGCGCTC AGCCCCCGAG GTATATGTGT TCCCACCACC AGAGGAGGAG AGCGAGGACA AACGCACACT CACCTGTTTG ATCCAGAACT TCTTCCCTGA GGATATCTCT GTGCAGTGGC TGGGGGATGG CAACTGATC TCAAACAGCC AGCACAGTAC CACAACACCC CTGAAATCCA ATGGCTCCAA TCAAGGCTTC TTCATCTTCA GTCGCCTAGA GGTCGCCAAG ACACTCTGGA CACAGAGAAA ACAGTTCACC TGCCAAGTGA TCCATGAGGC ACTTCAGAAA CCCAGGAAAC TGGAGAAAAC AATATCCACA AGCCTTGGTA ACACCTCCCT CCGTCCCTCC TAGGCCTCCA TGTAGCTGTG GTGGGGAAGG TGGATGACAG ACATCCGCTC ACTGTTGTAA CACCAGGAAG CTACCCCAAT AAACACTCAG TGCCTGATTA GAGCCCTGGG TGCTGTCTCT TGGGGAAGGC AGGTTATGGG CAGAAATATC TTGGCCTGAA AGAAGGGACA CCCCAGAGA AGGACAGGAG TGAAGCATGG CTCACCCATC TGTCTATGTG TTGAATATTT AACAAATAGG ACATCACAGG ACTTCAGCAT AGTCCTTCAG CATACCCCTG GTCCTTCCTG CTCTTCACTG GATATCATGC ACCTGATCTC TAGAGATGCA GCTAAAATGA GCCAGTCTGA GAAGCCTCAG CACCCACCTC TCGGTCTTGC AAGCTCCTGC TCCCAGGCTT TCCTGGATAC TAAACCCCTT CAGGTAGAGA AACAGCCAAA GTCAACATCT AGGACGCAGG ACTCAACATG GTCCTGCTCC TTCCCTCTCT ACTCAACAGC	10B

SEQ ID NO	Sequence	Figure
	CATTGAGGCT GAGCCCACCG CCCCAACCGC CTGCCTTGCC AAATGATCAC GCCAGGCCTG GTGCTCCTCG ACTTACTACC TAGACTCACT CCAACCCAAA TTCATCCCAA GGACCAGAAT GGGCTGCCAG CCTCATAACAG TCAGGTTCCT CCATCTATGA CATGTTTTCA CACACATGCA CACACACACA CACACACACA CACACACACA GAGCTAGGCT TCATTGAGCT CTCTGGTTTA GCAATAGCCC AAAGCAAGCC ATACATCCAT CCCAGTTCCA GAAGGATAAG AAAACCAGAA CCAAGACACA CCCACACCTA TTCCATACCC AACCACCAGC ACATATGGCT TACACACCTG AGATCAGTGG CTCCCATCAT GTACACACAC ATGCACACAA AGGAGACCAT ACATACCCAT CATTTCAGGA GGTAAGTATC TAACCTTTGG ATCTGAGATA CCTCTGAGGA ACACCAATGG CAGAGTCGAC CAGCACCTCA GCCTCCAGAC TAAATCCTTA CATTTTGGCC CACCCCAAGC CATGAGAGAT GGAGGAGGGT AGAGGCCTGA GCTGCGGGAA AGCAGAGACA GGAAGATGGG CTGTTTGGTG AGAGTAGTAA ACCAGACAAT GGGGAGACTA AGGCAGGAGT AGAGCCCCTA CAAGGCCAG AGTCTGCTTT AGAGTCCATG TGTCTTGACC TGCCCCTCAG ATGCCACAAC CAAGATTCTT GGTTCAGAG CATGCATGCA GGCCCTAGAA ATGGACCTAT GAGCTCAGAG CCTTCCTAGA GAGCCCTGGG TACTCTCTGA ACAAAGGCA ATTCTGTGTA GAGGCATCCT GTGGCCAAAG ACCCTAAGAC AGTCATACAC ACACACAACA CACACAACAC AGGTAGGCTT TATCATGCTC TTTGATTTAG CAATAGCCCT GTTGATGGTG GGGGATACTG GGTCAGTGTG GGCACCGGAG TAGAAAGAGG GAATGAACAG TCAGTGGGGA AAGGACATCT GCCTCTAGGG CTGAACAGAG ACTGGAGCAG TCTCAGAGCA GGTGGGATGG GGACCTCTGC CACTCTAGCT TCATCAGAAC TGCATGAGAC AAATATGGGG CCTACCCCTT CCCCACTGTC ACCTGGAGTS NNMCTGGGGA AGCTAACTGG CTGGTCCCAC CCCATCCCAG AGCTAGACCT CCAGGACCTA TGTATTGAAG AGGTGGAGGG CGAGGAGCTG GAAGAGCTGT GGACCAGTAT TTGTGTCTTC ATCACCTGT TCCTGCTCAG TGTGAGCTAT GGGGCCACTG TCACCGTCCT CAAGGTGGGA TCCTGCACCT CAGCGGGTGG GTCTGGGAGG GCTAGGCCAA GCCGCAGAGC CATCCTCACA TACSNNMACC TTTCCCCCAG GTGAAGTGGG TCTTCTCCAC ACCGATGCAG GATACACCCC AGACCTTCCA AGACTATGCC AACATCCTCC AGACCAGGGC ATAGGTGCGA TGCCAGCACC CATGCAGGCC TGCAGCCATG TGTGCTTGAG CCTCCTGAGG TGCTGTGTTG CCCGGGTGAT AGGAGGGAGC AGAGACCCCT AAAGGCACCA ACGTTGATGA GATATCAGCA TCCCAGAAAG TTGCAGCTCA GAGCACCTAG GTGGGCTGTC CTACACAGAT ACTTTGAGAC AAAGCTTAGA AGAACATCTA TCCCTCATCG ATTTGACCTA CCAGATGCTA GCCACCTGGG CTAATCCCAG GTCTATGGGC ATCAGGACCA CTCCATTTTG ACTGAATAAC CACAAAAACA CAAGAACTCA GAGTCTAGAG TTCCCACTAG ACCCCACCTA GAGCACAGAG TCAAAGCTGG GACACTCAGA ATCAACCCTA AGTCCAGACG CTGGCTCCTC AGAAGG	

SEQ ID NO	Sequence	Figure
11	CTCTGGCCCTGCTTATTGTTG	11A
12	CCTGATAGAGGCTGTGAGAAAGGAAGGACC	11B
13	AGACCTGGGAATGTATGGTT	11C
14	TAGGTTAGACTTATTTATATCACTGCATGC	11D
15	GTTGAGAGCCCTAGTAAGCG	11E
16	TTGAGAGCCCTAGTAAGCG	11F
17	TGAGCTCAGCTATGCTACGCGTGTTG	11G
18	GCCCGATTGGCTCTACCTACCCAGTCTGGC	11H
19	gggaagagat agaaggaagg ctaggtgggg caagacgagg gaactaaagc cactgtgctg ctggggacac tgtggacact gatggacaga aagggagtga tcagtctgtg gacaggaggg ggaggggcaa ggatgatgct gacagagagt cacagtggag tccgtagcag gaaagagaga gagcgcccag tgtagtccta aggccttagga agttgcaact gcctcctctc cttccagagg atcactcact gccatctagc atagaactca gatgaccag aaccagcagc tcagcccaac ctgtgtgtca cagaagaatc aggcccagtc aggcctagaca caaaggctct tggccctcat gctgtgaggg aggtacacac tgggggcaca ccacaaacag ttggagcaga ggcttctcac ccctattttt ccctctgaac aatagttgct tccagggaac tctgcattta cccctcaggc tcccacccat gtctgttagg ctgaaggcca agcctgtcac ctgagacaga cagtggatct gaaagacaga aggccgtgca agaccacaat tcccttgaat ctcacactct gtcttcccaa agttcctaac tgcactctgac ctttctgggc cagcctctca gcctgcctgg ctctgccact atcaggaaga tctctaatat cttccaaatg caattaaaca cgctcctgtg aaagtcagac ttggcaaagc ctaagtcctt tcggtccctt tcagtgggac caacgactct gagcagccag ggtccaaggg atggggctct cattttcttc cccaaatctc tgtgtgcctc tctcaagact caagactcac aagcaaaatt agtggctcct atagcttgta tgtatgtttt cttggaactc ctaggaacca tgggcctaca gagacatcag agtgtagagg gaatccctga acccagaaga tgaccttgct ctacaaagct gcagctgaga cagacactac	12

SEQ ID NO	Sequence	Figure
	tagtacccca tgaaagctgc tgagccaaag cccagccctc acaccatctt taccctcatc cctcccctca gtgcagacat agaccacagg cctggaagag acgttagctg tttctacaca gctccgtgaa acccagtcac aaccagatg cgctctgtcc ttctggactc cttgccagag tagcaggtag aggacctcaa gctgaaagat aatcacttgt gagtgggcac caggggaaggc cactgtccct cgcatgccag ctccaaagct gatacaggaa ctaggggtgcc tctatcagag gccctgcaat gtcatatctg gccacacaggc tgttcctctt tgtgcaccat taataactta caaagtgaca gccacactcc cctgaagggc tgccaaagga acagaaaaag caatggctag ggtctagtcc tgcttcaggg cagtgcactc ccaaaggggc aggcattggtg actgcacaca cgcacatgca aggcctttaat acgagagcta tgcaaggaga cctgggatca gacgatggag aatagagagc cttgaccaga gtgtgcaggt gtgtctccta gaaagaggcc tcacctgaga ccccactgtg ccttagtcaa cttcccaaga acagaatcaa aagggaaactt ccaaggctgc taaggccggg ggttcccacc ccacttttag ctgagggcac tgaggcagag cggcccctag gtactaccat ctgggcatga attaatggtt actagagatt cacaacgcct gggagcctgc acagggggca gaagatggct tcgaataaga acagtctggc cagccactca cttatcagag gacctcaggt attacaaccc atgggaccct gagcaaaagg gtttgcctaa ggagaaggga caaacagggt acaggggtcct gggtggggaa ggggacacct gggctgcctt ctaatgtgga cagtctcttg gtcaccgaat gtccttcagc tatcacttcc ctgcactaag gcacacaggt attagaaact gctatagcta tccatgaaga caggggactg tggatctcaa ccagagaggg ctgaaccaag ataaactgaa tatgttgtga gaaactcaaa aactgcagga gaggctggag aggaatcggc cagcaagcca tcagacaaaa atgcaatgac aaatgtcaga tccagcaaat gacagcaagg aattgccctg tgatgaacta acaaccaaga ggactgtcca cagctgggct gaccaggca gcactgggct aaattgggtg ggatctgtgc tgccctgggc tggatatgagc caggatgagc caagtgaagt gggctggact agtttgggct ggactggcct ggagtaggct aaaccagttt aaactagagt aggctgggct gaggtgaatc agactaggct agactagtct gagctggggt aagcagagct gtgctgggct ggtatgagct ggtccaagtt gggctaaaca gagctgggct aggctagtat gagctgggtc gaactacact aagcaggact aggctgggct gagctgagct ggactggctg gacttggctg agatgtgttg agctgggtta agtatggctg ggctgggctg gcctgggctg ggctggactg gattggtatg agctgggtcca agttgggcta agcagagctg ggccaggctg gtatgagctg gtctaaactg aactaagtag ggctgggcta agctgagctg gtctacacta gcctgacctg agctagggta ggctggactg ggctgagcta agttgcactg ggcaggggtg ggctggaccg agctgatttg agctgggatg ggctgagatg ggttcagcag gcctaagcag gcctagctgg gtttagctag atttagctag gcaaggctga	

SEQ ID NO	Sequence	Figure
	gctaggctgg gcggggcggg gctaggctgg gcagggctgg actgagctag cttttgtata ttcggttgaa atgggttggt ctggtctgga ctgaactgac tgagctgggc tagcctgagc tcgatggggg gtatactcag ctgagatggg ctggtctggc tagactgaac tggattgggc taggctgagc taggctgacc tgaactggcc tggctctgggc tggactgggc agggctggtc tcagctagac tacactgagt taacctgggc tggaccatac tgggttaaac taggttgcac tggctgggtt agacttggtt gagctgggct tggctgagct gagtcaagat ggtctgagtt gatttgagtt ggctaagcta agctgagcta cactgaacta ggcaaggctg ggctggaaag gtctgggtta agttaggagg gacttggctt ggcttagctg ggccaagcta ggctgaactg ggctgaactg agctgagctg ggctgagctg ggctgagctg ggctgagctg ggcaaggcta aactggaatg gactgaattg gcctaagatg ggcccggcta agctaagtaa ggctgccttg aactgagcag gactggcctg gcctggattg acctggcatg agcttaactt gactagacta gtctatcttg ggtgaactgg gctaagcagg actaatctgg cctgatctga gctagactga actaggctaa gctgagctga gtttagcttg gctgaactgg gctgggctgc actgaactgt attgagctat gtagaactga gctgggtcttg tctgaggttg gttgggcttg tctgggctga accagattgc actagactga gcttagcttg acctggctga gctggactgc attgtgctaa actggctctc tttagaccga gcttagcttg actggactga gctaggtttg gtgggctgat ctaagctgag ctaggctggc ctcacctgag gaatgctgtg ctgtgctgag ctgaactaaa ctgagctcag ctaagggaagt gtgagctaga ctgagctgag ctaggctggg ttgggctgaa ctgagctacc ttgggtggac taggctgagc tgagctgggt tgagctgagc tatagatttg gttggacttg actggatttg gctaaactga actggttttg ggtaggcttg gatgagctgg actgagctag gctgtacttg tctgagctaa actaagttga gtggggctaa gaggagctga gtgaggcttg gctggaatga gctaggctag ggttgtgagc tagggttgta ctggtctaag ctgagtttag ctgagagagg ctgggctaga cttccataag gtggctgagt catactacag tgcactgagc tgtgttgagc ttaacttgga ttaagtggaa tgggttgagc tggctgaact gggctgaact gagataaact agactgagct gggacacgct gggacgagct ggaacgagct agaattactg ttctaactctg atctgggctg aggtaaactg ggcctgggtg agctctacta ggctaagtag agttgagcta tgagatgaaa gtaagctgag ttgggcagtt ctgactattt ctaggctggt ctgggctgac ctgaactggg tgggggttgag ctgaatgaag taggctgtgc tgagctctgc caggctggat gagtttattg atctgagttg gactggcctg ggctgcacta aatgggactg agatgagatt ggccaggcta ggaggggttg agcaaggcta agctaagttg ttctggacta ggccaaacta ttagaggtct tttggtttag ttgaactttc tgggtactgaa ctaaactgtc tttgaagatag aatgctcaaa attatttgtg ggtgttttaa ctgtcctcaa	

SEQ ID NO	Sequence	Figure
	agaagattgt cctggttgtag gatacaacaa cagctactag ccagactggg taggtagagc caatcggggc tagcaggaat atcctgtgct ttctgaggac ctggcacaga gctgagctga gccccctctc caggagaatg tcctgggcat gtggacactc tagagcatca aggtggcttc tgaagtgggt gtattctcta tgtgctttct gggatccacg gaggtcatct tggaggcaga aactgtgca ggtagccta tggtaaagca gagagcctca tgtatctgaa acccaaagat ccgatgaatt gccattgtaa gtttgcctct tcatccaaac tcgtgcccag ctctcctgga agccccctgt ctaagccagc taggggcagt gagtgagcaa agcctgatgg ggtgtaagga atcaggggga tccctaggtc tgtgtttggg tttagtgaat aaagacaaga cccagaagga atctatgacc aacagcccta ggaaacaaga atctcaccat tctgtcctca atgtgtccca aaacagattt aatgtgtctc accaagaaac tgggtgtcct gggaaagctc tgaatcccca ggccccaaga gtggggacag aagagacaat ggcaattcat cggatctctg ggccaccaag ccctgtgggg tacctatgtc ctggacataa aggacaacct agtccctctg tcaacattac atagcctacc ttaaagctac tccattcatc ctgagaccat aatggcttcc agtctgccac ccagctctca tgcttcattt ctggacattc cctagatggg gtcactgtca cctggtctaa aggacagaca ggagatacct cacacatatc caaaaattt cccaatcaag aaagagggca agtttgctc tttatccaaa cttgtgcccc gctctcctgg aagcccctgt gctaagccag cgaggggcag tgagtgagca aagcctggcg ggggtgaagg aatcagggag ctccctaggt ctgtgtttgg gtttagagaa taaagacaag acccagaatg aatctaacca tctgtctcct agactggaat ggggtcccca gagccctgct cctgtcacag ctgcccttaa tcagttcccc atgtctgcagg catgcagtga tataaataag tctaacctag gtccttcctt tctcacagcc tctatcagga accctcagct ctacccttg aagccctgta aaggcactgc ttccatgacc ctgggctgcc tggtaaagga ctacttccct ggtcctgtga ctgtgacctg gtattcagac tcctgaaca tgagcactgt gaacttccct gcccttgggt ctgaactcaa ggtcaccacc agccaagtga ccagctgggg caagtcagcc aagaacttca catgccacgt gacacatcct ccatcattca acgaaagtag gactatccta ggtaagtagg gatgggctga cagttacact gtgtattctc ccttgagat ggaacagttt ctgtctaata aggaacttgt cacaatttcc tttcatagag gacttcataa gagatttttt tttctacttc tatcatgttt agtgctccaa atagattttt aaaactgggt gagtgcataa tacttttagc ctcagaagac atcatgtata tttaagaggc atttaactat tgtaaattat tctgatgact ttaaaaaaag ttaatgctga gttgtatatt tttaataaaa ttttattagt ttagtttaaa aaaagaaaag aaaattatta attttattta aaaatctcct atatttaaaa aaaaagaga aaaaagcaga gctgggctgg ctacagttac cacaagaaca tggtcagagg aggaaggac tcttatacat acctatgaca	

SEQ ID NO	Sequence	Figure
	ggagaacggg agaccaaca tactcggggg cctaccttca gagaacacaa ggccagggca atactcacag ctcatgtgtc gaccctgccc tagttcgacc tgtcaacatc actgagccca ccttggagct actccattca tcctgcgacc ccaatgcttt ccactccacc atccagctgt actgcttcat ttatggccac atcctaaatg atgtctctgt cagctggcag atct	
20	taatggacga tcgggagata actgatacac ttgcacaaac tggttctaate aaggaggaag gcaaactagc ctctacctgc agtaaactca acatcactga gcagcaatgg atgtctgaaa gcaccttcac ctgcaaggtc acctccaag gcgtagacta tttggcccac actcggagat gccaggtag gtctacactc gcctgatgtc cagacctcag agtcctgagg gaaaggcagg ctctcacaca gcccttcctc cccgacagat catgagccac ggggtgtgat tacctacctg atcccaccca gcccctgga cctgtatcaa aacggtgctc ccaagctt	N/A

N/A = not applicable

CITATION LIST**Patent Literature**

[193] Karasuyama *et al.*, US 6118044 – September 12, 2000 - *Transgenic non-human animal allergy models*

Non-Patent Literature

[194] Gerstein *et al.*, Isotype switching of an immunoglobulin heavy chain transgene occurs by DNA recombination between different chromosomes, *Cell* (1990) 63:537-548

[195] Liu *et al.*, A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Research* (2003) vol. 13 (3) pp. 476-84.

[196] Pan *et al.*, Characterization of Human $\gamma 4$ Switch Region Polymorphisms Suggests a Meiotic Recombinational Hot Spot Within the Ig Locus: Influence of S Region Length on IgG4 Production, *J. Immunol.* (1998) 161:3520-3526.

[197] Schmitz, J and Radbruch, A, *Immunoglobulin Class Switching* in Encyclopedia of Immunology, Delves and Roitt (eds.), pages 1302-1306.

[198] Szurek *et al.*, Complete nucleotide sequence of the murine gamma-3 switch region and analysis of switch recombination in two gamma-3 expressing hybridomas, *J. Immunol.* 135:620-626 (1985).

[199] Warming *et al.*, *Mol. Cell. Biol.* (2006) 26 (18):6913-22) for subsequent use in embryonic stem (ES) cell targeting, resulting in "pBlight-DTA-IgE".

[200] Waterston *et al.*, *Initial sequencing and comparative analysis of the mouse genome*, *Nature*. (2002) 420(6915):520-62.

[201] Zarrin *et al.*, Influence of switch region length on immunoglobulin class switch recombination, *Proc Natl Acad Sci* (2005) 102(7):2466-2470.

[202] Zarrin *et al.*, Antibody Class Switching Mediated by Yeast Endonuclease-Generated DNA Breaks, *Science* (2007) 315:377-381

[203] Zarrin *et al.*, Sgamma3 switch sequences function in place of endogenous Sgamma1 to mediate antibody class switching, (2008) *J. Exp. Med.* 205, 1567

CLAIMS

What is claimed is:

1. A targeting vector comprising:
 - a. a fragment of DNA homologous to the 5' end of the switch region to be altered (the 5' arm/acceptor) is selected from the group consisting of at least 1500 nucleotides, at least 1800 nucleotides, at least 2000 nucleotides, at least 2200 nucleotides and at least 2400 nucleotides corresponding to Nucleotides 25470628 to 25468161 of NCBI Accession number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J);
 - b. a selectable gene marker;
 - c. a desired/donor DNA sequence encoding a donor switch region; and
 - d. a second fragment of DNA homologous to the 3' end of the switch region to be altered (the 3' arm/acceptor) is selected from the group consisting of at least 1500 nucleotides, at least 1800 nucleotides, at least 2000 nucleotides, at least 2200 nucleotides, at least 2400 nucleotides and at least 2800 nucleotides corresponding to Nucleotides 25470628 to 25468161 of NCBI Accession Number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J).
2. The targeting vector of Claim 1 wherein the 5' arm comprises SEQ ID NO:4 or 5.
3. The targeting vector of Claim 1 wherein the 5' arm is homologous to a region 3' of the endogenous I ϵ and 5' of the endogenous S ϵ .
4. The targeting vector of Claim 1 wherein the 3' arm comprises SEQ ID NO:7 or 8.
5. The targeting vector of Claim 1 wherein the selectable gene marker is selected from the group consisting of Neomycin and thymidine kinase.
6. The targeting vector of Claim 1 wherein the selectable gene marker is Neomycin.
7. The targeting vector of Claim 1 wherein the selectable gene marker is flanked by loxp sites.
8. The targeting vector of Claim 1 wherein the desired switch region is from a mouse.
9. The targeting vector of Claim 1 wherein the desired switch region is selected from S μ , S γ 1, S γ 2a, S γ 2b and S γ 3.
10. The targeting vector of Claim 1 wherein the desired switch region is the HindIII/NheI fragment containing most of mouse Sm region.

11. The targeting vector of Claim 1 wherein the desired switch region comprises Nucleotides corresponding to 25617172 to 25615761 of NCBI Accession Number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J).
12. A method for producing an altered embryonic stem cell *in vitro*, comprising the steps of:
 - a. Altering the genomic DNA in said cell to enhance the probability of CSR to express the C ϵ selected from
 - i. increasing the S ϵ length by adding at least one additional S ϵ copy in tandem with the endogenous S ϵ region;
 - ii. S ϵ region substitution; and
 - b. Selecting the cell for correctly altered genomic DNA.
13. The method according to Claim 12 wherein the alteration is a substitution of a switch region selected from S μ , S γ 1, S γ 2a, S γ 2b and S γ 3 for the S ϵ region.
14. The method according to Claim 12 wherein the alteration is a substitution of a S μ region for the S ϵ region.
15. A method for producing an altered embryonic stem cell *in vitro*, comprising the steps of:
 - a. Using the vector according to Claim 1 to exchange the S μ for the S ϵ region
 - b. Selecting the cell for correctly altered genomic DNA.
16. The method according to Claim 15 wherein the alteration is a substitution of a switch region selected from S μ , S γ 1, S γ 2a, S γ 2b and S γ 3 for the S ϵ region.
17. The method according to Claim 15 wherein the alteration is a substitution of a S μ region for the S ϵ region.
18. The method of claim 15, wherein the ESC are from a mouse strain selected from BALB/c or C57BL/6.
19. A non-human animal wherein
 - a. At least one allele of the IgH locus has been altered to enhance the rate of IgE expression/production/secretion/ relative to a non-altered allele; and
 - b. Has an IgE profile selected from the group consisting of
 - i. The IgE fraction of all serum antibodies is greater than 0.04%;
 - ii. The IgE serum concentration is above 4,000 ng/ml
 - iii. The IgG/IgE ratio is less than 10.
20. A non-human mammal having a genome which has been altered to express an IgE molecule at a level greater than 4000 ng/ml.
21. A non-human mammal having an IgG/IgE ratio that is between 0.1 and 10.

22. A non-human mammal having an unchallenged (i.e., resting) IgE serum concentration of between 100 ng/mL and 10000 ng/mL.
23. A non-human mammal having a challenged (i.e., activated) IgE serum concentration of between 1000 ng/mL and 1000000 ng/mL.
24. The animal model of claim 19, wherein the animal model is a nonhuman vertebrate.
25. The animal model of claim 19, wherein the animal model is a mouse, rat, guinea pig, rabbit, or primate.
26. The non-human animal/mammal model of Claim 19, wherein the genome of said non-animal has had the S ϵ region of the IgH locus altered to express/produce more IgE.
27. The non-human animal/mammal model of claim 19, wherein the alteration is by gene targeting.
28. A method of testing an allergy therapy using the animal model of Claim 19 comprising exposing said animal to an allergen prior to, simultaneous with or after the administration of said method of treatment for allergic disorders and evaluating the IgE response.
29. The method of claim 28 wherein the IgE response is less than without the allergy therapy.
30. The method of Claim 28, wherein the test animal and the control animal are littermates.
31. Use of a compound identified by the method of claim 28 as a medicament for the treatment of an allergy.
32. A cell line obtainable from the animal model of Claim 19.
33. A cell isolated from an animal model of Claim 19.
34. A process for making a non-human animal model, said process comprising:
 - a. microinjecting linearized fragments of plasmids encoding SEQ ID NO:6 (S μ) into a fertilized egg of a mouse such that the fragment is incorporated in the genomic DNA upstream from and operably linked to the C ϵ -encoding region,
 - b. transferring said fertilized egg to the oviduct of a female mouse which has previously been treated to induce pseudopregnancy, and
 - c. allowing said egg to develop in the uterus of the female mouse.
35. A recombinant mouse comprising in its germline a modified genome wherein said modification comprises at least one allele of the IgH locus altered to enhance the rate of IgE production.

36. The recombinant mouse of Claim 35 wherein the alteration comprises a replacing the S ϵ with the S μ region or a functional portion thereof.
37. The recombinant mouse of Claim 35 wherein the S μ functional portion is between at least 1kb and 10kb in length.

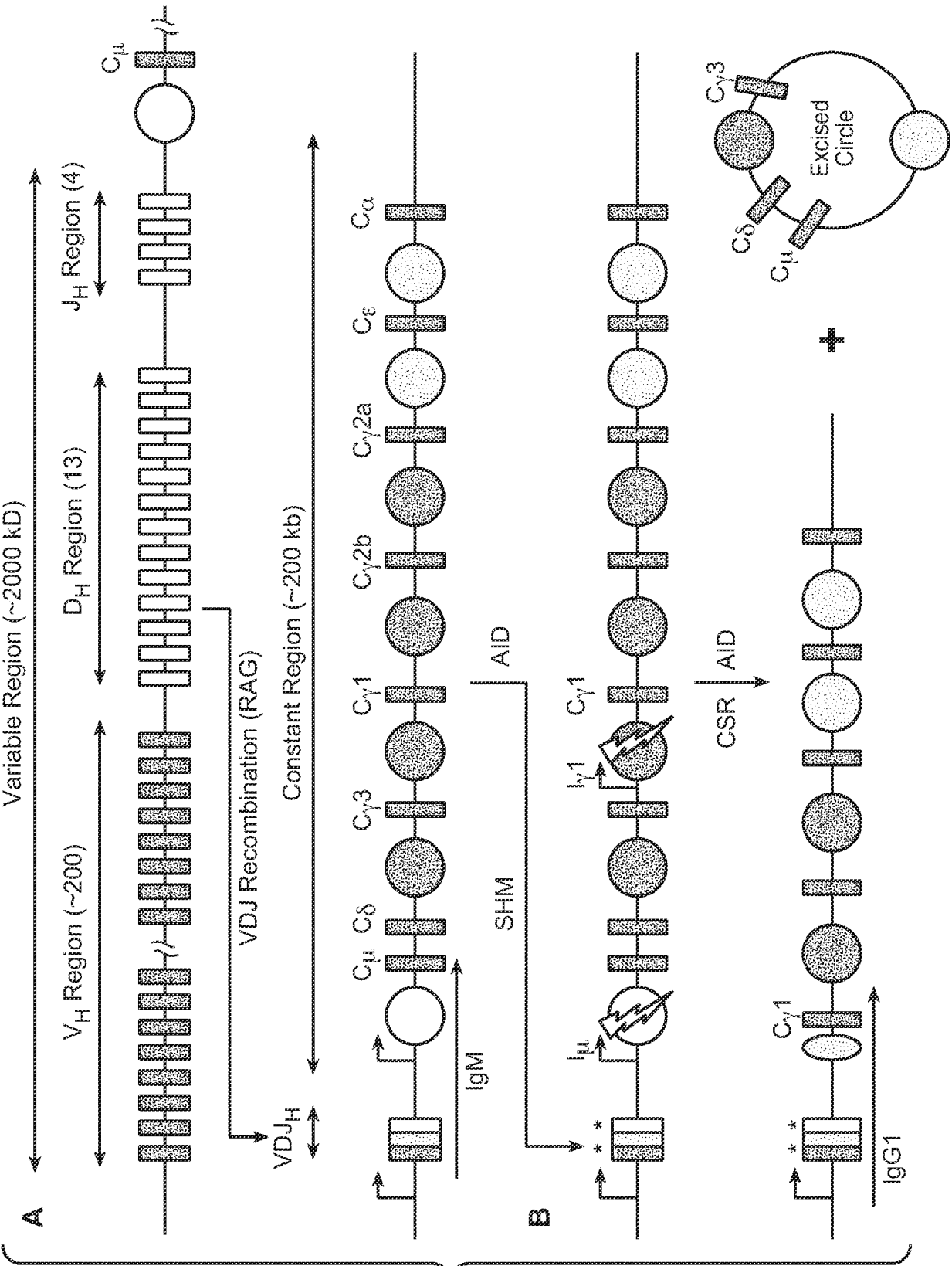
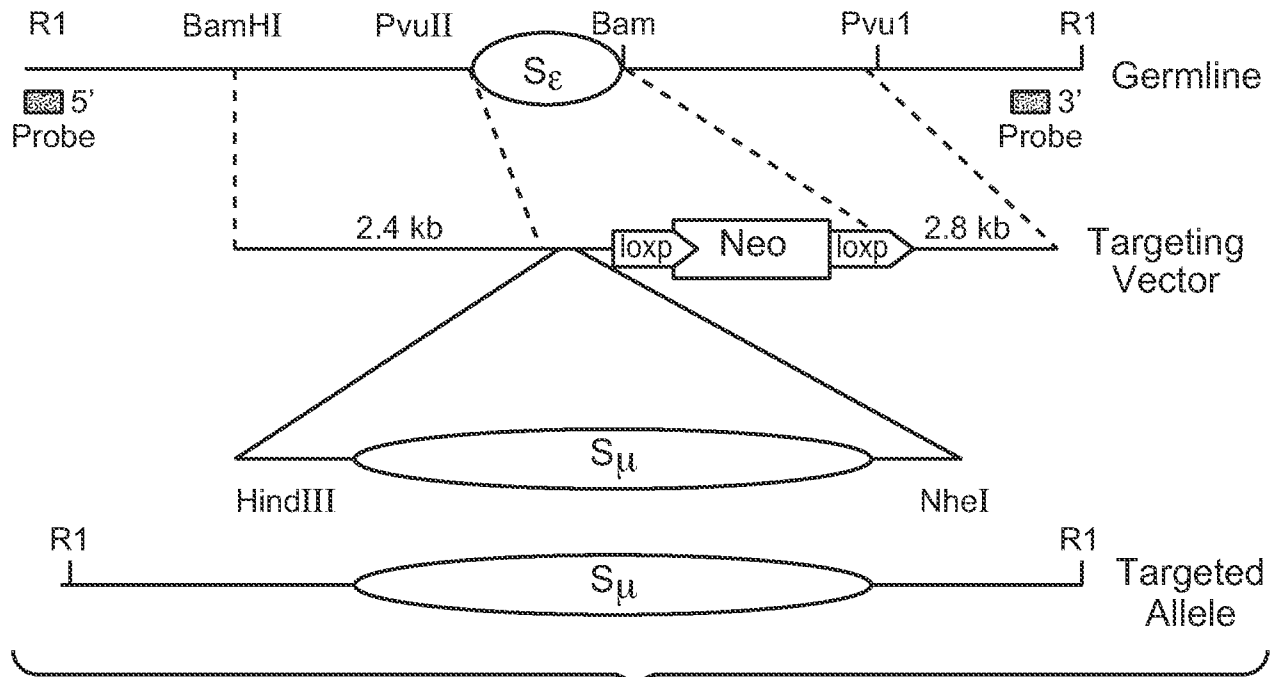
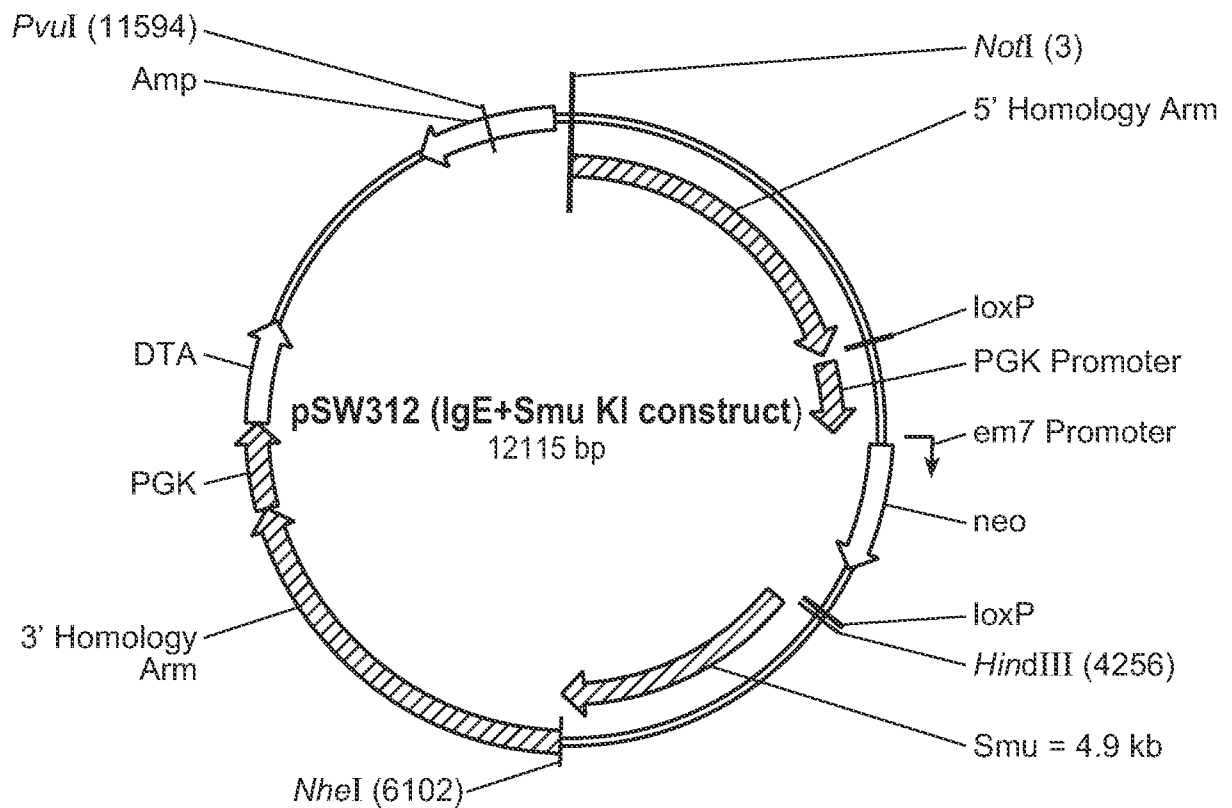
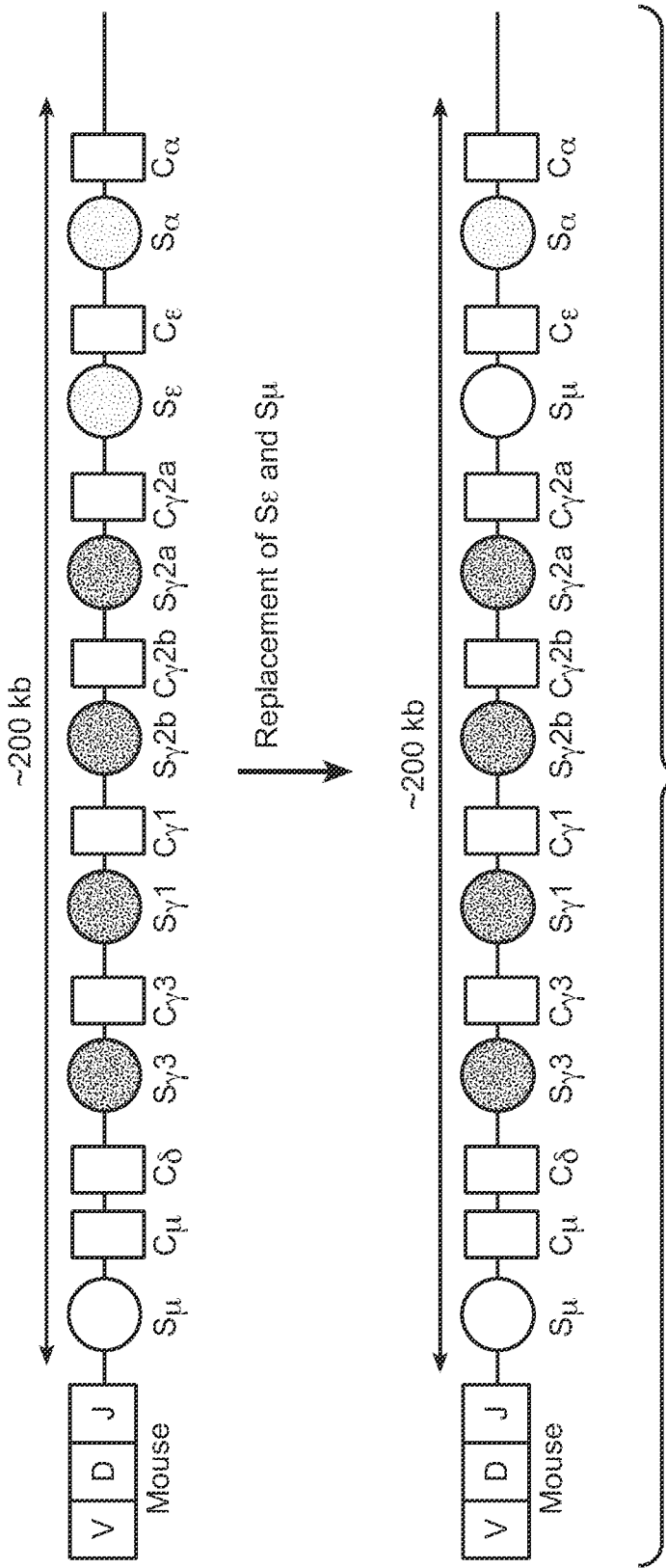
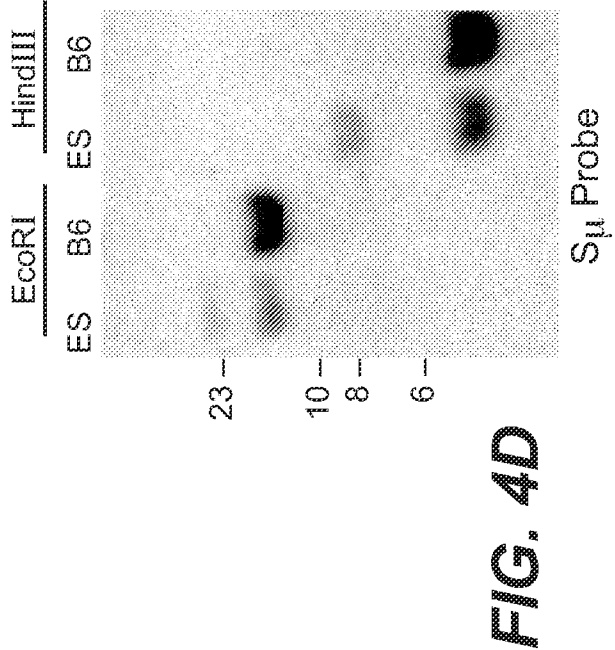
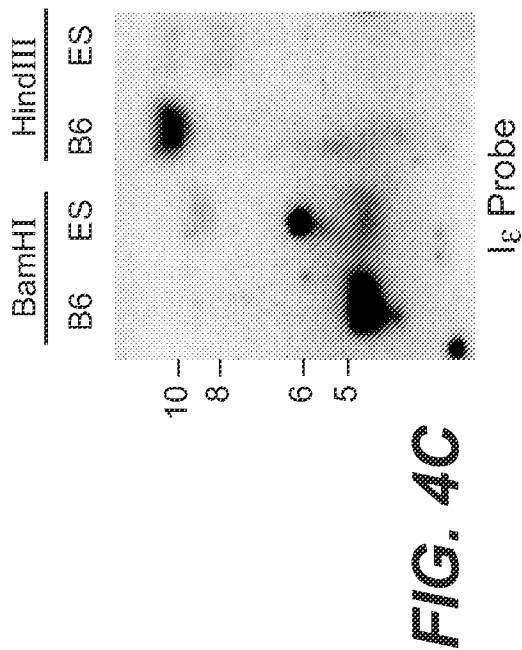
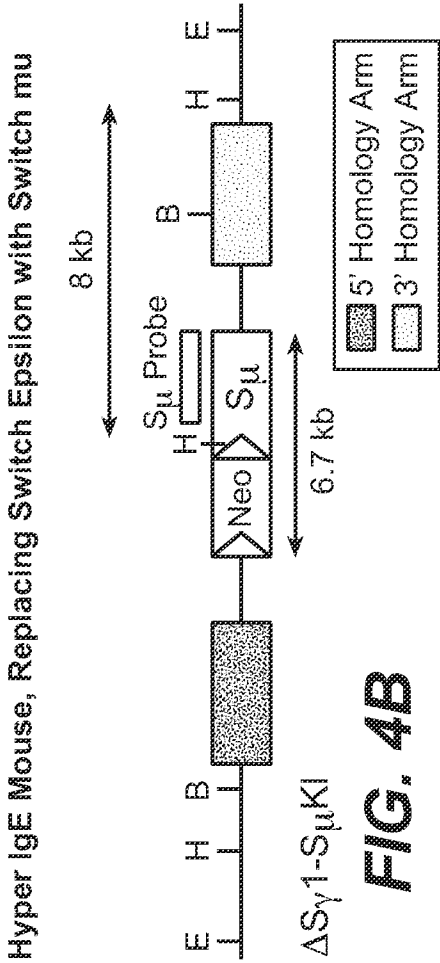
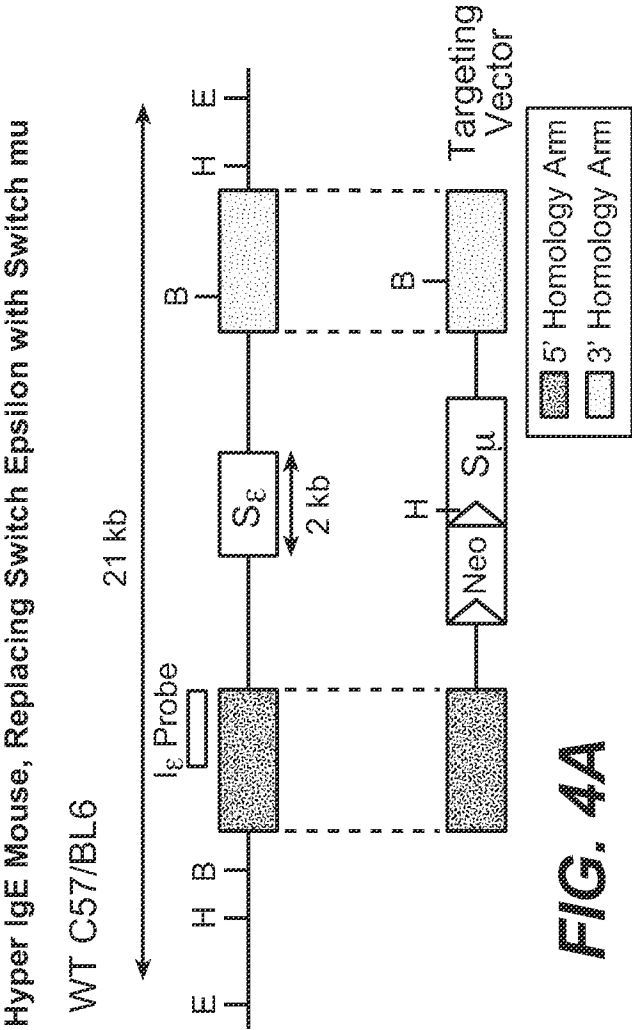


FIG. 1

2 / 20

**FIG. 2A****FIG. 2B**





5 / 20

FIG. 5A A)
gggctgggctg (SEQ ID NO:1)

FIG. 5B B)
gagctgagct (SEQ ID NO:2)

A) Deletion fragment (2055 bp) (SEQ ID NO:3):

tgggttaagc	agagctgtgc	tgggctggta	tgagctggtc	caagttgggc	50
taaacagagc	tgggccaggc	tagtatgagc	tggtctgaac	tacactaagc	100
aggactaggc	tgggctgagc	tgagctggac	tggctggact	tggtctgagat	150
gtgttgagct	gggttaagta	tggctgggct	gggctggcct	gggctgggct	200
ggactggatt	ggtatgagct	ggtccaagtt	gggctaagca	gagctgggcc	250
aggctggtat	gagctgggtct	aaactgaact	aagtagggct	gggctaagct	300
gagctgggtct	acactagcct	gacctgagct	agggtaggct	ggactgggct	350
gagctaagtt	gcactgggca	ggggtgggct	ggaccgagct	gatttgagct	400
gggatgggct	gagatgggtt	cagcaggcct	aagcaggcct	agctggggtt	450
agctagatth	agctaggcaa	ggctgagcta	ggctgggcgg	ggcggggcta	500
ggctgggcag	ggctggactg	agctagctth	tgtatatctg	ggtgaaatgg	550
ggttggtctg	tctggactga	actgactgag	ctgggctagc	ctgagctcga	600
tgggggggtat	actcagctga	gatgggctgg	tctggctaga	ctgaactgga	650
ttgggctagg	ctgagctagg	ctgacctgaa	ctggcctggg	ctgggctgga	700
ctgggcaggg	ctgggtctcag	ctagactaca	ctgagttaac	ctgggctgga	750
ccatactggg	ttaaactagg	ttgcactggc	tgggttagac	ttggctgagc	800
tgggcttggc	tgagctgagt	caagatggtc	tgagttgatt	tgagttggct	850
aagctaagct	gagctacact	gaactaggca	aggctgggct	ggaaaggctct	900
gggttaagtt	aggagggact	tggcttggtc	tagctgggcc	aagctaggct	950
gaactgggct	gaactgagct	gagctgggct	gagctgggct	gagctgggct	1000
gagctgggca	aggctaaact	ggaatggact	gaattggcct	aagatgggcc	1050
cggctaagct	aagtaaggct	gccctgaact	gagcaggact	ggcctggcct	1100
ggattgacct	ggcatgagct	taacttgact	agactagtct	atcttggttg	1150
aactgggcta	agcaggacta	atctggcctg	atctgagcta	gactgaacta	1200
ggctaagctg	agctgagttt	agcttggttg	aactgggctg	ggctgcactg	1250
aactgtattg	agctatgtag	aactgagctg	gtcttgcttg	aggtgggttg	1300
ggctggtctg	ggctgaacca	gattgcacta	gactgagctt	agctggacct	1350
ggctgagctg	gactgcattg	tgcataaactg	gctctcttta	gaccgagctt	1400
agctggactg	gactgagcta	ggttggttg	gctgatctaa	gctgagctag	1450
gctggtctca	cctgaggaat	gctgtgctgt	gctgagctga	actaaactga	1500
gctcagctaa	ggaagtgtga	gctagactga	gctgagctag	gctgggttg	1550
gctgaactga	gctaccttg	gtggactagg	ctgagctgag	ctgggttgag	1600
ctgagctata	gatttggttg	gactggactg	gattgggcta	aactgaactg	1650
gtttggggta	ggctgggatg	agctggactg	agctaggctg	tactggtctg	1700
agctaaacta	agttgagtgg	ggctaagagg	agctgagtga	ggctgggctg	1750
gaatgagcta	ggctagggtt	gtgagctagg	gttgtagctg	tctaagctga	1800
gtttagctga	gagaggctgg	gctagacttc	cataagggtg	ctgagtcata	1850
ctacagtga	ctgagctgtg	ttgagcttaa	cttggaattaa	gtggaatggg	1900
ttgagctggc	tgaactgggc	tgaactgaga	taaactagac	tgagctggga	1950
cacgctggga	cgagctggaa	cgagctagaa	ttactgttct	aatctgatct	2000
gggctgaggt	aaactgggcc	tgggttagact	ctactaggct	aagtagagtt	2050
gagct					2055

FIG. 6

6 / 20

A) 5'arm (for 129 mice) 2471 bp (SEQ ID NO:4)

```

1  GATCCCTGTG AAGCCCTGGG CCATGGGAAG AGATAGAAGG AAGGCTAGGT GGGGCAAGAC 60
61 GAGGGAAC TA AAGCCACTGT GCTGCTGGGG AACTGTGGA CACTgatgga cagaaagga 120
121 gtgatcagtc tgtggacagg agggggaggg gCAAGGATGA TGCTGACAGA GAGTCACAGT 180
181 GGAGTCCGTA GCAGGAAAGA GAGAGAGCGC CCAGTGTAGT CCTAAGGCTT AGGAAGTTGC 240
241 AACTGCCTCC TCTCCTTCCA GAGGATCACT CACTGCCACC TAGCATAGAA CTCAGAGGAC 300
301 CCAGAACCAG CAGCTCAGCC CAACCTGTGT GTCACAGAAG AATCAGGCCC GGTGAGGCTA 360
361 GACACAAAGG CTCTTGGCCC TCATGCTGTG AGGGAGGTAC AACTGGGAGG CACACCACAA 420
421 ACAGTTGGAG CAGAGGCTTC TCGCCCCCTAT TTTTCCCTCT GAACAATAGT TGCTTCCAGG 480
481 GAACTCTGCA TTTACCCCTC AGGCTCCCAC CCATGTCTAT TAGGCTGAAG GCCAAGCCTG 540
541 TCACCTCAGA CAGACAGTGT ATCTGAAAGA CAGAAGGCCG TGCAAGACCA CAATTCCCTT 600
601 GAATCTCACA CTCTGTCTTC CCAAAGTTCC TAACTGCATC TGACCTTTCT GGGCCAGCCT 660
661 CTCAGCCTGC CTGGCTCTGC CACTATCAGG AAGATCTCTA ATATCTTCCA AATGCAATTA 720
721 AACACGCTCC TGTGAAAGTC AGACTTGGCA TAGCCTAAGT CCCTTCGGTC CCTTTCCTG 780
781 GGACCAACGA CCCTGAGCAG CCAGGGTCCA AGGGATGGGG CTCTCATTTT CTTCCCCAAA 840
841 TCTCTGTGTG CCTCTCTCAA GACTCAAGAC TCACAAGCAA AATTAGTGGC TCCTATAGTT 900
901 TGTATGTATG TTTTCTTAGA ACTCCTAGGA ACCATGGGCC TACAGAGACA TCAGAGTGTA 960
961 GAGGGAATCC CTGAACCCAG AAGATGACCT TGCTCTACAA AGCTGCAGCT GAGACAGACA 1020
1021 CTACTAGTAC CCCATGAAAG CTGCTGAGCC AAAGCCCAGC CCTCACACCA TCTTTACCTT 1080
1081 CATCCCTCCC CTCAGTGCAG ACATAGACCA CAGGCCTGGA AGAGACGTTA GCTGTTTCTA 1140
1141 CACAGCTCCG TGAAACCCAG TCACAACCCA GATGTGCTCT GTCCCTCTGG ACTCCTTGCC 1200
1201 AGAGTAGCAG GTAGAGGACC TCAAGCTGAA AGATAATCAC TTGTGAGTGG GCACCAGGGA 1260
1261 AGGCCACTGT CCCTCGCATG CCAGCTCCAA AGCTGATACA GGAAGTAGGG TGCCCTATATC 1320
1321 AGAGGCCCTG CAATGTCATA TCTGGCCAC AGGCTGTTCC TCTTTGTGCA CCATTAATAA 1380
1381 CTTACAAAGT GACAGCCACA CTCCCCTGAA GGGCTGCCAA AGGAACAGAA AAAGCAATGG 1440
1441 CGAGGGTCTA GTCCTGCCTC AGGGCAGTGA CACTCCAAAG GGGCAGGCAT GGTGACTGCA 1500
1501 CGCASNNCAC ACATGCAAGG CTTTAATACG AGAGCTATGC AAGGAGACCT GGGATCAGAC 1560
1561 GATGGAGAAT AGAGAGCCTT GACCAGAGTG TGCAGGTGTG TCTCCTAGAA AGAGGCCCTCA 1620
1621 CCTGAGACCC CACTGTGCCT TAGTCAACTT CCCAAGAACA GAATCAAAAG GGAAGTTCCA 1680
1681 AGGCTGCTAA GGCCGGGGGT TCCCACCCCA CTTTGTAGCTG AGGGCACTGA GGCAGAGCGG 1740
1741 CCCCTAGGTA CTACCATCTG GGCATGAATT AATGGTTACT AGAGATTAC AACGCCTGGG 1800
1801 AGCCTGCACA GGGGGCAGAA GATGGCTTCG AATAAGAACA GTCTGGCCAG CCACTCACTT 1860
1861 ATCAGAGGAC CTCAGGTATT ACAACCCATG GGACCTGAG CAAAAGGGTT TGCCTAAGGA 1920
1921 GAAGGGACAA ACAGGTTACA GGGTCCTGGG TGGGGAAGGG GACACCTGGG CTGCCTTCTA 1980
1981 ATGTGGACAG TCTCTTGACC ACCGAATGTC CTTCAGCTAT CACTTCCCTG CACTAAGGCA 2040
2041 CACAGGTATT AGAAACTGCT ATAGCTATTC ATGAAGACGG GGGACTGTGG ATCTCAACCA 2100
2101 GAGAGGGCTG AACCAAGATA AACTGAATAT GTTGTGAGAA ACTCAAAAAC TGCAGGAGAG 2160
2161 GCTGGAGAGG AATCGGCCAG CAAGCCATCA GACAAGAATG CAATGACAAA TGTCAGATCC 2220
2221 AGCAAATGAC AGCAAGGAAT TGCCCTGTGA TGAAC TAACA ACCAAGAGGA CTGTCCACAG 2280
2281 CTGGGCTGAC CCAGGCAGCA CTGGGCTAAA TTGGGTGGGA TCTGTGCTGC CCTGGGCTGG 2340
2341 TATGAGCCAG GATGAGCCAA GTGAAGTGGG CTGGACTAGT TTGGGCTGGA CTGGCCTGGA 2400
2401 GTAGGCTAAA CCAGTTTAAA CTAGACTAGG CTGGGCTGAG GTGAATCAGA CTAGGCTAGA 2460
2461 CTAGTCTGAG C 2471

```

FIG. 7A

7 / 20

**B) 5' arm (for C57Bl/6J strain) 2467 bp (SEQ ID NO:5)
corresponding to Nucleotides 25470628 to 25468161 of NCBI NT_166318:**

GATCCCTGTG	AAGCCCTGGG	CCATGGGAAG	AGATAGAAGG	AAGGCTAGGT	GGGGCAAGAC	25470569
GAGGGAAC TA	AAGCCACTGT	GCTGCTGGGG	ACACTGTGGA	CACTGATGGA	CAGAAAGGGA	25470509
GTGATCAGTC	TGTGGACAGG	AGGGGGAGGG	GCAAGGATGA	TGCTGACAGA	GAGTCACAGT	25470449
GGAGTCCGTA	GCAGGAAAGA	GAGAGAGCGC	CCAGTGTAGT	CCTAAGGCTT	AGGAAGTTGC	25470389
AACTGCCTCC	TCTCCTTCCA	GAGGATCACT	CACTGCCATC	TAGCATAGAA	CTCAGATGAC	25470329
CCAGAACCAG	CAGCTCAGCC	CAACCTGTGT	GTCACAGAAG	AATCAGGCCC	AGTCAGGCTA	25470269
GACACAAAGG	CTCTTGCCCC	TCATGCTGTG	AGGGAGGTAC	ACACTGGGGG	CACACCACAA	25470209
ACAGTTGGAG	CAGAGGCTTC	TCACCCCTAT	TTTTCCCTCT	GAACAATAGT	TGCTTCCAGG	25470149
GAACTCTGCA	TTTACCCCTC	AGGCTCCCAC	CCATGTCTGT	TAGGCTGAAG	GCCAAGCCTG	25470089
TCACCTCAGA	CAGACAGTGG	ATCTGAAAGA	CAGAAGGCCG	TGCAAGACCA	CAATTCCCTT	25470029
GAATCTCACA	CTCTGTCTTC	CCAAAGTTCC	TAAGTGCATC	TGACCTTTCT	GGGCCAGCCT	25469969
CTCAGCCTGC	CTGGCTCTGC	CACTATCAGG	AAGATCTCTA	ATATCTTCCA	AATGCAATTA	25469909
AACACGCTCC	TGTGAAAGTC	AGACTTGGCA	AAGCCTAAGT	CCCTTCGGTC	CCTTTCAGTG	25469849
GGACCAACGA	CTCTGAGCAG	CCAGGGTCCA	AGGGATGGGG	CTCTCATTTT	CTTCCCCAAA	25469789
TCTCTGTGTG	CCTCTCTCAA	GACTCAAGAC	TCACAAGCAA	AATTAGTGGC	TCCTATAGCT	25469729
TGTATGTATG	TTTTCTTGGA	ACTCCTAGGA	ACCATGGGCC	TACAGAGACA	TCAGAGTGTA	25469669
GAGGGAATCC	CTGAACCCAG	AAGATGACCT	TGCTCTACAA	AGCTGCAGCT	GAGACAGACA	25469609
CTACTAGTAC	CCCATGAAAG	CTGCTGAGCC	AAAGCCCAGC	CCTCACACCA	TCTTTACCTT	25469549
CATCCCTCCC	CTCAGTGCAG	ACATAGACCA	CAGGCCTGGA	AGAGACGTTA	GCTGTTTCTA	25469489
CACAGCTCCG	TGAAACCCAG	TCACAACCCA	GATGCGCTCT	GTCCTTCTGG	ACTCCTTGCC	25469429
AGAGTAGCAG	G TAGAGGACC	TCAAGCTGAA	AGATAATCAC	TTGTGAGTGG	GCACCAGGGA	25469369
AGGCCACTGT	CCCTCGCATG	CCAGCTCCAA	AGCTGATACA	GGAAGTAGGG	TGCCTCTATC	25469309
AGAGGCCCTG	CAATGTCATA	TCTGGCCCAC	AGGCTGTTCC	TCTTTGTGCA	CCATTAATAA	25469249
CTTACAAAGT	GACAGCCACA	CTCCCCTGAA	GGGCTGCCAA	AGGAACAGAA	AAAGCAATGG	25469189
CTAGGGTCTA	GTCCTGCTTC	AGGGCAGTGA	CACTCCAAAG	GGGCAGGCAT	GGTGACTGCA	25469129
CACA---CGC	ACATGCAAGG	CTTTAATACG	AGAGCTATGC	AAGGAGACCT	GGGATCAGAC	25469072
GATGGAGAAT	AGAGAGCCTT	GACCAGAGTG	TGCAGGTGTG	TCTCCTAGAA	AGAGGCCTCA	25469012
CCTGAGACCC	CACTGTGCCT	TAGTCAACTT	CCCAAGAACA	GAATCAAAAAG	GGAAC TTCCA	25468952
AGGCTGCTAA	GGCCGGGGGT	TCCCACCCCA	CTTTTAGCTG	AGGGCACTGA	GGCAGAGCGG	25468892
CCCCTAGGTA	CTACCATCTG	GGCATGAATT	AATGGTTACT	AGAGATTAC	AACGCCTGGG	25468832
AGCCTGCACA	GGGGGCAGAA	GATGGCTTCG	AATAAGAACA	GTCTGGCCAG	CCACTCACTT	25468772
ATCAGAGGAC	CTCAGGTATT	ACAACCCATG	GGACCCTGAG	CAAAAGGGTT	TGCCTAAGGA	25468712
GAAGGGACAA	ACAGGTTACA	GGGTCCTGGG	TGGGGAAGGG	GACACCTGGG	CTGCCTTCTA	25468652
ATGTGGACAG	TCTCTTGGTC	ACCGAATGTC	CTTCAGCTAT	CACTTCCCTG	CACTAAGGCA	25468592
CACAGGTATT	AGAAACTGCT	ATAGCTATCC	ATGAAGACAG	GGGACTGTGG	ATCTCAACCA	25468532
GAGAGGGCTG	AACCAAGATA	AACTGAATAT	GTTGTGAGAA	ACTCAAAAAC	TGCAGGAGAG	25468472
GCTGGAGAGG	AATCGGCCAG	CAAGCCATCA	GACAAAAATG	CAATGACAAA	TGTCAGATCC	25468412
AGCAAATGAC	AGCAAGGAAT	TGCCCTGTGA	TGAACTAACA	ACCAAGAGGA	CTGTCCACAG	25468352
CTGGGCTGAC	CCAGGCAGCA	CTGGGCTAAA	TTGGGTGGGA	TCTGTGCTGC	CCTGGGCTGG	25468292
TATGAGCCAG	GATGAGCCAA	GTGAAGTGGG	CTGGACTAGT	TTGGGCTGGA	CTGGCCTGGA	25468232
GTAGGCTAAA	CCAGTTTAAA	CTAGAGTAGG	CTGGGCTGAG	GTGAATCAGA	CTAGGCTAGA	25468172
CTAGTCTGAG	C					25468161

FIG. 7B

8 / 20

A) Nucleotides 25617172 to 25615761 of NCBI Accession Number NT_166318 (Mus musculus chromosome 12 genomic contig, strain C57BL/6J)(in caps) (1141 bp) (SEQ ID NO:6):

```

25615741 agctcacccc agctcagctc AGCTCACCCC AGCTCAGCCC AGCTCAGCCC AGCTCAGCCC
25615801 AGCTCAGCCC AGCTCAGCCC AGCTCAGCTC AGCTCAGCCC AGCTCAGCCC AGCTCACCCC
25615861 AGCTCAGCTC AGCTCACCCC AGCTCAGCCC TGCTCAGCCC AGCTCAGCTC ACCCCAGCTC
25615921 AGCTCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCAGCCC
25615981 AGCTCAGCTC AGCTCACCCC AGCTCAGCTC ACCCCAGCTC AGCCCAGCTC AGCCCAGCTC
25616041 AGCCCAGCTC ACCCCAGCTC AGCCCAGCTC AGCTCAGCTC ACCCCAGCTC AGCTCAGCTC
25616101 AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC
25616161 AGCTCAGCTC AGCCCAGCTC ACCCCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCACCCC
25616221 AGCTCAGCTC AGCTCAGCTC AGCTCACCCC AGCTCAGCCC AGCTCACCCC AGCTCAGCCC
25616281 AGCTCAGCTC ACTCCAGCTC AGCTCAGCTC ACCCCAGCTC ACCCCAGCTC AGCTCAGCTC
25616341 AGCTCACCCC AGCTCAGCTC AGCTCAGCTC AGCTCAGCTC ACCCCAGCTC AGCTCAGCTC
25616401 ACCCCAGCTC AGCTCACCCC AGCTCAGCTC AGCTCACCCC AGCTCAGCTC ACCCCAGCTC
25616461 ACCCCAGCTC AGCTCAGCTC ACCCCAGCTC AGCTCAGCTC ACCCCAGCTC AGCTCACCCC
25616521 AGCTCAGCCC AGCTCAGCCC AGCTCAGCCC AGCTCACCCC AGCTCAGCCC AGCTCAGCTC
25616581 ACTCCAGCTC AGCTCAGCTC ACCCCAGCTC ATCCCAGCTT ACCCCAGCTC AGCTCAGCTC
25616641 ACCCCAGCTC AGCCCAGCTC ACCCTAGCTC AGCCAAGCTC AGCTCAGCTC ACCCCAGCTT
25616701 AGCTCAGCTC ACCCAGCTCA GCTCACCCCTA GCTCAGCTCA GTCTAGCTCA GCCCAGCTCA
25616761 GCTCACCCCA TCTCACCCCA TCTCAGCTAC TCCAGAGTAT CTCATTTTCAG ATCAGCTCAC
25616821 CCCAACACAG CGTAGCATAG CTGAGCTCAC CCCAGCTCAT CTCAGCTCAG AACAGTCCAG
25616881 TGTAGGCAGT AGAGTTTAGC TCTATTCAAC CTAGATTAAT GAAGTTCATT CCAGTTTGGC
25616941 TCATCTCGGT TAAGCCAGCC TAGTTTAGCT TAGCGGCCCA GCTCATTCCA GTTCATTACA
25617001 GTCTACTTCA TTTTGGCTCA AGCCCAGCTT TGCTTACCTC AGAGTAATCA CCTCAGTTTA
25617061 GCGCATTTTA GAAGCACTCA GAGAAGCCCA CCCATCTCAG CTCAGCTGTG CTTTTTAGAG
25617121 CCTCGCTTAC TAGGGCTCTC AACCTTGTTT CCTTAATTTT GCTCAGCAAG CTttatgagt

```

FIG. 8

9 / 20

A) 3' arm (129 mouse sequence) (SEQ ID NO:7)

TATGAGATGA	AAGTAAGCTG	AGTTGGGCAG	TTCTAGACTA	TTCTAGGCTG	TTCTGGGCTG	60
ACCTGAAGCTG	GGTGGGGTTG	AGCTGAATGA	AGTAGGCTGT	GCTGAGCTCT	GCCAGGCTGG	120
ATGAGTTTAT	TGATCTGAGT	TGGACTGGCC	TGGGCTGCAC	TAAATGGGAC	TGAGATGAGA	180
TTGGCCAGGC	TAGGAGGGAT	TGAGCAAGGC	TAAGCTAAGT	TGTTCTGGAC	TAGGCCAAAC	240
TATTAGAGGT	CTTTTGGTTT	AGTTGAACTT	TCTGGTACTG	AACTAAACTG	TCTTTAAGAT	300
AGAATGCTCA	AAATTATTTG	TGGGTGTTTT	AACTGTCCTC	AAAGAAGATT	GTCTGTGTGT	360
AGGATACAAC	AACAGCTACT	AGCCAGACTG	GGTAGGTAGA	GCCAATCGGG	CCTAGCAGGA	420
ATATCCTGTG	CTTTCTGAGG	ACCTGGCACA	GAGCTGAGCT	GAGCCCCTCT	CTCAGGAGAA	480
TGTCCTGGGC	ATGTGGACAC	TCTAGAGCAT	CAAGGTGGCT	TCTGAAGTGG	TTGTATTCTC	540
TATGTGCTTT	CTGGGATCCA	CGGAGGTCAT	CTTGGAGGCA	GAACACTGTG	CAGGTTAGCC	600
TATGGTAAAG	CAGAGAGCCT	CATGTATCTG	AAACCCAAAG	ATCCGATTAA	TTGCCATTGT	660
AAGTTTGCCCT	CTTCATCCAA	ACTCGTGCCC	AGCTCTCCTG	GAAGCCCCTG	TGCTAAGCCA	720
GCTAGGGGCA	GTGAGTGAGC	AAAGCCTGAT	GGGGTGTAAG	GAATCAGGGG	GATCCCTAGG	780
TCTGTGTTTG	GGTTTAGTGA	ATAAAGACAA	GACCCAGAAG	GAATCTATGA	CCAACAGCCC	840
TAGGAAACAA	GAATCTCACC	ATTCTGTCCCT	CAATGTGTCC	CAAAACAGAT	TTAATGTGTC	900
TCACCAAGAA	ACTGGTGGTC	CTGGGAAAGC	TCTGAATCCC	CAGGCCCCAA	GAGTGGGGAC	960
AGAAGAGACA	ATGGCAATTC	ATCGGATCTC	TGGGCCACCA	AGCCCTGTGG	GGTACCTATG	1020
TCCTGGACAT	AAAGGACAAC	CTAGTCCCTC	TGTCAACATT	ACATAGCCTA	CCTTAAAGCT	1080
ACTCCATTCA	TCCTGAGACC	ATAATGGCTT	CCAGTCTGCC	ACCCAGCTCT	CATGCTTCAT	1140
TTCTGGACAT	TCCCTAGATG	GCGTCACTGT	CACCTGGTCT	AAAGGACAGA	CAGGAGATAC	1200
CTCACACATA	TCCACAAAAT	TTCCCAATCA	AGAAAGAGGG	CAAGTTTGCC	TCTTTATCCA	1260
AACTTGTGCC	CAGCTCTCCT	GGAAGCCCCCT	GTGCTAAGCC	AGCGAGGGGC	AGTGAGTGAG	1320
CAAAGCCTGG	CGGGGTGTAA	GGAATCAGGG	AGCTCCCTAG	GTCTGTGTTT	GGGTTTAGAG	1380
AATAAAGACA	AGACCCAGAA	GGAATCTAAC	CATCTGTCTC	CTAGACTGGA	ATGGGGTCCC	1440
CAGAGCCCTG	CTCCTGTAC	AGCTGCCCTT	AATCAGTTCC	CCATGCTGCA	GSNNGCATGC	1500
AGTGATATAA	ATAAGTCTAA	CCTAGGTCCT	TCCTTTCTCA	CAGCCTCTAT	CAGGAACCCCT	1560
CAGCTCTACC	CCTTGAAGCC	CTGTAAAGGC	ACTGCTTCCA	TGACCCTGGG	CTGCCTGGTA	1620
AAGGACTACT	TCCCTGGTCC	TGTGACTGTG	ACCTGGTATT	CAGACTCCCT	GAACATGAGC	1680
ACTGTGAACT	TCCCTGCCCT	CGGTTCTGAA	CTCAAGGTCA	CCACCAGCCA	AGTGACCAGC	1740
TGGGGCAAGT	CAGCCAAGAA	CTTCACATGC	CACGTGACAC	ATCCTCCATC	ATTCAACGAA	1800
AGTAGGACTA	TCCTAGGTAA	GTAGGGATGG	GCTGacagtt	acactgtgta	ttctcccttg	1860
gagatggaac	agtttctgtc	taatcaggaa	cttgtcacaa	tttcctttca	tagaggactt	1920
cataagagat	ttttttt-ct	acttctatca	tgtttagtga	tccaaataga	ttttaaaaaac	1979
tggttgagtg	catattactt	ttagcctcag	aagacatcat	gtatatttaa	gaggcattta	2039
actattgtaa	attattctga	tgacttttaa	aaatgttcoat	gctgagttgt	atattttttaa	2099
ataaattttta	ttagttagt	ttaaaaaaag	aaaagaaaat	tattaatttt	attaaaaaat	2159
ctcctatatt	taaaaaaaaa	agagaaaaaG	GCAGAGCTGG	GCTGGCTACA	GTTACCACAA	2219
GAACATGGTC	AGAGGAGGAA	GGGACTCTTA	TACATACCTA	TGACAGGAGA	ACGGGAGACC	2279
CAACATACTC	GGGGGCCTAC	CTTCAGAGAA	CACAAGGCCA	GGGCAATACT	CACAGSNNCT	2339
CATTGTTTGA	CCCTGCCCTA	GTTTCGACCTG	TCAACATCAC	TGAGCCCACC	TTGGAGCTAC	2399
TCCATTTCATC	CTGCGACCCC	AATGCATTCC	ACTCCACCAT	CCAGCTGTAC	TGCTTCATTT	2459
ATGGCCACAT	CCTAAATGAT	GTCTCTGTCA	GCTGGCTAAT	GGACGATCGG	GAGATAACTG	2519
ATACACTTGC	ACAAACTGTT	CTAATCAAGG	AGGAAGGCAA	ACTAGCCTCT	ACCTGCAGTA	2579
AACTCAACAT	CACTGAGCAG	CAATGGATGT	CTGAAAGCAC	CTTCACCTGC	AAGGTCACCT	2639
CCCAAGGCGT	AGACTATTTG	GCCCACACTC	GGAGATGCC	AGGTAGGTCT	ACACTCGCCT	2699
GATGCCCAGA	CCTCAGAGTC	CTGAGGGAAA	GGCAGGCTCT	CACACAGCCC	TTCTCCCSN	2759
NCGACAGATC	ATGAGCCACG	GGGTGTGATT	ACCTACCTGA	TCCCACCCAG	CCCCCTGGAC	2819
CTGTATCAAA	ACGGTGCTCC	CAA				2842

FIG. 9A

10 / 20

B) 3' arm (C57Bl/6J sequence) (SEQ ID NO:8) 25466106 to 25463273 of NCBI NT_166318

TATGAGATGA	AAGTAAGCTG	AGTTGGGCAG	TTCTAGACTA	TTCTAGGCTG	TTCTGGGCTG	25466047
ACCTGAACTG	GGTGGGGTTG	AGCTGAATGA	AGTAGGCTGT	GCTGAGCTCT	GCCAGGCTGG	25465987
ATGAGTTTAT	TGATCTGAGT	TGGACTGGCC	TGGGCTGCAC	TAAATGGGAC	TGAGATGAGA	25465927
TTGGCCAGGC	TAGGAGGGGT	TGAGCAAGGC	TAAGCTAAGT	TGTTCTGGAC	TAGGCCAAAC	25465867
TATTAGAGGT	CTTTTGGTTT	AGTTGAACTT	TCTGGTACTG	AACTAAACTG	TCTTTAAGAT	25465807
AGAATGCTCA	AAATTATTTG	TGGGTGTTTT	AACTGTCCTC	AAAGAAGATT	GTCCTGTTGT	25465747
AGGATACAAC	AACAGCTACT	AGCCAGACTG	GGTAGGTAGA	GCCAATCGGG	CCTAGCAGGA	25465687
ATATCCTGTG	CTTTCTGAGG	ACCTGGCACA	GAGCTGAGCT	GAGCCCCCTC	CTCAGGAGAA	25465627
TGTCCTGGGC	ATGTGGACAC	TCTAGAGCAT	CAAGGTGGCT	TCTGAAGTGG	TTGTATTCTC	25465567
TATGTGCTTT	CTGGGATCCA	CGGAGGTCAT	CTTGGAGGCA	GAACACTGTG	CAGGTTAGCC	25465507
TATGGTAAAG	CAGAGAGCCT	CATGTATCTG	AAACCCAAAG	ATCCGATGAA	TTGCCATTGT	25465447
AAGTTTGCCT	CTTCATCCAA	ACTCGTGCCC	AGCTCTCCTG	GAAGCCCCCTG	TGCTAAGCCA	25465387
GCTAGGGGCA	GTGAGTGAGC	AAAGCCTGAT	GGGGTGTAAG	GAATCAGGGG	GATCCCTAGG	25465327
TCTGTGTTTT	GGTTTAGTGA	ATAAAGACAA	GACCCAGAAG	GAATCTATGA	CCAACAGCCC	25465267
TAGGAAACAA	GAATCTCACC	ATTCTGTCTT	CAATGTGTCC	CAAAACAGAT	TTAATGTGTC	25465207
TCACCAAGAA	ACTGGTGGTC	CTGGGAAAGC	TCTGAATCCC	CAGGCCCCAA	GAGTGGGGAC	25465147
AGAAGAGACA	ATGGCAATTC	ATCGGATCTC	TGGGCCACCA	AGCCCTGTGG	GGTACCTATG	25465087
TCCTGGACAT	AAAGGACAAC	CTAGTCCCTC	TGTCAACATT	ACATAGCCTA	CCTTAAAGCT	25465027
ACTCCATTCA	TCCTGAGACC	ATAATGGCTT	CCAGTCTGCC	ACCCAGCTCT	CATGCTTCAT	25464967
TTCTGGACAT	TCCCTAGATG	GTGTCACTGT	CACCTGGTCT	AAAGGACAGA	CAGGAGATAC	25464907
CTCACACATA	TCCACAAAAT	TTCCCAATCA	AGAAAGAGGG	CAAGTTTGCC	TCTTTATCCA	25464847
AACTTGTTGCC	CAGCTCTCCT	GGAAGCCCCCT	GTGCTAAGCC	AGCGAGGGGC	AGTGAGTGAG	25464787
CAAAGCCTGG	CGGGGTGTAA	GGAATCAGGG	AGCTCCCTAG	GTCTGTGTTT	GGGTTTAGAG	25464727
AATAAAGACA	AGACCCAGAA	TGAATCTAAC	CATCTGTCTC	CTAGACTGGA	ATGGGGTCCC	25464667
CAGAGCCCTG	CTCCTGTGAC	AGCTGCCCTT	AATCAGTTCC	CCATGCTGCA	G---GCATGC	25464610
AGTGATATAA	ATAAGTCTAA	CCTAGGTCTT	TCCTTTCTCA	CAGCCTCTAT	CAGGAACCCCT	25464550
CAGCTCTACC	CCTTGAAGCC	CTGTAAAGGC	ACTGCTTCCA	TGACCCTGGG	CTGCCTGGTA	25464490
AAGGACTACT	TCCCTGGTCC	TGTGACTGTG	ACCTGGTATT	CAGACTCCCT	GAACATGAGC	25464430
ACTGTGAACT	TCCCTGCCCT	TGGTTCTGAA	CTCAAGGTCA	CCACCAGCCA	AGTGACCAGC	25464370
TGGGGCAAGT	CAGCCAAGAA	CTTCACATGC	CACGTGACAC	ATCCTCCATC	ATTCAACGAA	25464310
AGTAGGACTA	TCCTAGGTAA	GTAGGGATGG	GCTGACAGTT	ACACTGTGTA	TTCTCCCTTG	25464250
GAGATGGAAC	AGTTTCTGTC	TAATCAGGAA	CTTGTCACAA	TTTCCTTTCA	TAGAGGACTT	25464190
CATAAGAGAT	TTTTTTTTTCT	ACTTCTATCA	TGTTTAGTGC	TCCAAATAGA	TTTTTAAAC	25464130
TGGTTGAGTG	CATATTACTT	TTAGCCTCAG	AAGACATCAT	GTATATTTAA	GAGGCATTTA	25464070
ACTATTGTAA	ATTATTCTGA	TGACTTTAAA	AAAAGTTAAT	GCTGAGTTGT	ATATTTTAA	25464010
ATAAATTTTA	TTAGTTTAGT	TTAAAAAAAG	AAAAGAAAAT	TATTAATTTT	ATTTAAAAAT	25463950
CTCCTATATT	TAAAAAAAAG	AGAGAAAAAA	GCAGAGCTGG	GCTGGCTACA	GTTACCACAA	25463890
GAACATGGTC	AGAGGAGGAA	GGGACTCTTA	TACATACCTA	TGACAGGAGA	ACGGGAGACC	25463830
CAACATACTC	GGGGGCCTAC	CTTCAGAGAA	CACAAGGCCA	GGGCAATACT	CACAG---CT	25463773
CATTGTTTGA	CCCTGCCCTA	GTTCGACCTG	TCAACATCAC	TGAGCCCACC	TTGGAGCTAC	25463713
TCCATTTCATC	CTGCGACCCC	AATGCTTTCC	ACTCCACCAT	CCAGCTGTAC	TGCTTCATTT	25463653
ATGGCCACAT	CCTAAATGAT	GTCTCTGTCA	GCTGGCTAAT	GGACGATCGG	GAGATAACTG	25463593
ATACACTTGC	ACAAACTGTT	CTAATCAAGG	AGGAAGGCAA	ACTAGCCTCT	ACCTGCAGTA	25463533
AACTCAACAT	CACTGAGCAG	CAATGGATGT	CTGAAAGCAC	CTTCACCTGC	AAGGTCACCT	25463473
CCCAAGGCGT	AGACTATTTG	GCCCACACTC	GGAGATGCCC	AGGTAGGTCT	ACACTCGCCT	25463413
GATGTCCAGA	CCTCAGAGTC	CTGAGGGAAA	GGCAGGCTCT	CACACAGCCC	TTCTCTCC--	25463355
-CGACAGATC	ATGAGCCACG	GGGTGTGATT	ACCTACCTGA	TCCCACCCAG	CCCCCTGGAC	25463296
CTGTATCAAA	ACGGTGCTCC	CAA				25463273

FIG. 9B

11 / 20

A) 3.7 kb upstream of BamHI (used to design 5' probe) (SEQ ID NO:9):

CTCTCCCTGT	GGACCACAAA	AGTTTATATT	CTTCCTACAT	ACAGTGCCCC	50
TCCCCCATG	CCAACATCCC	AAAGTCTCAT	CCTTCCAGCA	TTCAGCTCTG	100
CCCCAAGTCT	CCTCCTAAGG	TCTGCTAGTC	AAGTAAGGGT	CAGATTGTGT	150
GAGGTTTGT	TTGAGATAGG	ATTCAGTCCA	CCTAGGCCAT	AGCTGTCAGG	200
AGGAAGGGGA	AGGAGAGAGG	CACAGAAGGG	AGAGGTATAC	CGTGATGAAC	250
TGGGCAGACT	GATAACATGC	TGTAGAGCCA	AAAGCTGAGG	GCAAGTGGGG	300
TCCCCTCCCT	CTCATGCTAA	GGTGACAGTT	TCTAAGGGAG	AGCAAGGGAT	350
TTGGAGAAAG	AAGTGAAGGT	TTGGTTCAGC	ACTGGCCTTC	CTGGTCCAGC	400
ACACTGCCCC	TGCCTCAAAC	TTTGACACATA	CAGATCCCCC	ACTGTACTCC	450
CCTCCTGCAT	TTTCCCCACT	ACCTTCAGCA	CACCACCAAC	CTCTCTTCCA	500
TGACTGCCTC	CATCCCACCT	AGCATGGAGC	CCCCTCCTG	TGTGAGCCTA	550
GTTCACTCAA	TGACCATGGG	TGTCCATCTT	CCAATGAAAC	ATGAGCTCCA	600
TGGACAGGAA	TATCCCTCCA	GACCCATGTT	CCTGCAGTTC	TATCTAACTG	650
TTGGGCATTT	ATGATGAAGT	CACACCAGGT	CCCTCATTTCC	TAACTAACCT	700
TCTAATCTGG	CACAGTTATC	TGCTGGGAAC	TAAGAGTGTG	GTCAAAGTAA	750
GATATGATGC	TGGCCGACCA	GTAGGTCCAT	GTGCTGGCTT	TCAACTCTAA	800
ATCTTCCCCA	CGCCTACCAT	GTAGCCACAG	GATCTTTCCC	CAAGGCAGGG	850
TACAGATAGC	ACATAGAGGA	AGGAGGCCAA	GTGGATGGGG	CTCTCCCGTA	900
GCAGGTGTGG	GAGAGGCAGG	TTGCACACAT	GTGTGAATCC	ATATGGTTCT	950
GAGGTTTGGG	GGGTTTTTTT	GTCCCAAAAT	ATCATGTTCC	AGAAGTACTT	1000
GCTCCTTTGA	CCTATACACC	AGAGAAGAGA	CCAAAACTG	TGGTAGACAC	1050
AGGAGCAAGA	ACAAAACCAT	GCTGTTGTTT	TTCTACAGGC	AAAAGTTGTT	1100
GTCACCCCTTA	TTCCTGGCAA	AGCTATGTCG	TCAAATTGCT	AGTCCTGGTT	1150
GTAATTACAA	ATTTAATAAT	ATATATAAAT	ATATGTGCTA	GACAACAGTT	1200
AAAGAAATAA	GGACAATGTT	GGAGGGAGGA	AATGAAAGTG	GGAAATGATA	1250
CAATAATTAT	ATTTTCATCC	CAAAGATAAA	GATATATGTT	TTATTTCTGG	1300
TCCTGGGCTA	AGGATATAGT	TCAATTGTTA	AAGTACTTAT	ATAGCATGCA	1350
GGAAGCCCTG	GATCTGATCT	CCATAAATAA	ACCAACTGTG	GTCATTCATA	1400
CCTGTAATTC	CAGTACTCCA	AAGGCAGACA	CAGGAAGATG	GGGGCTTTGA	1450
AGTCATTCTT	GGCTACATAG	CAAATTAGAG	ACCAGCCTTA	TCTTTAATAA	1500
TAATAATAAT	CCTGACATTC	CACTTGAAGT	AAATGTATCC	AGTAATTGTA	1550
CCTGATACCA	TGCCATGTTA	GATTTTATTT	CAGGGTTTCA	GAGAAAAGTA	1600
CCCAGGGTTT	TCCAGAGACG	CACAAGTAGT	GGAAGACAGC	AGACTGAGGA	1650
CTGGTAGATG	AACGCATGAA	CAACTTAGGA	CAGAGCTGGA	GGACCATAAG	1700
GCTGATAGGT	AGGTAGAAAC	AAGGATAACA	CATAATAGGC	TTACAGGTTT	1750
TATGGTTCAC	CTTGACTGGA	CTAAGGGATG	GAAGGCCTGT	GGAGGTGTCT	1800
GCAAAGGATT	TTCCAGAAGT	GTTAGGTCTT	GGGGACTCTG	ATTTAACCGC	1850
ATGGTGCTGA	ACAGTAGGAA	GAAGGCCAGG	TGAAAGAAGC	AGGTTACTGT	1900
GGGTAAGCCC	GGGTAATTCT	ATCAGATCCT	GGCACTGTAT	TCCTGTTATC	1950
TCTACTTCCC	ATCAGTTGAG	AAACAAACAG	CCTCTTCCAC	ACAACCCCGC	2000
TGCCAAGATG	GCCCTAAGCA	CATAGGGCCA	AACAAAAGTA	AATCTTTTCC	2050
CCCTTATGTT	TCTTTGAGGT	ATCAGGTCAT	CCTAGCTCCC	GGCTCCAAGT	2100
CACACAGAGG	GCTCCTATGA	GCAAGCATTT	CCAACCTCTAC	CCTTTTTTTT	2150
TGTCACAAAC	CGCTGGCTAT	TTGAGACATT	TCAGACAGCT	AAAAGATGGC	2200
TCCCTTAGCA	CTGATGGCTG	AGTTCAAGAG	GCTCACCTGC	TGGCTCGCAA	2250
ACCCAGAGGT	AACCTGGGTT	TGTGTTGCTT	TAGGCATAGA	GAATGCTGAC	2300
ATCCAGCGGC	TACGTGACTG	CTTCTGGGTT	TTGGAACCTG	TTCTTCAGTA	2350
CCATCCCCTG	CATCCAACCT	TTCTCAGCTT	GTAATCTTCT	CAATATTTCC	2400
ACCGTTCTTC	ATCACCTAG	ATTCTTGCAG	ATGCTGCCCT	CGATGGCTCA	2450

FIG. 10A-1

12 / 20

CTGGCTCCTC	TACTCCCTGC	CACTATCACC	CTCGGGGCCA	GAAACCTGGA	2500
GCCATGTCTG	CTCTTTGCAC	AGTAACACAG	CCTACTCTAC	CCATGACAAA	2550
GACCATAAGA	AGACTTGGAG	ACCATTTAGG	AAAAGCCTTT	ATCATGGCCT	2600
AATGCTGCAC	ACGTGGATCA	GGAGAAGCCT	CAAAATATAG	TAGGGGGCAC	2650
ACTGTAGAGA	CAGAAATAGAG	TCCATGATAC	GCTCATACAT	GGATTATACT	2700
TCCAACAAGC	ACTGCCCTGT	TTGTGCTCAT	CTCCTGGTTC	GACCAAGCAC	2750
AGTCTTCCCA	TATGAACCCA	TCACAAGCCC	TGCAGAATCA	CAGATCACAG	2800
GTCTTAGATA	GGACCAGCTT	TCTTTCTGAC	AATAACCAGG	ATTTATTTGT	2850
TATTTCTTTT	TATTGTATTT	ATTTTATATC	ATAATTTTAC	ATCCCTCTCA	2900
CTGCCCCCTC	CCAGTCATTC	CCTTCTGTTT	TCCTCTCCCC	ACTCCCCCTC	2950
CCTTTCTCCT	CTAAGCCAGT	TAAGCCCCCT	CTGTGTACCC	CCCACCCTAA	3000
TAATCAGGAG	TTTTGAGCCA	CCAGAGATGT	TCTTCCTCCT	CTCTGACCTT	3050
GCTGAGAGCC	TCTATGCCAA	GGTCCTCTCG	AGCTGCATGT	GAAGTCACTT	3100
GGAAGTCGTA	GGTGAAGTGG	AGTTTTCCAG	CTACAGTGCA	GGCTGGAGCC	3150
CTGGTAACTA	GAACAAGGCT	GTAGTTTCAG	CAGCAGCCAT	GATTGCAGGA	3200
TACCTTGCA	CTCAAATATG	GCCTCCTTGG	GGCTCTGTGA	GGTATTCAAA	3250
GCATCTAGAA	TCCCATGATG	ACAGTTCTAC	CAGTCCCTAA	AAGAAACCTA	3300
AGACGACTAG	ATATAAGGAA	AGACCCACCT	GAGTGCATCA	AAAGGTCAAA	3350
TCAGCCTGGC	GCTCAACAGC	TCATTTTACA	TGAAGAAAAG	GTGAACACTA	3400
CCCTATTCCC	AATAAAGACA	TGTTGTTACA	CTTACACTAA	CATCCTTGGC	3450
AGCCCTTAGC	AGATGATCCT	AGGGAGAGCT	GAGCAGTCTC	ATCTACCTCA	3500
CCTCCACCCA	GGCATCAAGT	TAACACTGTT	CTAAGGTGCA	CTTCTGAAAC	3550
TTACAGAGTT	GGGGTAGCAG	TCAGACCTTT	CCCTGACCCC	CAAGATATGA	3600
TCACACCCAC	AACCACATAC	ATGAGTTCGC	AGACACTAAC	CGACACAGTG	3650
GATCTTAGAC	CTGGCCCATT	CCGGAATAGA	TCACTGTCAC	AGTCACTTGA	3700
GTGAAGGAGC	CACCCAAGGG	AATGGCTAAA	GGACTG		3736

FIG. 10A-2

13 / 20

B) PVUI to ECORI fragment (used for 3' probe design) (SEQ ID NO:10):

CGGGAGATAA	CTGATACACT	TGCACAAACT	GTTCTAATCA	AGGAGGAAGG	50
CAAAC TAGCC	TCTACCTGCA	GTAAACTCAA	CATCACTGAG	CAGCAATGGA	100
TGTCTGAAAG	CACCTTCACC	TGCAAGGTCA	CCTCCCAAGG	CGTAGACTAT	150
TTGGCCACACA	CTCGGAGATG	CCCAGGTAGG	TCTACACTCG	CCTGATGCCC	200
AGACCTCAGA	GTCCTGAGGG	AAAGGCAGGC	TCTCACACAG	CCCTTCCTCC	250
CSNNCGACAG	ATCATGAGCC	ACGGGGTGTG	ATTACCTACC	TGATCCCACC	300
CAGCCCCCTG	GACCTGTATC	AAAACGGTGC	TCCCAAGCTT	ACCTGTCTGG	350
TGGTGGACCT	GGAAAGCGAG	AAGAATGTCA	ATGTGACGTG	GAACCAAGAG	400
AAGAAGACTT	CAGTCTCAGC	ATCCCAGTGG	TACACTAAGC	ACCACAATAA	450
CGCCACAACCT	AGTATCACCT	CCATCCTGCC	TGTAGTTGCC	AAGGACTGGA	500
TTGAAGGCTA	CGGCTATCAG	TGCATAGTGG	ACCACCCTGA	TTTTCCCAAG	550
CCCATTGTGC	GTTCCATCAC	CAAGACCCCA	GGTGAGTACA	GGAGGTGGAG	600
AGTGGGCCAG	CCCTSNNSMT	CTTCATGTTT	AGAGAACATG	GTTAACTGGT	650
TAAGTCATGT	CTGCCCACAG	GCCAGCGCTC	AGCCCCGAG	GTATATGTGT	700
TCCCACCACC	AGAGGAGGAG	AGCGAGGACA	AACGCACACT	CACCTGTTTG	750
ATCCAGAACT	TCTTCCCTGA	GGATATCTCT	GTGCAGTGGC	TGGGGGATGG	800
CAAAC TGATC	TCAAACAGCC	AGCACAGTAC	CACAACACCC	CTGAAATCCA	850
ATGGCTCCAA	TCAAGGCTTC	TTCATCTTCA	GTCGCCTAGA	GGTCGCCAAG	900
ACACTCTGGA	CACAGAGAAA	ACAGTTCACC	TGCCAAGTGA	TCCATGAGGC	950
ACTTCAGAAA	CCCAGGAAAC	TGGAGAAAAC	AATATCCACA	AGCCTTGGTA	1000
ACACCTCCCT	CCGTCCCTCC	TAGGCCTCCA	TGTAGCTGTG	GTGGGGAAGG	1050
TGGATGACAG	ACATCCGCTC	ACTGTTGTAA	CACCAGGAAG	CTACCCCAAT	1100
AAACACTCAG	TGCCTGATTA	GAGCCCTGGG	TGCCTGTTCT	TGGGGAAGGC	1150
AGGTTATGGG	CAGAAATATC	TTGGCCTGAA	AGAAGGGACA	CCCCAAGAGA	1200
AGGACAGGAG	TGAAGCATGG	CTCACCCATC	TGTCTATGTG	TTGAATATTT	1250
AACAAATAGG	ACATCACAGG	ACTTCAGCAT	AGTCCTTCAG	CATACCCCTG	1300
GTCCTTCCTG	CTCTTCACTG	GATATCATGC	ACCTGATCTC	TAGAGATGCA	1350
GCTAAAATGA	GCCAGTCTGA	GAAGCCTCAG	CACCCACCTC	TCGGTCTTGC	1400
AAGCTCCTGC	TCCCAGGCTT	TCCTGGATAC	TAAACCCCTT	CAGGTAGAGA	1450
AACAGCCAAA	GTCAACATCT	AGGACGCAGG	ACTCAACATG	GTCCTGCTCC	1500
TTCCCTCTCT	ACTCAACAGC	CATTGAGGCT	GAGCCCACCG	CCCCAACCGC	1550
CTGCCTTGCC	AAATGATCAC	GCCAGGCCTG	GTGCTCCTCG	ACTTACTACC	1600
TAGACTCACT	CCAACCCAAA	TTCATCCCAA	GGACCAGAAT	GGGCTGCCAG	1650
CCTCATAACAG	TCAGGTTCCC	CCATCTATGA	CATGTTTTCA	CACACATGCA	1700
CACACACACA	CACACACACA	CACACACACA	GAGCTAGGCT	TCATTGAGCT	1750
CTCTGGTTTA	GCAATAGCCC	AAAGCAAGCC	ATACATCCAT	CCCAGTTCCA	1800
GAAGGATAAG	AAAACCAGAA	CCAAGACACA	CCCACACCTA	TTCCATACCC	1850
AACCACCAGC	ACATATGGCT	TACACACCTG	AGATCAGTGG	CTCCCATCAT	1900
GTACACACAC	ATGCACACAA	AGGAGACCAT	ACATACCCAT	CATTTCCAGA	1950
GGTAAGTATC	TAACCTTTGG	ATCTGAGATA	CCTCTGAGGA	ACACCAATGG	2000
CAGAGTCGAC	CAGCACCTCA	GCCTCCAGAC	TAAATCCTTA	CATTTTGGCC	2050
CACCCCAAGC	CATGAGAGAT	GGAGGAGGGT	AGAGGCCTGA	GCTGCGGGAA	2100
AGCAGAGACA	GGAAGATGGG	CTGTTTGGTG	AGAGTAGTAA	ACCAGACAAT	2150
GGGGAGACTA	AGGCAGGAGT	AGAGCCCCTA	CAAGGCCAG	AGTCTGCTTT	2200
AGAGTCCATG	TGTCCTGACC	TGCCCCTCAG	ATGCCACAAC	CAAGATTTCT	2250
GGTTCCAGAG	CATGCATGCA	GGCCCTAGAA	ATGGACCTAT	GAGCTCAGAG	2300
CCTTCCTAGA	GAGCCCTGGG	TACTCTCTGA	ACAAAAGGCA	ATTCTGTGTA	2350
GAGGCATCCT	GTGGCCAAAG	ACCTTAAGAC	AGTCATACAC	ACACACAACA	2400
CACACAACAC	AGGTAGGCTT	TATCATGCTC	TTTGATTTAG	CAATAGCCCT	2450
GTGATGGTG	GGGGATACTG	GGTCACTGTG	GGCACCGGAG	TAGAAAGAGG	2500

FIG. 10B-1

14 / 20

GAATGAACAG	TCAGTGGGGA	AAGGACATCT	GCCTCTAGGG	CTGAACAGAG	2550
ACTGGAGCAG	TCTCAGAGCA	GGTGGGATGG	GGACCTCTGC	CACTCTAGCT	2600
TCATCAGAAC	TGCATGAGAC	AAATATGGGG	CCTACCCCCT	CCCCACTGTC	2650
ACCTGGAGTS	NNMCTGGGGA	AGCTAACTGG	CTGGTCCCAC	CCCATCCCAG	2700
AGCTAGACCT	CCAGGACCTA	TGTATTGAAG	AGGTGGAGGG	CGAGGAGCTG	2750
GAAGAGCTGT	GGACCAGTAT	TTGTGTCTTC	ATCACCCCTGT	TCCTGCTCAG	2800
TGTGAGCTAT	GGGGCCACTG	TCACCGTCCT	CAAGGTGGGA	TCCTGCACCT	2850
CAGCGGGTGG	GTCTGGGAGG	GCTAGGCCAA	GCCGCAGAGC	CATCCTCACA	2900
TACSNNMACC	TTTCCCCCAG	GTGAAGTGGG	TCTTCTCCAC	ACCGATGCAG	2950
GATACACCCC	AGACCTTCCA	AGACTATGCC	AACATCCTCC	AGACCAGGGC	3000
ATAGGTGCGA	TGCCAGCACC	CATGCAGGCC	TGCAGCCATG	TGTGCTTGAG	3050
CCTCCTGAGG	TGCCTGTTTG	CCCGGGTGAT	AGGAGGGAGC	AGAGACCCCT	3100
AAAGGCACCA	ACGTTGATGA	GATATCAGCA	TCCCAGAAAG	TTGCAGCTCA	3150
GAGCACCTAG	GTGGGCTGTC	CTACACAGAT	ACTTTGAGAC	AAAGCTTAGA	3200
AGAACATCTA	TCCCTCATCG	ATTTGACCTA	CCAGATGCTA	GCCACCTGGG	3250
CTAATCCCA	GTCTATGGGC	ATCAGGACCA	CTCCATTTTG	ACTGAATAAC	3300
CACAAAAACA	CAAGAACTCA	GAGTCTAGAG	TTCCCCTAG	ACCCACCTA	3350
GAGCACAGAG	TCAAAGCTGG	GACACTCAGA	ATCAACCCTA	AGTCCAGACG	3400
CTGGCTCCTC	AGAAGG				3416

FIG. 10B-2

15 / 20

- FIG. 11A** A) I-mu Forward-1 (21 bp):
5' -CTCTGGCCCTGCTTATTGTTG-3' (SEQ ID NO:11)
- FIG. 11B** B) C-epsilon Reverse-1 (30 bp):
5' -CCTGATAGAGGCTGTGAGAAAGGAAGGACC-3' (SEQ ID NO:12)
- FIG. 11C** C) E-mu Forward-2 (20 bp):
5' -AGACCTGGGAATGTATGGTT-3' (SEQ ID NO:13)
- FIG. 11D** D) C-epsilon Reverse-2 (30 bp):
5' -TAGGTTAGACTTATTTATATCACTGCATGC-3' (SEQ ID NO:14)
- FIG. 11E** E) Forward: SM5' (20 bp):
5' -GTTGAGAGCCCTAGTAAGCG-3' (SEQ ID NO:15)
- FIG. 11F** F) 9225F (19 bp):
5' -TTGAGAGCCCTAGTAAGCG-3' (SEQ ID NO:16)
- FIG. 11G** G) 9518F (26 bp):
5' -TGAGCTCAGCTATGCTACGCGTGTTG-3' (SEQ ID NO:17)
- FIG. 11H** H) Reverse (30 bp):
5' -GCCCCGATTGGCTCTACCTACCCAGTCTGGC-3' (SEQ ID NO:18)

16 / 20

Retrieved IgE C57BL/6 genomic sequence (Se region to be deleted shown in bold, underlined font) from BAC RP23-135L12 (invitrogen) (SEQ ID NO:19):

gggaagagat	agaaggaagg	ctaggtgggg	caagacgagg	gaactaaagc	50
cactgtgctg	ctggggacac	tgtggacact	gatggacaga	aagggagtga	100
tcagtctgtg	gacaggaggg	ggaggggcaa	ggatgatgct	gacagagagt	150
cacagtggag	tccgtagcag	gaaagagaga	gagcgcccag	tgtagtccta	200
aggcttagga	agttgcaact	gcctcctctc	cttccagagg	atcactcact	250
gccatctagc	atagaactca	gatgacccag	aaccagcagc	tcagcccaac	300
ctgtgtgtca	cagaagaatc	aggcccagtc	aggctagaca	caaaggctct	350
tggccctcat	gctgtgaggg	aggtaacacac	tgggggcaca	ccacaaacag	400
ttggagcaga	ggctttctcac	ccctatTTTT	ccctctgaac	aatagttgct	450
tccagggaac	tctgcattta	cccctcaggc	tcccacccat	gtctgttagg	500
ctgaaggcca	agcctgtcac	ctcagacaga	cagtggatct	gaaagacaga	550
aggcogtgca	agaccacaat	tcccttgaat	ctcacactct	gtcttcccaa	600
agttcctaac	tgcattctgac	ctttctgggc	cagcctctca	gcctgcctgg	650
ctctgccact	atcaggaaga	tctctaatat	cttccaaatg	caattaaaca	700
cgctcctgtg	aaagtcagac	ttggcaaagc	ctaagtccct	tcggtccctt	750
tcagtgggac	caacgactct	gagcagccag	ggtccaaggg	atggggctct	800
cattttcttc	cccaaattctc	tgtgtgcctc	tctcaagact	caagactcac	850
aagcaaaatt	agtggctcct	atagcttgta	tgtatgtttt	cttggaaactc	900
ctaggaacca	tgggcctaca	gagacatcag	agtgtagagg	gaatccctga	950
accagaaga	tgaccttgct	ctacaaagct	gcagctgaga	cagacactac	1000
tagtacccca	tgaaagctgc	tgagccaaag	cccagccctc	acaccatctt	1050
taccctcatc	cctccctca	gtgcagacat	agaccacagg	cctggaagag	1100
acgttagctg	tttctacaca	gctccgtgaa	accagtcac	aaccagatg	1150
cgctctgtcc	ttctggactc	cttgccagag	tagcaggtag	aggacctcaa	1200
gctgaaagat	aatcacttgt	gagtgggcac	caggggaaggc	cactgtccct	1250
cgcatgccag	ctccaaagct	gatacaggaa	ctagggtgcc	tctatcagag	1300
gccctgcaat	gtcatatctg	gccacaggc	tgttcctctt	tgtgcaccat	1350
taataactta	caaagtgaca	gocacactcc	cctgaagggc	tgccaaagga	1400
acagaaaaag	caatggctag	ggtctagtc	tgcttcaggg	cagtgcact	1450
ccaaaggggc	aggcatggtg	actgcacaca	cgcacatgca	aggctttaat	1500
acgagagcta	tgcaaggaga	cctgggatca	gacgatggag	aatagagagc	1550
cttgaccaga	gtgtgcaggt	gtgtctccta	gaaagaggcc	tcacctgaga	1600
ccccactgtg	ccttagtcaa	cttcccaaga	acagaatcaa	aagggaactt	1650
ccaaggctgc	taaggccggg	ggttcccacc	ccacttttag	ctgagggcac	1700
tgaggcagag	cggcccctag	gtactacct	ctgggcacga	attaatggtt	1750
actagagatt	cacaacgcct	gggagcctgc	acagggggca	gaagatggct	1800
tcgaataaga	acagtctggc	cagccactca	cttatcagag	gacctcaggt	1850
attacaaccc	atgggacct	gagcaaaagg	gtttgcctaa	ggagaaggga	1900
caaacagggt	acagggtcct	gggtggggaa	ggggacacct	gggctgcctt	1950
ctaattgtga	cagtctcttg	gtcaccgaat	gtccttcagc	tatcacttcc	2000
ctgcactaag	gcacacaggt	attagaaact	gctatagcta	tccatgaaga	2050
caggggactg	tggatctcaa	ccagagaggg	ctgaaccaag	ataaactgaa	2100
tatgtttgtga	gaaactcaaa	aactgcagga	gaggctggag	aggaatcggc	2150
cagcaagcca	tcagacaaaa	atgcaatgac	aaatgtcaga	tccagcaaat	2200
gacagcaagg	aattgccctg	tgatgaacta	acaaccaaga	ggactgtcca	2250
cagctgggct	gaccacaggca	gcactgggct	aaattgggtg	ggatctgtgc	2300
tgccctgggc	tggtatgagc	caggatgagc	caagtgaagt	gggctggact	2350
agtttgggct	ggactggcct	ggagtaggct	aaaccagttt	aaactagagt	2400

FIG. 12-1

17 / 20

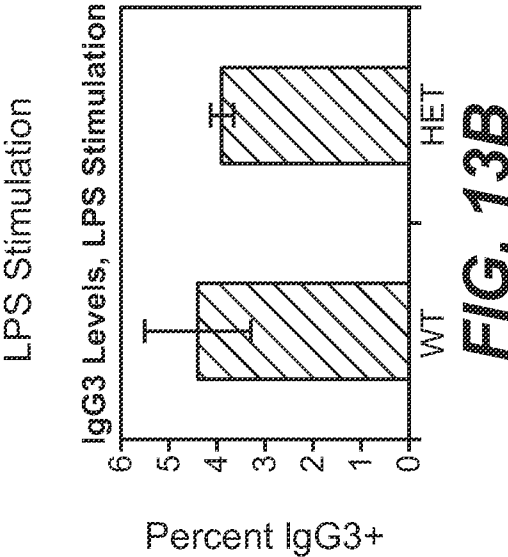
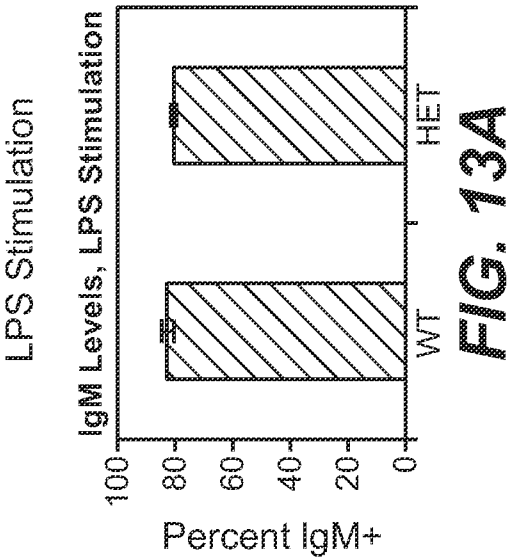
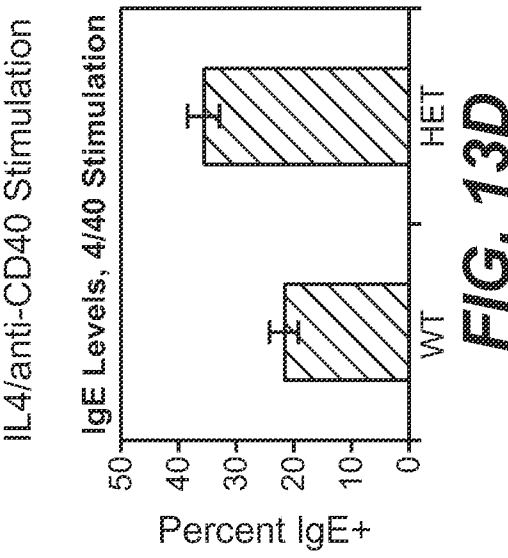
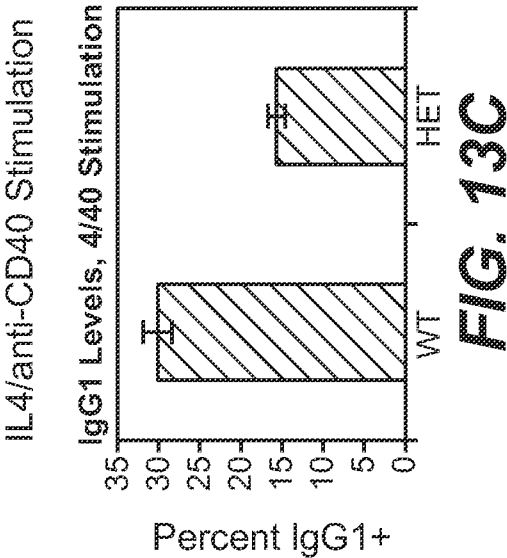
aggctgggct gaggtgaatc agactaggct agactagtct gagctgggtt	2450
aagcagagct gtgctgggct ggtatgagct ggtccaagtt gggctaaaca	2500
gagctgggcc aggctagtat gagctggtct gaactacact aagcaggact	2550
aggctgggct gagctgagct ggactggctg gacttggctg agatgtgttq	2600
agctgggtta agtatggctg ggctgggctg gcctgggctg ggctggactg	2650
gattggtatg agctggtcca agttgggcta agcagagctg ggccaggctg	2700
gtatgagctg gtctaaactg aactaagtag ggctgggcta agctgagctg	2750
gtctacacta gcctgacctg agctagggta ggctggactg ggctgagcta	2800
agttgcactg ggcaggggtg ggctggaccg agctgatttg agctgggatg	2850
ggctgagatg ggttcagcag gcctaagcag gcctagctgg gtttagctag	2900
atttagctag gcaaggctga gctaggctgg gcggggcggg gctaggctgg	2950
gcagggctgg actgagctag cttttgtata ttcggttgaa atgggttgg	3000
ctggtctgga ctgaactgac tgagctgggc tagcctgagc tcgatggggg	3050
gtatactcag ctgagatggg ctggtctggc tagactgaac tggattgggc	3100
taggctgagc taggctgacc tgaactggcc tggctctggc tggactggc	3150
agggctggtc tcagctagac tacactgagt taacctgggc tggaccatac	3200
tgggttaaac taggttgac tggctgggt agacttggct gagctgggct	3250
tggctgagct gagtcaagat ggtctgagtt gatttgagtt ggctaagcta	3300
agctgagcta cactgaacta ggcaaggctg ggctggaaaq gtctgggtta	3350
agttaggagg gacttggctt ggcttagctg ggccaagcta ggctgaactg	3400
ggctgaactg agctgagctg ggctgagctg ggctgagctg ggctgagctg	3450
ggcaaggcta aactggaatg gactgaattg gcctaagatg ggcccggcta	3500
agctaagtaa ggctggcctg aactgagcag gactggcctg gcctggattg	3550
acctggcatg agcttaactt gactagacta gtctatcttg ggtgaactgg	3600
gctaagcagg actaatctgg cctgatctga gctagactga actaggctaa	3650
gctgagctga gtttagcttg gctgaactgg gctgggctgc actgaactgt	3700
attgagctat gtagaactga gctggtcttg tctgaggtgg gttgggctgg	3750
tctgggctga accagattgc actagactga gcttagctgg acctggctga	3800
gctggactgc attgtgctaa actggctctc tttagaccga gcttagctgg	3850
actggactga gctaagtttg gtgggctgat ctaagctgag ctaggctggt	3900
ctcacctgag gaatgctgtg ctgtgctgag ctgaactaaa ctgagctcag	3950
ctaagggaagt gtgagctaga ctgagctgag ctaggctggg ttgggctgaa	4000
ctgagctacc ttgggtggac taggctgagc tgagctgggt tgagctgagc	4050
tatagatttg gttggactgg actggattgg gctaaactga actggttttg	4100
ggtaggctgg gatgagctgg actgagctag gctgtactgg tctgagctaa	4150
actaagttga gtggggctaa gaggagctga gtgaggctgg gctggaatga	4200
gctaggctag ggttgtgagc tagggttgta ctggtctaag ctgagtttag	4250
ctgagagagg ctgggctaga cttccataag gtggctgagt catactacag	4300
tgcactgagc tgtgttgagc ttaacttgga ttaagtggaa tgggttgagc	4350
tggctgaact gggctgaact gagataaact agactgagct gggacacgct	4400
gggacgagct ggaacgagct agaattactg ttctaactctg atctgggctg	4450
aggtaaactg ggcctgggtg agctctacta ggctaagtag agttgagcta	4500
tgagatgaaa gtaagctgag ttgggcagtt ctgactatt ctaggctggt	4550
ctgggctgac ctgaactggg tgggggttgag ctgaatgaag taggctgtgc	4600
tgagctctgc caggctggat gagtttattg atctgagttg gactggcctg	4650
ggctgcacta aatgggactg agatgagatt ggccaggcta ggagggttg	4700
agcaaggcta agctaagttg ttctggacta ggccaaacta ttagaggtct	4750
tttggttttag ttgaactttc tggtagctgaa ctaaactgtc tttaagatag	4800
aatgctcaaa attatttgtg ggtgttttaa ctgtcctcaa agaagattgt	4850
cctgtttagtag gatacaacaa cagctactag ccagactggg taggtagagc	4900

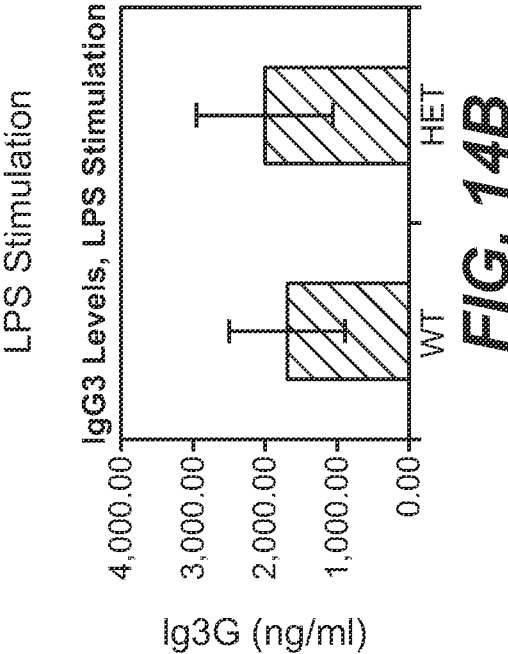
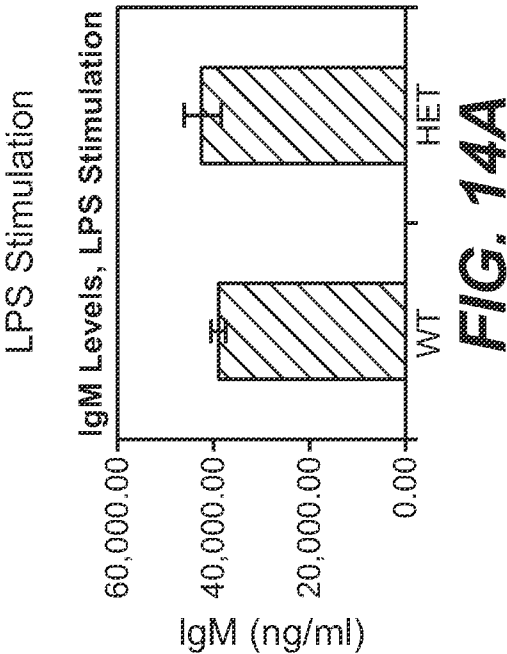
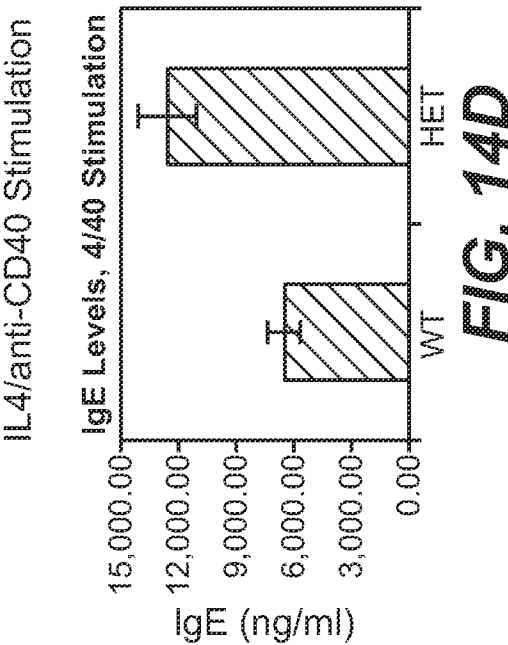
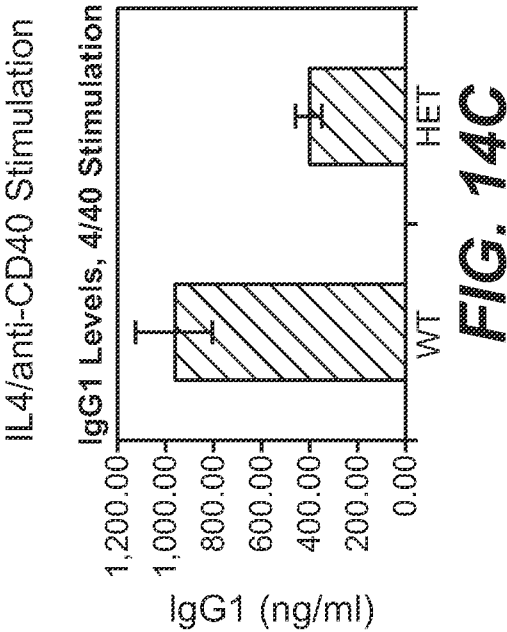
FIG. 12-2

18 / 20

caatcggggcc	tagcaggaat	atcctgtgct	ttctgaggac	ctggcacaga	4950
gctgagctga	gcccctctct	caggagaatg	tcctgggcat	gtggacactc	5000
tagagcatca	aggtggcttc	tgaagtgggt	gtattctcta	tgtgctttct	5050
gggatccacg	gaggtcatct	tggaggcaga	acactgtgca	ggttagccta	5100
tggtaaagca	gagagcctca	tgtatctgaa	acccaaagat	ccgatgaatt	5150
gccattgtaa	gtttgcctct	tcattccaaac	tcgtgccag	ctctcctgga	5200
agcccctgtg	ctaagccagc	taggggcagt	gagtgaagca	agcctgatgg	5250
ggtgtaagga	atcaggggga	tccctagggt	tgtgtttggg	tttagtgaat	5300
aaagacaaga	cccagaagga	atctatgacc	aacagcccta	ggaaacaaga	5350
atctcaccat	tctgtcctca	atgtgtccca	aaacagattt	aatgtgtctc	5400
accaagaaac	tgggtgctct	gggaaagctc	tgaatcccca	ggccccaaga	5450
gtggggacag	aagagacaat	ggcaattcat	cggatctctg	ggccaccaag	5500
ccctgtgggg	tacctatgtc	ctggacataa	aggacaacct	agtcctctctg	5550
tcaacattac	atagcctacc	ttaaagctac	tccattcctc	ctgagaccat	5600
aatggcttcc	agtctgccac	ccagctctca	tgttctcattt	ctggacattc	5650
cctagatggg	gtcactgtca	cctgggtctaa	aggacagaca	ggagatacct	5700
cacacatatc	cacaaaattt	cccaatcaag	aaagagggca	agtttgcctc	5750
tttatccaaa	cttgtgcccc	gctctcctgg	aagcccctgt	gctaagccag	5800
cgaggggcag	tgagtgaagca	aagcctggcg	gggtgtaagg	aatcagggag	5850
ctccctaggt	ctgtgtttgg	gttttagagaa	taaagacaag	accagaatg	5900
aatctaacca	tctgtctcct	agactggaat	gggttcccca	gagccctgct	5950
cctgtcacag	ctgcccttaa	tcagttcccc	atgctgcagg	catgcagtga	6000
tataaataag	tctaacctag	gtccttccct	tctcacagcc	tctatcagga	6050
accctcagct	ctaccccttg	aagccctgta	aaggcactgc	ttccatgacc	6100
ctgggctgcc	tggtaaagga	ctacttccct	ggctcctgtga	ctgtgacctg	6150
gtattcagac	tccttgaaca	tgagcaactgt	gaacttccct	gcccttggtt	6200
ctgaactcaa	ggtcaccacc	agccaagtga	ccagctgggg	caagtcagcc	6250
aagaacttca	catgccacgt	gacacatcct	ccatcattca	acgaaagtag	6300
gactatccta	ggtaagtagg	gatgggctga	cagttacact	gtgtattctc	6350
ccttgagat	ggaacagttt	ctgtctaatc	aggaacttgt	cacaatttcc	6400
tttcatagag	gacttcataa	gagatttttt	tttctacttc	tatcatgttt	6450
agtgtcccaa	atagattttt	aaaactgggt	gagtgcata	tacttttagc	6500
ctcagaagac	atcatgtata	tttaagaggc	atttaactat	tgtaaattat	6550
tctgatgact	ttaaaaaaag	ttaatgctga	gttgtatatt	tttaaaataaa	6600
ttttattagt	ttagttttaa	aaaagaaaag	aaaattatta	atttttattta	6650
aaaatctcct	atattttaaa	aaaaaagaga	aaaaagcaga	gctgggctgg	6700
ctacagttac	cacaagaaca	tggtcagagg	aggaaggagc	tcttatacat	6750
acctatgaca	ggagaacggg	agacccaaca	tactcggggg	cctaccttca	6800
gagaacacaa	ggccagggca	atactcacag	ctcattgttc	gacctgtccc	6850
tagttcgacc	tgtaaacatc	actgagccca	ccttgagact	actccattca	6900
tcctgcgacc	ccaatgcttt	ccactccacc	atccagctgt	actgcttcat	6950
ttatggccac	atcctaaatg	atgtctctgt	cagctggcag	atct	6994

FIG. 12-3





INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/025507

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/85 A01K67/027
ADD. G01M33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZARRIN A.A. ET AL.: "S[gamma]3 switch sequences function in place of endogenous S[gamma]1 to mediate antibody class switching." J. EXP. MED., vol. 205, no. 7, 7 July 2008 (2008-07-07), pages 1567-1572, XP002581013 the whole document ----- -/--	1-11, 15-18, 34

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

4 May 2010

Date of mailing of the international search report

14/06/2010

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Galli, Ivo

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/025507

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AAVV: "Mus musculus chromosome 12 genomic contig, strain C57BL/6J, nucleotides 25468161-25470628" 20 June 2007 (2007-06-20), XP002581014 Retrieved from the Internet: URL: http://www.ncbi.nlm.nih.gov/nuccore/NT_166318.1?report=fasta&log\$=seqview&from=25468161&to=25470628 [retrieved on 2010-05-04] cited in the application the whole document	1-11, 15-18,34
X	ZARRIN A.A. ET AL.: "Influence of switch region length on immunoglobulin class switch recombination" PROC. NATL. ACAD. SCI. USA, vol. 102, no. 7, 15 February 2005 (2005-02-15), pages 2466-2470, XP002581015 the whole document	12
X	LAURENCIKIENE J., TAMOSIUNAS V. & SEVERINSON E.: "Regulation of [epsilon] germline transcription and switch region mutations by IgH locus 3' enhancers in transgenic mice" IMMUNOBIOLOG., vol. 109, no. 1, 1 January 2007 (2007-01-01), pages 159-167, XP002581016 the whole document	19-27, 32,33,35
Y A	LEE G.R. & FLAVELL R.A.: "Transgenic mice which overproduce Th2 cytokines develop spontaneous atopic dermatitis and asthma" INTL. IMMUNOL., vol. 16, no. 8, 28 June 2004 (2004-06-28), pages 1155-1160, XP002581017 the whole document	28-30 12-14, 36,37
X	SINGER S.M., UMETSU D.T. & MCDEVITT H.O.: "High copy number I-Ab transgenes induce production of IgE through an interleukin 4-dependent mechanism" PROC. NATL. ACAD. SCI. USA, vol. 93, 1 April 1996 (1996-04-01), pages 2947-2952, XP002581018 the whole document	20-23
A		12-14

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/025507

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MANIS J P ET AL: "Mechanism and control of class-switch recombination" TRENDS IN IMMUNOLOGY, ELSEVIER, RAHWAY, NJ, US LNKD- DOI:10.1016/S1471-4906(01)02111-1, vol. 23, no. 1, 1 January 2002 (2002-01-01), pages 31-39, XP004332191 ISSN: 1471-4906 -----	1-30, 32-34

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2010/025507

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a. (means)
☐ on paper
☒ in electronic form
 - b. (time)
☐ in the international application as filed
☒ together with the international application in electronic form
☐ subsequently to this Authority for the purpose of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 31(completely); 12-30, 32-37(partially)

NO SEARCH

Present claim 31 encompasses compounds defined only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained. Moreover, no such compounds have been disclosed (Art. 5 PCT). The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed. Undue experimentation would be required to screen compounds randomly. The non-compliance with the substantive provisions is to such an extent that no meaningful search of claim 31 could be carried out at all (Article 17(2) PCT). LIMITED SEARCH The present application contains 37 claims, of which 14 are independent (see in particular claims 12-30, 32-37). These claims are not in compliance with the provisions of clarity and conciseness of Article 6 PCT. Moreover, claims 19-23, 34, 35 are drafted in terms of results to be achieved. The description does not provide support and disclosure in the sense of Article 6 and 5 PCT for that subject-matter with the exception of the disclosed substitution of S[μ] for S[ϵ]; there is also no common general knowledge of this kind available to the person skilled in the art. The non-compliance of claims 12-30, 32-37 with the substantive provisions is to such an extent that the search had to be limited pursuant to PCT Guidelines 9.19, 9.20 and 9.25, to that subject-matter which, as far as can be understood, could reasonably be expected to be claimed later in the procedure. To wit: (i) Transgenic models for enhanced IgE production, in particular involving the presence of a modified IgH gene (ii) Increasing the (probability of a) switch to a given IgH subtype by increasing the length of the corresponding switch region, with focus on S- ϵ (iii) Increasing the (probability of a) switch to a given IgH subtype by changing the corresponding switch region with another donor switch region, with focus on changing S- μ for S- ϵ .

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2010/025507

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 31(completely); 12-30, 32-37(partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.