

(51) International Patent Classification:
C12N 15/09 (2006.01)(21) International Application Number:
PCT/US2009/058494(22) International Filing Date:
25 September 2009 (25.09.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/100,075 25 September 2008 (25.09.2008) US
61/231,555 5 August 2009 (05.08.2009) US(71) Applicants (for all designated States except US):
TRANSGENRX, INC. [US/US]; P. O. Box 25088, Baton Rouge, LA 70894 (US). **THE BOARD OF SUPERVISORS OF LOUISIANA STATE UNIVERSITY AND AGRICULTURAL AND MECHANICAL COLLEGE** [US/US]; P. O. Box 25055, Baton Rouge, LA 70894-5055 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **FIORETTI, William, C.** [US/US]; 5015 Addison Circle, #408, Addison, TX 75001 (US). **COOPER, Richard, K.** [US/US]; 622 Woodview Court, Baton Rouge, LA 70810 (US).(74) Agents: **PRATT, John** et al.; 1100 Peachtree Street NE, Suite 2800, Atlanta, GA 30309 (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, 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:

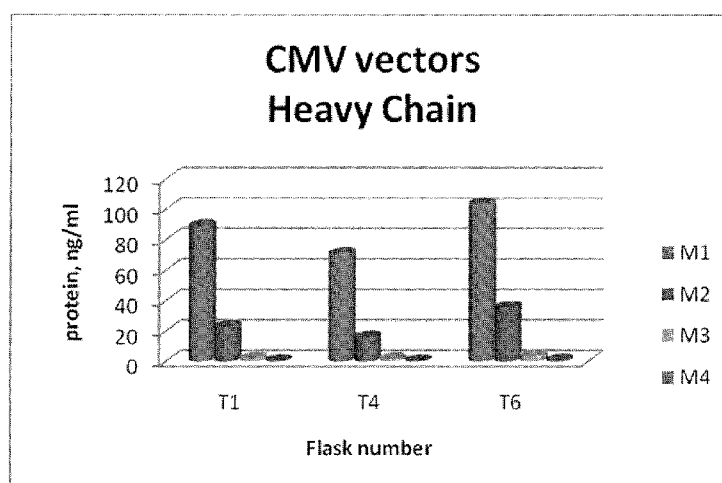
— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

[Continued on next page]

(54) Title: NOVEL VECTORS FOR PRODUCTION OF ANTIBODIES

FIG. 6

A.



(57) **Abstract:** Novel compositions for the production of the light and heavy chains of an antibody are provided. These novel compositions are also used to produce germline transgenic birds that can successfully pass the transgene encoding the antibody to their offspring. The compositions comprise components of vectors, such as a vector backbone, a promoter, and a gene of interest that encodes the light or heavy chain of an antibody, and the vectors comprising these components. In certain embodiments, these vectors are transposon-based vectors. Also provided are methods of making these compositions and methods of using these compositions for the production of the light and heavy chains of an antibody. In one embodiment, the antibody is a human monoclonal antibody.



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

NOVEL VECTORS FOR PRODUCTION OF ANTIBODIES

FIELD OF THE INVENTION

The present disclosure relates to compositions and methods for the production of an antibody, for example, a monoclonal antibody (mAb). In particular, the disclosure relates to transposon based vectors and their use in methods for the efficient expression of an antibody, for example, a monoclonal antibody.

BACKGROUND OF THE INVENTION

Antibodies, or immunoglobulins, are proteins produced by cells of the immune system to identify and neutralize foreign substances, such as bacteria, viruses, or improperly proliferating native cells. An antibody consists of two larger “heavy” chain polypeptides and two smaller “light” chain polypeptides. Both the heavy and the light chain polypeptides contain a constant region and a variable region. It is the variable region that allows each antibody to specifically recognize a different target (*i.e.*, antigen). The recognition of a foreign antigen by a specific antibody allows other parts of the immune system to recognize and attack the foreign antigen. In addition, an antibody can directly neutralize a bacterial or viral cell by binding to a part of the bacterial or viral cell that is needed for infection.

Antibodies are useful both for therapeutic and diagnostic functions. For example, anti-Rhesus factor D (anti-RhD) antibodies are regularly given as a prenatal therapy to mothers who are Rhesus factor negative (Rh^-) to prevent the mother’s body from attacking the Rhesus factor positive (Rh^+) cells of a baby that she is carrying. Antibodies also are currently being used for a number of other therapeutic applications, such as for the treatment of rheumatoid arthritis, multiple sclerosis, psoriasis, transplant rejection, cardiovascular disease, inflammatory diseases (*e.g.*, autoimmune-related disorders), respiratory syncytial virus infections, macular degeneration, and many forms of cancer including non-Hodgkin’s lymphoma, colorectal cancer, head and neck cancer, chronic lymphocytic leukemia, and breast cancer. Some immune deficiencies, such as X-linked agammaglobulinemia and hypogammaglobulinemia also are treated with antibodies. In addition, antibodies are used to detect bacterial or viral infections or to detect which particular allele of a protein is being produced by a patient.

The manufacture of therapeutic and/or diagnostic proteins such as antibodies, is an expensive process. Companies using recombinant techniques to manufacture these proteins are working at capacity and usually have a long waiting list to access their fermentation facilities.

What is needed, therefore, is a new, efficient, and economical approach to make antibodies *in vitro* or *in vivo*.

SUMMARY

5 The present invention addresses these needs by providing novel compositions which can be used to transfect cells for production of antibodies *in vivo* and *in vitro*, and which can be used for the production of transgenic animals that can transmit the gene encoding an antibody to their offspring. These novel compositions include components of vectors such as a vector backbone (SEQ ID NOs:1-13), a novel promoter (SEQ ID NOs:14-15), and a gene of interest that encodes
10 for the light chain and/or heavy chain protein subunits of an antibody. The present vectors comprise an insulator element located between the transposon insertion sequences and the multicloning site on the vector. In one embodiment, the insulator element is selected from the group consisting of an HS4 element, a lysozyme replicator element, a combination of a lysozyme replicator element and an HS4 element, and a matrix attachment region element. The vectors
15 comprising each of these components are shown in SEQ ID NOs:17-27. In one embodiment, the gene of interest encodes a kappa chain or a lambda chain of an antibody. In one embodiment, the disclosed vectors are transposon-based vectors. The present invention also provides methods of making these compositions and methods of using these compositions for the production of antibodies. In certain embodiments, the antibody is a human monoclonal antibody. In one
20 embodiment, the antibody is a human RM2 monoclonal antibody or herceptin. Some of these vectors have been used to transfect germline cells of birds through cardiac injection. The transgene in these vectors has been successfully passed through two generations of offspring, demonstrating stable integration and inheritance of the transgene.

 It is to be understood that different cells may be transfected *in vitro* with one of the
25 presently disclosed compositions, provided the cells contain protein synthetic biochemical pathways for the expression of the gene of interest. For example, both prokaryotic cells and eukaryotic cells may be transfected with one of the disclosed compositions. In certain embodiments, animal or plant cells are transfected. Animal cells are preferred cells and include, for example, mammalian cells and avian cells. Animal cells that may be transfected include, but
30 are not limited to, Chinese hamster ovary (CHO) cells, CHO-K1 cells, chicken embryonic fibroblasts, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, A20 cells, EL4 cells, HepG2 cells, J744A cells, Jurkat cells, P388D1 cells, RC-4B/c cells, SK-N-SH cells, Sp2/mIL-6 cells, SW480 cells, 3T6 Swiss cells, human ARPT-19 (human pigmented retinal epithelial) cells, LMH cells, LMH2a cells, tubular gland cells, or hybridomas.

In one embodiment, avian cells are transfected with one of the disclosed compositions. In a specific embodiment, avian hepatocytes, hepatocyte-related cells, or tubular gland cells are transfected. In certain embodiments, chicken cells are transfected with one of the disclosed compositions. In one embodiment, chicken tubular gland cells, chicken embryonic fibroblasts, 5 chicken LMH2A cells, or chicken LMH cells are transfected with one of the disclosed compositions. Chicken LMH and LMH2A cells are chicken hepatoma cell lines; LMH2A cells have been transformed to express estrogen receptors on their cell surface.

In other embodiments, mammalian cells are transfected with one of the disclosed compositions. In one embodiment, Chinese hamster ovary (CHO) cells, ARPT-19 cells, chicken 10 embryonic fibroblasts, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, or hybridomas are transfected for antibody production. In a specific embodiment, CHO-K1 cells or ARPT-19 cells are transfected with one of the disclosed compositions.

The present invention provides compositions and methods for efficient production of antibodies. These methods enable production of large quantities of bioactive antibodies. In some 15 embodiments, the antibodies are produced at a level of between about 25 g protein/month and about 4 kg protein/month.

These vectors may also be used *in vivo* to transfect germline cells in animals such as birds which can be bred and which then pass the antibody transgene through several generations. These vectors also may be used for the production of antibodies *in vivo*, for example, for deposition in 20 an egg.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the structure of two different hybrid promoters. Figure 1A is a schematic of the Version 1 CMV/Oval promoter 1 (ChOvp/CMVenh/CMVp; SEQ ID NO:14). Figure 1B is 25 a schematic of the Version 2 CMV/Oval promoter (SEQ ID NO:15; ChSDRE/CMVenh/ChNRE/CMVp).

Figure 2 is a schematic showing vector #148 (SEQ ID NO:17) used for expression of an RM2 monoclonal antibody.

Figure 3 is a schematic showing vector #210 (SEQ ID NO:18) used for expression of an 30 RM2 monoclonal antibody.

Figure 4 is a schematic showing vector #212 (SEQ ID NO:19) used for expression of an RM2 monoclonal antibody.

Figure 5 is a schematic showing vector #226 (SEQ ID NO:20) used for expression of an RM2 monoclonal antibody.

Figure 6 is a graph showing the results of an enzyme linked immunosorbent assay (ELISA) demonstrating the efficient expression of the RM2 monoclonal antibody light chain (Panel B) and heavy chain (Panel A) in LMH2A cells using the expression vectors described herein. T1, T4, and T6 correspond to cells transfected with SEQ ID NOs:17, 18, and 19, respectively. Control flasks also were run, but exhibited readings that were too low to detect (data not shown). M1, M2, M3, and M4 are samples taken 3 days, 8 days, 11 days, and 14 days post transfection, respectively. The bars from left to right in each group correspond to M1, M2, M3, and M4 samples, respectively.

Figure 7 is a graph showing the results of an enzyme linked immunosorbent assay (ELISA) demonstrating the efficient expression of the RM2 monoclonal antibody light chain (LC) and heavy chain (HC) in LMH and LMH2A (2A's) cells using vector #226 (SEQ ID NO:20) described herein. M1, M2, M3, and M4 represent samples taken at 2, 6, 9 and 12 days later, respectively.

Figure 8 is a graph showing the results of an ELISA demonstrating the efficient expression of the RM2 monoclonal antibody heavy chain in LMH (left three groups) and LMH2A (right three groups) cells using vector #226 (SEQ ID NO:20) described herein. M1, M2, and M3 represent samples taken at 3, 7, and 10 days later, respectively, and are shown in each group from left (M1) to right (M3).

Figure 9 is a graph showing the results of an ELISA demonstrating the efficient expression of the RM2 monoclonal antibody light chain in LMH (left three groups) and LMH2A (right three groups) cells using vector #226 (SEQ ID NO:20) described herein. M1, M2, and M3 represent samples taken at 3, 7, and 10 days later, respectively, and are shown in each group from left (M1) to right (M3).

Figure 10 is a graph showing the results of an ELISA demonstrating the efficient expression of the RM2 monoclonal antibody light chain (ng/ml) in LMH and LMH2A cells using the expression vectors described herein (#275 (SEQ ID NO:21) and (#273 (SEQ ID NO:23)). M1, M2, and M3 represent samples taken at 3, 6, and 9 days post-transfection, respectively, and are shown in each group from left (M1) to right (M3).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

The present invention provides novel vectors and vector components for use in antibody production. In one embodiment, the vectors are used for transfecting cells for antibody production *in vitro*. In another embodiment, the vectors are used for transfecting cells for antibody production *in vivo*. The present invention provides methods to make these vector

components, methods to make the vectors themselves, and methods for using these vectors to transfect cells such that the transfected cells produce antibodies *in vitro*. These vectors also may be used *in vivo* to transfect germline cells in animals, such as birds, which can be bred and which then pass the transgene through several generations. These vectors may be used *in vivo* to transfect cells such as secretory cells in birds which may produce the antibodies, for example, for deposition into an egg of the bird.

It is to be understood that different cells may be transfected *in vitro* with one of the presently disclosed compositions, provided the cells contain protein synthetic biochemical pathways for the expression of the gene of interest. For example, both prokaryotic cells and eukaryotic cells may be transfected with one of the disclosed compositions. In certain embodiments, animal or plant cells are transfected. Animal cells are preferred cells and include, for example, mammalian cells and avian cells. Animal cells that may be transfected include, but are not limited to, Chinese hamster ovary (CHO) cells, CHO-K1 cells, chicken embryonic fibroblasts, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, A20 cells, EL4 cells, HepG2 cells, J744A cells, Jurkat cells, P388D1 cells, RC-4B/c cells, SK-N-SH cells, Sp2/mIL-6 cells, SW480 cells, 3T6 Swiss cells, human ARPT-19 (human pigmented retinal epithelial) cells, LMH cells, LMH2a cells, tubular gland cells, or hybridomas. Avian cells include, but are not limited to, LMH, LMH2a cells, chicken embryonic fibroblasts, and tubular gland cells. In one embodiment, avian cells are transfected with one of the disclosed compositions. In a specific embodiment, avian hepatocytes, hepatocyte-related cells, or tubular gland cells are transfected. In certain embodiments, chicken cells are transfected with one of the disclosed compositions. In one embodiment, chicken tubular gland cells, chicken embryonic fibroblasts, chicken LMH2A or chicken LMH cells are transfected with one of the disclosed compositions. Chicken LMH and LMH2A cells are chicken hepatoma cell lines; LMH2A cells have been transformed to express estrogen receptors on their cell surface.

In other embodiments, mammalian cells are transfected with one of the disclosed compositions. In one embodiment, Chinese hamster ovary (CHO) cells, ARPT-19 cells, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, or hybridomas are transfected for antibody production. In a specific embodiment, CHO-K1 cells or ARPT-19 cells are transfected with one of the disclosed compositions.

As used herein, the terms “antibody” or “antibodies” refer to a light chain and heavy chain protein of an antibody that are encoded by a gene or genes that are either a naturally occurring gene or a codon-optimized gene. As used herein, the term “codon-optimized” means that the DNA sequence has been changed such that where several different codons code for the

same amino acid residue, the sequence selected for the gene is the one that is most often utilized by the cell in which the gene is being expressed. For example, in some embodiments, the antibody light and heavy chain genes are expressed in LMH2A cells and include codon sequences that are preferred in that cell type. In certain embodiments, the antibody light chain and heavy chain genes are human antibody light chain and heavy chain genes. In certain embodiments, the light chain is either a kappa chain or a lambda chain.

In one embodiment, the genes encode RM2 monoclonal antibody heavy and light chain subunits and are shown at nucleotides 9274-10644 and 6623-7270 of SEQ ID NO:17, nucleotides 9268-10638 and 6617-7264 of SEQ ID NO:18, nucleotides 9719-11089 and 7068-7715 of SEQ ID NO:19, nucleotides 9719-11089 and 7068-7715 of SEQ ID NO:20, and nucleotides 11766-13133 and 7918-8565 of SEQ ID NO:21, respectively.

In one embodiment, the vectors contain a gene of interest encoding an antibody, for the production of such antibody by transfected cells *in vitro*. In one embodiment, the vectors of the present invention contain a gene encoding a heavy chain of an antibody for the production of such protein by transfected cells *in vitro*. In another embodiment, the vectors contain a gene encoding a light chain of an antibody for the production of such protein by transfected cells *in vitro*. In another embodiment, the vectors contain a gene encoding a monoclonal antibody for the production of such protein by transfected cells *in vitro*.

Immunoglobulins are one class of desired globulin molecules and include, but are not limited to, IgG, IgM, IgA, IgD, IgE, IgY, lambda chains, kappa chains and fragments thereof; bi-specific antibodies, and fragments thereof; scFv fragments, Fc fragments, and Fab fragments as well as dimeric, trimeric and oligomeric forms of antibody fragments. Desired antibodies include, but are not limited to, naturally occurring antibodies, animal-specific antibodies, human antibodies, humanized antibodies, autoantibodies and hybrid antibodies. Genes encoding modified versions of naturally occurring antibodies or fragments thereof and genes encoding artificially designed antibodies or fragments thereof may be incorporated into the transposon-based vectors of the present invention. Desired antibodies also include antibodies with the ability to bind specific ligands, for example, antibodies against proteins associated with cancer-related molecules, such as anti-her 2, or anti-CA125. Accordingly, the present invention encompasses a transposon-based vector containing one or more genes encoding a heavy immunoglobulin (Ig) chain and a light Ig chain. Further, more than one gene encoding for more than one antibody may be administered in one or more transposon-based vectors of the present invention. Such administration may be *in vivo* or *in vitro* to transfect cultured cells. In this manner, antibodies may be made in liver cells or another cell selected for transfection, such as fibroblasts and

released *in vitro*, or released locally, or gain access to the circulation *in vivo*. In one embodiment, a transposon-based vector contains a heavy Ig chain and a light Ig chain, both operably linked to a promoter.

Antibodies used as therapeutic reagents include but are not limited to antibodies for use in cancer immunotherapy against specific antigens, or for providing passive immunity to an animal against an infectious disease or a toxic agent. Antibodies may be made by the animal receiving the transposon-based vectors to facilitate the animal's immune response to a selected antigen.

Antibodies that may be made with the practice of the present invention include, but are not limited to primary antibodies, secondary antibodies, designer antibodies, anti-protein antibodies, anti-peptide antibodies, anti-DNA antibodies, anti-RNA antibodies, anti-hormone antibodies, anti-hypophysiotropic peptides, antibodies against non-natural antigens, anti-anterior pituitary hormone antibodies, anti-posterior pituitary hormone antibodies, anti-venom antibodies, anti-tumor marker antibodies, antibodies directed against epitopes associated with infectious disease, including, anti-viral, anti-bacterial, anti-protozoal, anti-fungal, anti-parasitic, anti-receptor, anti-lipid, anti-phospholipid, anti-growth factor, anti-cytokine, anti-monokine, anti-idiotypic, and anti-accessory (presentation) protein antibodies. Antibodies made with the present invention, as well as light chains or heavy chains, may also be used to inhibit enzyme activity.

Antibodies that may be produced using the present invention include, but are not limited to, antibodies made against the following proteins: Bovine γ -Globulin, Serum; Bovine IgG, Plasma; Chicken γ -Globulin, Serum; Human γ -Globulin, Serum; Human IgA, Plasma; Human IgA₁, Myeloma; Human IgA₂, Myeloma; Human IgA₂, Plasma; Human IgD, Plasma; Human IgE, Myeloma; Human IgG, Plasma; Human IgG, Fab Fragment, Plasma; Human IgG, F(ab')₂ Fragment, Plasma; Human IgG, Fc Fragment, Plasma; Human IgG₁, Myeloma; Human IgG₂, Myeloma; Human IgG₃, Myeloma; Human IgG₄, Myeloma; Human IgM, Myeloma; Human IgM, Plasma; Human Immunoglobulin, Light Chain κ , Urine; Human Immunoglobulin, Light Chains κ and λ , Plasma; Mouse γ -Globulin, Serum; Mouse IgG, Serum; Mouse IgM, Myeloma; Rabbit γ -Globulin, Serum; Rabbit IgG, Plasma; and Rat γ -Globulin, Serum. In one embodiment, the transposon-based vector comprises the coding sequence of light and heavy chains of a murine monoclonal antibody that shows specificity for human seminoprotein (GenBank Accession numbers AY129006 and AY129304 for the light and heavy chains, respectively).

A further non-limiting list of antibodies that recognize other antibodies is as follows: Anti-Chicken IgG, heavy (H) & light (L) Chain Specific (Sheep); Anti-Goat γ -Globulin (Donkey); Anti-Goat IgG, Fc Fragment Specific (Rabbit); Anti-Guinea Pig γ -Globulin (Goat); Anti-Human Ig, Light Chain, Type κ Specific; Anti-Human Ig, Light Chain, Type λ Specific;

Anti-Human IgA, α -Chain Specific (Goat); Anti-Human IgA, Fab Fragment Specific; Anti-Human IgA, Fc Fragment Specific; Anti-Human IgA, Secretory; Anti-Human IgE, ϵ -Chain Specific (Goat); Anti-Human IgE, Fc Fragment Specific; Anti-Human IgG, Fc Fragment Specific (Goat); Anti-Human IgG, γ -Chain Specific (Goat); Anti-Human IgG, Fc Fragment Specific; Anti-Human IgG, Fd Fragment Specific; Anti-Human IgG, H & L Chain Specific (Goat); Anti-Human IgG₁, Fc Fragment Specific; Anti-Human IgG₂, Fc Fragment Specific; Anti-Human IgG₂, Fd Fragment Specific; Anti-Human IgG₃, Hinge Specific; Anti-Human IgG₄, Fc Fragment Specific; Anti-Human IgM, Fc Fragment Specific; Anti-Human IgM, μ -Chain Specific; Anti-Mouse IgE, ϵ -Chain Specific; Anti-Mouse γ -Globulin (Goat); Anti-Mouse IgG, γ -Chain Specific (Goat); Anti-Mouse IgG, γ -Chain Specific (Goat) F(ab')₂ Fragment; Anti-Mouse IgG, H & L Chain Specific (Goat); Anti-Mouse IgM, μ -Chain Specific (Goat); Anti-Mouse IgM, H & L Chain Specific (Goat); Anti-Rabbit γ -Globulin (Goat); Anti-Rabbit IgG, Fc Fragment Specific (Goat); Anti-Rabbit IgG, H & L Chain Specific (Goat); Anti-Rat γ -Globulin (Goat); Anti-Rat IgG, H & L Chain Specific; Anti-Rhesus Monkey γ -Globulin (Goat); and, Anti-Sheep IgG, H & L Chain Specific.

Another non-limiting list of the antibodies that may be produced using the present invention is provided in product catalogs of companies such as Phoenix Pharmaceuticals, Inc. (Burlingame, CA), Peninsula Labs (San Carlos CA), SIGMA (St. Louis, MO), Cappel ICN (Irvine, CA), and Calbiochem (La Jolla, CA), which are all incorporated herein by reference in their entirety. The polynucleotide sequences encoding these antibodies may be obtained from the scientific literature, from patents, and from databases such as GenBank. Alternatively, one of ordinary skill in the art may design the polynucleotide sequence to be incorporated into the genome by choosing the codons that encode for each amino acid in the desired antibody. Antibodies made by the transgenic animals *in vivo* or transfected cells *in vitro* of the present invention include antibodies that may be used as therapeutic reagents, diagnostic reagents or laboratory reagents. Some of these antibodies include, but are not limited to, antibodies which bind the following ligands: adrenomedulin, amylin, calcitonin, amyloid, calcitonin gene-related peptide, cholecystokinin, gastrin, gastric inhibitory peptide, gastrin releasing peptide, interleukin, interferon, cortistatin, somatostatin, endothelin, sarafotoxin, glucagon, glucagon-like peptide, insulin, atrial natriuretic peptide, BNP, CNP, neurokinin, substance P, leptin, neuropeptide Y, melanin concentrating hormone, melanocyte stimulating hormone, orphanin, endorphin, dynorphin, enkephalin, enkephalin, leumorphin, peptide F, PACAP, PACAP-related peptide, parathyroid hormone, urocortin, corticotrophin releasing hormone, PHM, PHI, vasoactive intestinal polypeptide, secretin, ACTH, angiotensin, angiotensin, bombesin, endostatin,

bradykinin, FMRF amide, galanin, gonadotropin releasing hormone (GnRH) associated peptide, GnRH, growth hormone releasing hormone, inhibin, granulocyte-macrophage colony stimulating factor (GM-CSF), motilin, neurotensin, oxytocin, vasopressin, osteocalcin, pancreastatin, pancreatic polypeptide, peptide YY, proopiomelanocortin, transforming growth factor, vascular endothelial growth factor, vesicular monoamine transporter, vesicular acetylcholine transporter, ghrelin, NPW, NPB, C3d, prokineticin, thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, prolactin, growth hormone, beta-lipotropin, melatonin, kallikriens, kinins, prostaglandins, erythropoietin, p146, estrogen, testosterone, corticosteroids, mineralocorticoids, thyroid hormone, thymic hormones, connective tissue proteins, nuclear proteins, actin, avidin, activin, agrin, albumin, and prohormones, propeptides, splice variants, fragments and analogs thereof.

The following is yet another non-limiting list of antibodies that can be produced by the methods of present invention: abciximab (ReoPro), abciximab anti-platelet aggregation monoclonal antibody, anti-CD11a (hu1124), anti-CD18 antibody, anti-CD20 antibody, anti-cytomegalovirus (CMV) antibody, anti-digoxin antibody, anti-hepatitis B antibody, anti-HER-2 antibody, anti-idiotypic antibody to GD3 glycolipid, anti-IgE antibody, anti-IL-2R antibody, antimetastatic cancer antibody (mAb 17-1A), anti-rabies antibody, anti-respiratory syncytial virus (RSV) antibody, anti-Rh antibody, anti-TCR, anti-TNF antibody, anti-VEGF antibody and Fab fragment thereof, rattlesnake venom antibody, black widow spider venom antibody, coral snake venom antibody, antibody against very late antigen-4 (VLA-4), C225 humanized antibody to EGF receptor, chimeric (human & mouse) antibody against TNF α , antibody directed against GPIIb/IIIa receptor on human platelets, gamma globulin, anti-hepatitis B immunoglobulin, human anti-D immunoglobulin, human antibodies against *S aureus*, human tetanus immunoglobulin, humanized antibody against the epidermal growth receptor-2, humanized antibody against the α subunit of the interleukin-2 receptor, humanized antibody CTLA4IG, humanized antibody to the IL-2 R α -chain, humanized anti-CD40-ligand monoclonal antibody (5c8), humanized mAb against the epidermal growth receptor-2, humanized mAb to rous sarcoma virus, humanized recombinant antibody (IgG1k) against respiratory syncytial virus (RSV), lymphocyte immunoglobulin (anti-thymocyte antibody), lymphocyte immunoglobulin, mAb against factor VII, MDX-210 bi-specific antibody against HER-2, MDX-22, MDX-220 bi-specific antibody against TAG-72 on tumors, MDX-33 antibody to Fc γ R1 receptor, MDX-447 bi-specific antibody against EGF receptor, MDX-447 bispecific humanized antibody to EGF receptor, MDX-RA immunotoxin (ricin A linked) antibody, Medi-507 antibody (humanized form of BTI-322) against CD2 receptor on T-cells, monoclonal antibody LDP-02, muromonab-

CD3(OKT3) antibody, OKT3 ("muromomab-CD3") antibody, PRO 542 antibody, ReoPro ("abciximab") antibody, and TNF-IgG fusion protein. It is to be understood that wherever the term "humanized" appears in the present patent application with regard to an antibody or molecule, that an antibody or molecule may be designed to be specific for any animal using
5 selected polynucleotide sequences in the gene of interest included in the transposon-based vectors. Antibodies may be made against any selected antigen known to one of ordinary skill in the art.

The antibodies prepared using the methods of the present invention may also be designed to possess specific labels that may be detected through means known to one of ordinary skill in
10 the art so that their location and distribution can be assessed following gene therapy and expression of the antibodies. The antibodies may also be designed to possess specific sequences useful for purification through means known to one of ordinary skill in the art. Specialty antibodies designed for binding specific antigens may also be made in transgenic animals using the transposon-based vectors of the present invention.

As used herein, the proteins referred to herein encompass a protein that is encoded by a gene that is either a naturally occurring or a codon-optimized gene. As used herein, the term "codon-optimized" means that the DNA sequence has been changed such that where several different codons code for the same amino acid residue, the sequence selected for the gene is the one that is most often utilized by the cell in which the gene is being expressed. For example, in
15 some embodiments, the gene of interest is expressed in LMH or LMH2A cells and includes codon sequences that are preferred in that cell type.

In one embodiment, the vectors of the present invention contain a gene or genes (called a gene(s) of interest) encoding for a protein or protein of interest for the production of such protein by transfected cells *in vitro*. As used herein, the "protein of interest" is an antibody or antibody
25 light chain or heavy chain. In one embodiment, the vectors of the present invention contain a gene encoding a heavy chain of an antibody for the production of such protein by transfected cells *in vitro*. In one embodiment, the vectors of the present invention contain a gene encoding a light chain of an antibody for the production of such protein by transfected cells *in vitro*. In one embodiment, the vectors of the present invention contain a gene encoding a monoclonal antibody
30 for the production of such protein by transfected cells *in vitro*.

A. Vectors & Vector Components

The following paragraphs describe the novel vector components and vectors employed in the present invention.

1. *Backbone Vectors*

The backbone vectors provide the vector components minus the genes of interest (GOIs) that encode for the light and heavy chains of an antibody. In one embodiment, transposon-based vectors are used. The present vectors comprise an insulator element located between the transposon insertion sequences and the multicloning site on the vector. In one embodiment, the insulator element is selected from the group consisting of an HS4 element, a lysozyme replicator element, a combination of a lysozyme replicator element and an HS4 element, and a matrix attachment region element.

a. *Transposon-Based Vector Tn-MCS #5001 (p5001) (SEQ ID NO:1)*

Linear sequences were amplified using plasmid DNA from pBluescriptII sk(-) (Stratagene, La Jolla, CA), pGWIZ (Gene Therapy Systems, San Diego, CA), pNK2859 (Dr. Nancy Kleckner, Department of Biochemistry and Molecular Biology, Harvard University), and synthetic linear DNA constructed from specifically designed DNA Oligonucleotides (Integrated DNA Technologies, Coralville, IA). PCR was set up using the above referenced DNA as template, electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using Zymo Research's Clean Gel Recovery Kit (Orange, CA). The resulting products were cloned into the Invitrogen's PCR Blunt II Topo plasmid (Carlsbad, CA) according to the manufacturer's protocol.

After sequence verification, subsequent clones were selected and digested from the PCR Blunt II Topo Vector (Invitrogen Life Technologies, Carlsbad, CA) with corresponding enzymes (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. The linear pieces were ligated together using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated products were transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread to LB (Luria-Bertani) agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using Qiagen's Maxi-Prep Kit according to the manufacturer's protocol (Chatsworth, CA). The DNA was used as a sequencing template to

verify that the pieces were ligated together accurately to form the desired vector sequence. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that consisted of the desired sequence, the DNA was isolated for use in cloning in specific genes of interest.

5 b. *Preparation of Transposon-Based Vector TnX-MCS #5005 (p5005)*

This vector (SEQ ID NO:2) is a modification of p5001 (SEQ ID NO:1) described above in section 1.a. The MCS extension was designed to add unique restriction sites to the multiple cloning site of the pTn-MCS vector (SEQ ID NO:1), creating pTnX-MCS (SEQ ID NO:2), in order to increase the ligation efficiency of constructed cassettes into the backbone vector. The first step was to create a list of all non-cutting enzymes for the current pTn-MCS DNA sequence (SEQ ID NO:1). A linear sequence was designed using the list of enzymes and compressing the restriction site sequences together. Necessary restriction site sequences for XhoI and PspOMI (New England Biolabs, Beverly, MA) were then added to each end of this sequence for use in splicing this MCS extension into the pTn-MCS backbone (SEQ ID NO:1). The resulting sequence of 108 bases is SEQ ID NO:16 shown in the Appendix. A subset of these bases within this 108 base pair sequence corresponds to bases 4917-5012 in SEQ ID NO:4 (discussed below).

For construction, the sequence was split at the NarI restriction site and divided into two sections. Both 5' forward and 3' reverse oligonucleotides (Integrated DNA Technologies, San Diego, CA) were synthesized for each of the two sections. The 5' and 3' oligonucleotides for each section were annealed together, and the resulting synthetic DNA sections were digested with NarI then subsequently ligated together to form the 108 bp MCS extension (SEQ ID NO:16). PCR was set up on the ligation, electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The resulting product was cloned into the PCR Blunt II Topo Vector (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol.

After sequence verification of the MCS extension sequence (SEQ ID NO:16), a clone was selected and digested from the PCR Blunt II Topo Vector (Invitrogen Life Technologies, Carlsbad, CA) with XhoI and PspOMI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. The pTn-MCS vector (SEQ ID NO:1) also was digested with XhoI and PspOMI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol, purified as described above, and the two pieces were ligated together using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E.*

coli Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 mls of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the multiple cloning site extension, the DNA was isolated and used for cloning specific genes of interest.

c. Preparation of Transposon-Based Vector TnHS4FBV #5006 (p5006)

This vector (SEQ ID NO:3) is a modification of p5005 (SEQ ID NO:2) described above in section 1.b. The modification includes insertion of the HS4 beta globin insulator element on both the 5' and 3' ends of the multiple cloning site. The 1241 bp HS4 element was isolated from chicken genomic DNA and amplified through polymerase chain reaction (PCR) using conditions known to one skilled in the art. The PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size of the HS4 beta globin insulator element were excised from the agarose gel and purified using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified HS4 DNA was digested with restriction enzymes NotI, XhoI, PspOMI, and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. The digested DNA was then purified using a Zymo DNA Clean and Concentrator kit (Orange, CA). To insert the 5' HS4 element into the MCS of the p5005 vector (SEQ ID NO:2), HS4 DNA and vector p5005 (SEQ ID NO:2) were digested with NotI and XhoI restriction enzymes, purified as described above, and ligated using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. To insert the 3' HS4 element into the MCS of the p5005 vector (SEQ ID NO:2), HS4 and vector p5005 DNA (SEQ ID NO:2) were digested with PspOMI and MluI, purified, and ligated as described above. Ligated product was transformed into *E. coli* Top10

cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 mls of LB/amp broth and plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as sequencing template to verify that any changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both HS4 elements, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 500 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

d. *Preparation of Transposon-Based Vector pTn10 HS4FBV #5012*

This vector (SEQ ID NO:4) is a modification of p5006 (SEQ ID NO:3) described above under section 1.c. The modification includes a base pair substitution in the transposase gene at base pair 1998 of p5006. The corrected transposase gene was amplified by PCR from template DNA, using PCR conditions known to one skilled in the art. PCR product of the corrected transposase was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified transposase DNA was digested with restriction enzymes NruI and StuI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction digests using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the corrected transposase sequence into the MCS of the p5006 vector (SEQ ID NO:3), the transposase DNA and the p5006 vector (SEQ ID NO:3) were digested with NruI

and StuI, purified as described above, and ligated using a Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C before spreading onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth. The plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the corrected transposase sequence, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest was grown in 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

e. Preparation of Transposon-Based Vector pTn-10 MARFBV #5018

This vector (SEQ ID NO:5) is a modification of p5012 (SEQ ID NO:4) described above under section 1.d. The modification includes insertion of the chicken 5' Matrix Attachment Region (MAR) on both the 5' and 3' ends of the multiple cloning site. To accomplish this, the 1.7 kb MAR element was isolated from chicken genomic DNA and amplified by PCR. PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. DNA bands corresponding to the expected size were excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified MAR DNA was digested with restriction enzymes NotI, XhoI, PspOMI, and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested

DNA was purified from agarose using a Zymo DNA Clean and Concentrator kit (Zymo Research, Orange CA). To insert the 5' MAR element into the MCS of p5012, the purified MAR DNA and p5012 were digested with Not I and Xho I, purified as described above, and ligated using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. To insert the 3' MAR element into the MCS of p5012, the purified MAR DNA and p5012 were digested with PspOMI and MluI, purified, and ligated as described above. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C and then spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth, and plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both MAR elements, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

f. *Preparation of Transposon-Based Vector TnLysRep #5020*

The vector (SEQ ID NO:6) included the chicken lysozyme replicator (LysRep or LR2) insulator elements to prevent gene silencing. Each LysRep element was ligated 3' to the insertion sequences (IS) of the vector. To accomplish this ligation, a 930 bp fragment of the chicken LysRep element (GenBank # NW 060235) was amplified using PCR conditions known to one skilled in the art. Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the

expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified LysRep DNA was sequentially digested with restriction enzymes Not I and Xho I (5'end) and Mlu I and Apa I (3'end) (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the LysRep elements between the IS left and the MCS in pTnX-MCS (SEQ ID NO:2), the purified LysRep DNA and pTnX-MCS were digested with Not I and Xho I, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB media (broth or agar) plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C, and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the 5' LysRep DNA, the vector was digested with Mlu I and Apa I as was the purified LysRep DNA. The same procedures described above were used to ligate the LysRep DNA into the backbone and verify that it was correct. Once a clone was identified that contained both LysRep elements, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

g. Preparation of Transposon-Based Vector TnPuro #5019 (p5019)

This vector (SEQ ID NO:7) is a modification of p5012 (SEQ ID NO:4) described above in section 1.d. The modification includes insertion of the puromycin gene in the multiple cloning site adjacent to one of the HS4 insulator elements. To accomplish this ligation, the 602 bp puromycin gene was isolated from the vector pMOD Puro (Invivogen, Inc.) using PCR conditions known to one skilled in the art. Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on a U.V. transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified Puro DNA was digested with restriction enzyme Kas I (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the Puro gene into the MCS of p5012, the purified Puro DNA and p5012 were digested with Kas I, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB (broth or agar) plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both Puro gene, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using

a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

h. *Preparation of Transposon-Based Vector pTn-10 PuroMAR #5021 (p5021)*

This vector (SEQ ID NO:8) is a modification of p5018 (SEQ ID NO:5) described above in section 1.e. The modification includes insertion of the puromycin (puro) gene into the multiple cloning site adjacent to one of the MAR insulator elements. To accomplish this, the 602 bp puromycin gene was amplified by PCR from the vector pMOD Puro (Invitrogen Life Technologies, Carlsbad, CA). Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified DNA from the puromycin gene was digested with the restriction enzymes BsiWI and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from agarose using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the puro gene into the MCS of p5018, puro and p5018 were digested with BsiWI and MluI, purified as described above, and ligated using Stratagene's T4 Ligase Kit (La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. The plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was used as a sequencing template to verify that the changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the puro gene, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid of interest was grown in 500 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria

using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

i. *Preparation of Transposon-Based Vector TnGenMAR #5022 (p5022)*

5 This vector (SEQ ID NO:9) is a modification of p5021 (SEQ ID NO:8) described above under section 1.h. The modification includes insertion of the gentamycin gene in the multiple cloning site adjacent to one of the MAR insulator elements. To accomplish this ligation, the 1251 bp gentamycin gene was isolated from the vector pS65T-C1(Clontech Laboratories, using PCR conditions known to one skilled in the art. Amplified PCR product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified gentamycin DNA was digested with restriction enzyme BsiW I and Mlu I (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the gentamycin gene into the MCS of p5018, the purified gentamycin DNA and p5018 were digested with BsiW I and Mlu I, purified as described above, and ligated using a Stratagene T4 Ligase Kit (Stratagene, Inc. La Jolla, CA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* Top10 competent cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to Invitrogen's protocol. Transformed bacteria were incubated in 1 ml of SOC (GIBCO BRL, CAT# 15544-042) medium for 1 hour at 37°C before being spread to LB (broth or agar) plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C, and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System. Once a clone was identified that contained both Puro gene, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol.

5 Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

j. *Preparation of Low Expression CMV Tn PuroMAR Flanked Backbone #5024 (p5024)*

This vector (SEQ ID NO:10) is a modification of p5018 (SEQ ID NO:5), which includes the deletion of the CMV Enhancer region of the transposase cassette. The CMV enhancer was
10 removed from p5018 by digesting the backbone with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size of the backbone without the enhancer region was excised from the gel and purified
15 from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Backbone DNA from above was re-circularized using an Epicentre Fast Ligase Kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's protocol. The ligation was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were
20 incubated in 250 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet
25 transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in 5ml of LB/amp broth. Plasmid DNA was harvested using Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen Burnie, MD). The DNA was then used as a sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was
30 performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified containing the replacement promoter fragment, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 ml of LB broth (supplemented with an

appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

5 k. *Preparation of Low Expression CMV Tn PuroMAR Flanked Backbone #5025 (p5025)*

This vector (SEQ ID NO:11) is a modification of p5021 (SEQ ID NO:8), which includes the deletion of the CMV Enhancer of on the transposase cassette. The CMV enhancer was removed from p5021 by digesting the backbone with MscI and AfeI restriction enzymes (New
10 England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size of the backbone without the enhancer region was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

15 Backbone DNA from above was re-circularized using an Epicentre Fast Ligase Kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's protocol. The ligation was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 250 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C then spread
20 onto LB (Luria-Bertani) agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected
25 size were cultured in 5 ml of LB/amp broth. Plasmid DNA was harvested using Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen Burnie, MD). The DNA was then used as a sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was
30 identified containing the replacement promoter fragment, the DNA was isolated and used for cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from

the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

1. *Preparation of Low Expression SV40 promoter Tn PuroMAR Flanked Backbone #5026 (p5026)*

This vector (SEQ ID NO:12) is a modification of p5018 (SEQ ID NO:5), which includes the replacement of the CMV Enhanced promoter of the transposase cassette, with the SV40 promoter from pS65T-C1 (Clontech, Mountainview, CA). The CMV enhanced promoter was removed from p5018 by digesting the backbone with MscI and AfeI restriction enzymes. (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The SV40 promoter fragment was amplified to add the 5' and 3' cut sites, MscI and AscI, respectively. The PCR product was then cloned into pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA). Sequence verified DNA was then digested out of the pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA), with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified digestion product was ligated into the excised backbone DNA using Epicentre's Fast Ligase Kit (Madison, WI) according to the manufacturer's protocol. The ligation product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were incubated in 250 ml of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37° C before then spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in 5 ml of LB/amp broth. The plasmid DNA was harvested using a Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen

Burnie, MD). The DNA was then used as sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the replacement promoter fragment, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

m. Preparation of Low Expression SV40 promoter Tn PuroMAR Flanked Backbone #5027 (p5027)

This vector (SEQ ID NO:13) is a modification of p5021 (SEQ ID NO:8), which includes the replacement of the CMV Enhanced promoter of the transposase cassette, with the SV40 promoter from pS65T-C1 (Clontech, Mountainview, CA). The CMV enhanced promoter was removed from p5021 by digesting the backbone with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The SV40 promoter fragment was amplified to add the 5' and 3' cut sites, MscI and AscI, respectively. The PCR product was then cloned into pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA). Sequence verified DNA was then digested out of the pTopo Blunt II backbone (Invitrogen Life Technologies, Carlsbad, CA), with MscI and AfeI restriction enzymes (New England Biolabs, Beverly, MA). The digested product was electrophoresed, stained with Syber Safe DNA Gel Stain (Invitrogen Life Technologies, Carlsbad, CA), and visualized on a Visi-Blue transilluminator (UVP Laboratory Products, Upland, CA). A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA).

Purified digestion product was ligated into the excised backbone DNA using Epicentre's Fast Ligase Kit (Madison, WI) according to the manufacturer's protocol. The ligation product was transformed into *E. coli* Top10 cells (Invitrogen Life Technologies, Carlsbad, CA) using chemical transformation according to the manufacturer's protocol. Transformed cells were

incubated in 250 µl of SOC (GIBCO BRL, CAT# 15544-042) for 1 hour at 37°C before being spread onto LB agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on an ultraviolet transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in 5 ml of LB/amp broth. The plasmid DNA was harvested using a Fermentas' Gene Jet Plasmid Miniprep Kit according to the manufacturer's protocol (Glen Burnie, MD). The DNA was then used as sequencing template to verify that any changes made in the vector were desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System. Once a clone was identified that contained the replacement promoter fragment, the DNA was isolated for use in cloning in specific genes of interest.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in a minimum of 500 mL of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

2. Promoters

A second embodiment of this invention are hybrid promoters that consist of elements from the constitutive CMV promoter and the estrogen inducible ovalbumin promoter. The goal of designing these promoters was to couple the high rate of expression associated with the CMV promoter with the estrogen inducible function of the ovalbumin promoter. To accomplish this goal, two hybrid promoters, designated versions 1 and 2 (SEQ ID NOs:14 and 15, respectively)(Figure 1), were designed, built, and tested in cell culture. Both versions 1 and 2 provided high rates of expression.

a. Version 1 CMV/Oval promoter 1 = ChOvp/CMVenh/CMVp

Hybrid promoter version 1 (SEQ ID NO:14) was constructed by ligating the chicken ovalbumin promoter regulatory elements to the 5' end of the CMV enhancer and promoter. A schematic is shown in Figure 1A.

Hybrid promoter version 1 was made by PCR amplifying nucleotides 1090 to 1929 of the ovalbumin promoter (GenBank # J00895) from the chicken genome and cloning this DNA

fragment into the pTopo vector (Invitrogen, Carlsbad, CA). Likewise, nucleotides 245-918 of the CMV promoter and enhancer were removed from the pgWiz vector (ClonTech, Mountain View, CA) and cloned into the pTopo vector. By cloning each fragment into the multiple cloning site of the pTopo vector, an array of restriction enzyme sites were available on each end of the DNA fragments which greatly facilitated cloning without PCR amplification. Each fragment was sequenced to verify it was the correct DNA sequence. Once sequence verified, the pTopo clone containing the ovalbumin promoter fragment was digested with Xho I and EcoR I, and the product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The pTopo clone containing the CMV promoter was treated in the same manner to open up the plasmid 5' to the CMV promoter; these restriction enzymes also allowed directional cloning of the ovalbumin promoter fragment upstream of CMV.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

b. Version 2 CMV/Oval promoter = ChSDRE/CMVenh/ChNRE/CMVp

Hybrid promoter version 2 (SEQ ID NO:15) consisted of the steroid dependent response element (SDRE) ligated 5' to the CMV enhancer (enh) and the CMV enhancer and promoter separated by the chicken ovalbumin negative response element (NRE).

A schematic is shown in Figure 1B. Hybrid promoter version 2 was made by PCR amplifying the steroid dependent response element (SDRE), nucleotides 1100 to 1389, and nucleotides 1640 to 1909 of the negative response element (NRE) of the ovalbumin promoter (GenBank # J00895) from the chicken genome and cloning each DNA fragment into the pTopo vector. Likewise, nucleotides 245-843 of the CMV enhancer and nucleotides 844-915 of the CMV promoter were removed from the pgWiz vector and each cloned into the pTopo vector. By cloning each piece into the multiple cloning site of the pTopo vector, an array of restriction enzyme sites were available on each end of the DNA fragments which greatly facilitated cloning without PCR amplification.

Each fragment was sequenced to verify it was the correct DNA sequence. Once sequence verified, the pTopo clone containing the ovalbumin SDRE fragment was digested with Xho I and EcoR I to remove the SDRE, and the product was electrophoresed on a 1% agarose gel, stained

with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The pTopo clone containing the CMV enhancer was treated in the same manner to open up the plasmid 5' to the CMV enhancer; these
5 restriction enzymes also allowed directional cloning of the ovalbumin SDRE fragment upstream of CMV. The ovalbumin NRE was removed from pTopo using NgoM IV and Kpn I; the same restriction enzymes were used to digest the pTopo clone containing the CMV promoter to allow directional cloning of the NRE.

The DNA fragments were purified as described above. The new pTopo vectors
10 containing the ovalbumin SDRE/CMV enhancer and the NRE/CMV promoter were sequence verified for the correct DNA sequence. Once sequence verified, the pTopo clone containing the ovalbumin SDRE/CMV enhancer fragment was digested with Xho I and NgoM IV to remove the SDRE/CMV Enhancer, and the product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized on an ultraviolet transilluminator. A band corresponding to the
15 expected size was excised from the gel and purified from the agarose using a Zymo Clean Gel Recovery Kit (Zymo Research, Orange, CA). The pTopo clone containing the NRE/CMV promoter was treated in the same manner to open up the plasmid 5' to the CMV enhancer. These restriction enzymes also allowed directional cloning of the ovalbumin SDRE fragment upstream of CMV. The resulting promoter hybrid was sequence verified to insure that
20 it was correct.

All plasmid DNA was isolated by standard procedures. Briefly, *E. coli* containing the plasmid were grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol.
25 Plasmid DNA was resuspended in 500 µL of PCR-grade water and stored at -20°C until needed.

3. *Transposases and Insertion Sequences*

In a further embodiment of the present invention, the transposase found in the transposase-based vector is an altered target site (ATS) transposase and the insertion sequences
30 are those recognized by the ATS transposase. However, the transposase located in the transposase-based vectors is not limited to a modified ATS transposase and can be derived from any transposase. Transposases known in the prior art include those found in AC7, Tn5SEQ1, Tn916, Tn951, Tn1721, Tn 2410, Tn1681, Tn1, Tn2, Tn3, Tn4, Tn5, Tn6, Tn9, Tn10, Tn30, Tn101, Tn903, Tn501, Tn1000 ($\gamma\delta$), Tn1681, Tn2901, AC transposons, Mp transposons, Spm

transposons, En transposons, Dotted transposons, Mu transposons, Ds transposons, dSpm transposons and I transposons. According to the present invention, these transposases and their regulatory sequences are modified for improved functioning as follows: a) the addition one or more modified Kozak sequences comprising any one of SEQ ID NOs:28 to 37 at the 3' end of the promoter operably-linked to the transposase; b) a change of the codons for the first several amino acids of the transposase, wherein the third base of each codon was changed to an A or a T without changing the corresponding amino acid; c) the addition of one or more stop codons to enhance the termination of transposase synthesis; and/or, d) the addition of an effective polyA sequence operably-linked to the transposase to further enhance expression of the transposase gene.

Although not wanting to be bound by the following statement, it is believed that the modifications of the first several N-terminal codons of the transposase gene increase transcription of the transposase gene, in part, by increasing strand dissociation. It is preferable that between approximately 1 and 20, more preferably 3 and 15, and most preferably between 4 and 12 of the first N-terminal codons of the transposase are modified such that the third base of each codon is changed to an A or a T without changing the encoded amino acid. In one embodiment, the first ten N-terminal codons of the transposase gene are modified in this manner. It is also preferred that the transposase contain mutations that make it less specific for preferred insertion sites and thus increases the rate of transgene insertion as discussed in U.S. Patent No. 5,719,055.

In some embodiments, the transposon-based vectors are optimized for expression in a particular host by changing the methylation patterns of the vector DNA. For example, prokaryotic methylation may be reduced by using a methylation deficient organism for production of the transposon-based vector. The transposon-based vectors may also be methylated to resemble eukaryotic DNA for expression in a eukaryotic host.

Transposases and insertion sequences from other analogous eukaryotic transposon-based vectors that can also be modified and used are, for example, the *Drosophila* P element derived vectors disclosed in U.S. Patent No. 6,291,243; the *Drosophila* mariner element described in Sherman et al. (1998); or the sleeping beauty transposon. See also Hackett et al. (1999); D. Lampe et al., 1999. Proc. Natl. Acad. Sci. USA, 96:11428-11433; S. Fischer et al., 2001. Proc. Natl. Acad. Sci. USA, 98:6759-6764; L. Zagoraiou et al., 2001. Proc. Natl. Acad. Sci. USA, 98:11474-11478; and D. Berg et al. (Eds.), *Mobile DNA*, Amer. Soc. Microbiol. (Washington, D.C., 1989). However, it should be noted that bacterial transposon-based elements are preferred, as there is less likelihood that a eukaryotic transposase in the recipient species will recognize prokaryotic insertion sequences bracketing the transgene.

Many transposases recognize different insertion sequences, and therefore, it is to be understood that a transposase-based vector will contain insertion sequences recognized by the particular transposase also found in the transposase-based vector. In a preferred embodiment of the invention, the insertion sequences have been shortened to about 70 base pairs in length as compared to those found in wild-type transposons that typically contain insertion sequences of well over 100 base pairs.

While the examples provided below incorporate a “cut and insert” Tn10 based vector that is destroyed following the insertion event, the present invention also encompasses the use of a “rolling replication” type transposon-based vector. Use of a rolling replication type transposon allows multiple copies of the transposon/transgene to be made from a single transgene construct and the copies inserted. This type of transposon-based system thereby provides for insertion of multiple copies of a transgene into a single genome. A rolling replication type transposon-based vector may be preferred when the promoter operably-linked to gene of interest is endogenous to the host cell and present in a high copy number or highly expressed. However, use of a rolling replication system may require tight control to limit the insertion events to non-lethal levels. Tn1, Tn2, Tn3, Tn4, Tn5, Tn9, Tn21, Tn501, Tn551, Tn951, Tn1721, Tn2410 and Tn2603 are examples of a rolling replication type transposon, although Tn5 could be both a rolling replication and a cut and insert type transposon.

4. *Other Promoters and Enhancers*

The first promoter operably-linked to the transposase gene and the second promoter operably-linked to the gene of interest can be a constitutive promoter or an inducible promoter. Constitutive promoters include, but are not limited to, immediate early cytomegalovirus (CMV) promoter, herpes simplex virus 1 (HSV1) immediate early promoter, SV40 promoter, lysozyme promoter, early and late CMV promoters, early and late HSV promoters, β -actin promoter, tubulin promoter, Rous-Sarcoma virus (RSV) promoter, and heat-shock protein (HSP) promoter. Inducible promoters include tissue-specific promoters, developmentally-regulated promoters and chemically inducible promoters. Examples of tissue-specific promoters include the glucose-6-phosphatase (G6P) promoter, vitellogenin promoter, ovalbumin promoter, ovomucoid promoter, conalbumin promoter, ovotransferrin promoter, prolactin promoter, kidney uromodulin promoter, and placental lactogen promoter. The G6P promoter sequence may be deduced from a rat G6P gene untranslated upstream region provided in GenBank accession number U57552.1. Examples of developmentally-regulated promoters include the homeobox promoters and several hormone induced promoters. Examples of chemically inducible promoters include reproductive hormone

induced promoters and antibiotic inducible promoters such as the tetracycline inducible promoter and the zinc-inducible metallothionine promoter.

Other inducible promoter systems include the Lac operator repressor system inducible by IPTG (isopropyl beta-D-thiogalactoside) (Cronin, A. et al. 2001. *Genes and Development*, v. 15),
 5 ecdysone-based inducible systems (Hoppe, U. C. et al. 2000. *Mol. Ther.* 1:159-164); estrogen-based inducible systems (Brasemann, S. et al. 1993. *Proc. Natl. Acad. Sci.* 90:1657-1661); progesterone-based inducible systems using a chimeric regulator, GLVP, which is a hybrid protein consisting of the GAL4 binding domain and the herpes simplex virus transcriptional activation domain, VP16, and a truncated form of the human progesterone receptor that retains
 10 the ability to bind ligand and can be turned on by RU486 (Wang, et al. 1994. *Proc. Natl. Acad. Sci.* 91:8180-8184); CID-based inducible systems using chemical inducers of dimerization (CIDs) to regulate gene expression, such as a system wherein rapamycin induces dimerization of the cellular proteins FKBP12 and FRAP (Belshaw, P. J. et al. 1996. *J. Chem. Biol.* 3:731-738; Fan, L. et al. 1999. *Hum. Gene Ther.* 10:2273-2285; Shariat, S.F. et al. 2001. *Cancer Res.*
 15 61:2562-2571; Spencer, D.M. 1996. *Curr. Biol.* 6:839-847). Chemical substances that activate the chemically inducible promoters can be administered to the animal containing the transgene of interest via any method known to those of skill in the art.

Other examples of cell-specific and constitutive promoters include but are not limited to smooth-muscle SM22 promoter, including chimeric SM22alpha/telokin promoters (Hoggatt A.M. et al., 2002. *Circ Res.* 91(12):1151-9); ubiquitin C promoter (*Biochim Biophys Acta*, 2003. Jan. 3;1625(1):52-63); Hsf2 promoter; murine COMP (cartilage oligomeric matrix protein) promoter; early B cell-specific mb-1 promoter (Sigvardsson M., et al., 2002. *Mol. Cell Biol.* 22(24):8539-51); prostate specific antigen (PSA) promoter (Yoshimura I. et al., 2002, *J. Urol.* 168(6):2659-64); exorh promoter and pineal expression-promoting element (Asaoka Y., et al., 2002. *Proc.*
 25 *Natl. Acad. Sci.* 99(24):15456-61); neural and liver ceramidase gene promoters (Okino N. et al., 2002. *Biochem. Biophys. Res. Commun.* 299(1):160-6); PSP94 gene promoter/enhancer (Gabril M.Y. et al., 2002. *Gene Ther.* 9(23):1589-99); promoter of the human FAT/CD36 gene (Kuriki C., et al., 2002. *Biol. Pharm. Bull.* 25(11):1476-8); VL30 promoter (Staplin W.R. et al., 2002. *Blood* October 24, 2002); and, IL-10 promoter (Brenner S., et al., 2002. *J. Biol. Chem.* December
 30 18, 2002). Additional promoters are shown in Table 1.

Examples of avian promoters include, but are not limited to, promoters controlling expression of egg white proteins, such as ovalbumin, ovotransferrin (conalbumin), ovomucoid, lysozyme, ovomucin, g2 ovoglobulin, g3 ovoglobulin, ovoflavoprotein, ovostatin (ovomacroglobin), cystatin, avidin, thiamine-binding protein, glutamyl aminopeptidase minor

glycoprotein 1, minor glycoprotein 2; and promoters controlling expression of egg-yolk proteins, such as vitellogenin, very low-density lipoproteins, low density lipoprotein, cobalamin-binding protein, riboflavin-binding protein, biotin-binding protein (Awade, 1996. Z. Lebensm. Unters. Forsch. 202:1-14). An advantage of using the vitellogenin promoter is that it is active during the egg-laying stage of an animal's life-cycle, which allows for the production of the protein of interest to be temporally connected to the import of the protein of interest into the egg yolk when the protein of interest is equipped with an appropriate targeting sequence. As used herein, the "protein of interest" is an antibody. In some embodiments, the avian promoter is an oviduct-specific promoter. As used herein, the term "oviduct-specific promoter" includes, but is not limited to, ovalbumin; ovotransferrin (conalbumin); ovomucoid; 01, 02, 03, 04 or 05 avidin; ovomucin; g2 ovoglobulin; g3 ovoglobulin; ovoflavoprotein; and ovostatin (ovomacroglobin) promoters.

When germline transformation occurs via cardiovascular, intraovarian or intratesticular administration, or when hepatocytes are targeted for incorporation of components of a vector through non-germ line administration, liver-specific promoters may be operably-linked to the gene of interest to achieve liver-specific expression of the transgene. Liver-specific promoters of the present invention include, but are not limited to, the following promoters, vitellogenin promoter, G6P promoter, cholesterol-7-alpha-hydroxylase (CYP7A) promoter, phenylalanine hydroxylase (PAH) promoter, protein C gene promoter, insulin-like growth factor I (IGF-I) promoter, bilirubin UDP-glucuronosyltransferase promoter, aldolase B promoter, furin promoter, metallothionein promoter, albumin promoter, and insulin promoter.

Also included in this invention are modified promoters/enhancers wherein elements of a single promoter are duplicated, modified, or otherwise changed. In one embodiment, steroid hormone-binding domains of the ovalbumin promoter are moved from about -3.5 kb to within approximately the first 1000 base pairs of the gene of interest. Modifying an existing promoter with promoter/enhancer elements not found naturally in the promoter, as well as building an entirely synthetic promoter, or drawing promoter/enhancer elements from various genes together on a non-natural backbone, are all encompassed by the current invention.

Accordingly, it is to be understood that the promoters contained within the transposon-based vectors of the present invention may be entire promoter sequences or fragments of promoter sequences. The constitutive and inducible promoters contained within the transposon-based vectors may also be modified by the addition of one or more modified Kozak sequences comprising any one of SEQ ID NOs:28 to 37.

As indicated above, the present invention includes transposon-based vectors containing one or more enhancers. These enhancers may or may not be operably-linked to their native promoter and may be located at any distance from their operably-linked promoter. A promoter operably-linked to an enhancer and a promoter modified to eliminate repressive regulatory effects are referred to herein as an “enhanced promoter.” The enhancers contained within the transposon-based vectors may be enhancers found in birds, such as an ovalbumin enhancer, but are not limited to these types of enhancers. In one embodiment, an approximately 675 base pair enhancer element of an ovalbumin promoter is cloned upstream of an ovalbumin promoter with 300 base pairs of spacer DNA separating the enhancer and promoter. In one embodiment, the enhancer used as a part of the present invention comprises base pairs 1-675 of a chicken ovalbumin enhancer from GenBank accession #S82527.1. The polynucleotide sequence of this enhancer is provided in SEQ ID NO:38.

Also included in some of the transposon-based vectors of the present invention are cap sites and fragments of cap sites. In one embodiment, approximately 50 base pairs of a 5' untranslated region wherein the capsite resides are added on the 3' end of an enhanced promoter or promoter. An exemplary 5' untranslated region is provided in SEQ ID NO:39. A putative cap-site residing in this 5' untranslated region preferably comprises the polynucleotide sequence provided in SEQ ID NO:40.

In one embodiment of the present invention, the first promoter operably-linked to the transposase gene is a constitutive promoter and the second promoter operably-linked to the gene of interest is a cell specific promoter. In the second embodiment, use of the first constitutive promoter allows for constitutive activation of the transposase gene and incorporation of the gene of interest into virtually all cell types, including the germline of the recipient animal. Although the gene of interest is incorporated into the germline generally, the gene of interest may only be expressed in a tissue-specific manner to achieve gene therapy. A transposon-based vector having a constitutive promoter operably-linked to the transposase gene can be administered by any route, and in several embodiments, the vector is administered to the cardiovascular system, directly to an ovary, to an artery leading to the ovary or to a lymphatic system or fluid proximal to the ovary. In another embodiment, the transposon-based vector having a constitutive promoter operably-linked to the transposase gene can be administered to vessels supplying the liver, muscle, brain, lung, kidney, heart or any other desired organ, tissue or cellular target. In another embodiment, the transposon-based vector having a constitutive promoter operably-linked to the transposase gene can be administered to cells for culture *in vitro*.

It should be noted that cell- or tissue-specific expression as described herein does not require a complete absence of expression in cells or tissues other than the preferred cell or tissue. Instead, "cell-specific" or "tissue-specific" expression refers to a majority of the expression of a particular gene of interest in the preferred cell or tissue, respectively.

5 When incorporation of the gene of interest into the germline is not preferred, the first promoter operably-linked to the transposase gene can be a tissue-specific or cell-specific promoter. For example, transfection of a transposon-based vector containing a transposase gene operably-linked to a liver specific promoter such as the G6P promoter or vitellogenin promoter provides for activation of the transposase gene and incorporation of the gene of interest in the
10 cells of the liver *in vivo*, or *in vitro*, but not into the germline and other cells generally. In another example, transfection of a transposon-based vector containing a transposase gene operably-linked to an oviduct specific promoter such as the ovalbumin promoter provides for activation of the transposase gene and incorporation of the gene of interest in the cells of the oviduct *in vivo* or into oviduct cells *in vitro*, but not into the germline and other cells generally. In this
15 embodiment, the second promoter operably-linked to the gene of interest can be a constitutive promoter or an inducible promoter. In one embodiment, both the first promoter and the second promoter are an ovalbumin promoter. In embodiments wherein tissue-specific expression or incorporation is desired, it is preferred that the transposon-based vector is administered directly to the tissue of interest, to the cardiovascular system which provides blood supply to the tissue of
20 interest, to an artery leading to the organ or tissue of interest or to fluids surrounding the organ or tissue of interest. In one embodiment, the tissue of interest is the oviduct and administration is achieved by direct injection into the oviduct, into the cardiovascular system, or an artery leading to the oviduct. In another embodiment, the tissue of interest is the liver and administration is achieved by direct injection into the cardiovascular system, the portal vein or hepatic artery. In
25 another embodiment, the tissue of interest is cardiac muscle tissue in the heart and administration is achieved by direct injection into the coronary arteries or left cardiac ventricle. In another embodiment, the tissue of interest is neural tissue and administration is achieved by direct injection into the cardiovascular system, the left cardiac ventricle, a cerebrovascular or spinovascular artery. In yet another embodiment, the target is a solid tumor and the
30 administration is achieved by injection into a vessel supplying the tumor or by injection into the tumor.

Accordingly, cell specific promoters may be used to enhance transcription in selected tissues. In birds, for example, promoters that are found in cells of the fallopian tube, such as ovalbumin, conalbumin, ovomucoid and/or lysozyme, are used in the vectors to ensure

transcription of the gene of interest in the epithelial cells and tubular gland cells of the fallopian tube, leading to synthesis of the desired protein encoded by the gene and deposition into the egg white. In liver cells, the G6P promoter may be employed to drive transcription of the gene of interest for protein production. Proteins made in the liver of birds may be delivered to the egg yolk. Proteins made in transfected cells in vitro may be released into cell culture medium.

In order to achieve higher or more efficient expression of the transposase gene, the promoter and other regulatory sequences operably-linked to the transposase gene may be those derived from the host. These host specific regulatory sequences can be tissue specific as described above or can be of a constitutive nature.

10

Table 1

Reproductive tissue	Promoter	Ref.	Function/comments
testes, spermatogenesis	SPATA4	1	constitutive 30 d after birth in rat
placenta, glycoprotein	ERVWE1	2	URE, Upstream Regulatory Element is tissue spec. enhancer
breast epithelium and breast cancer	mammaglobin	6	specific to breast epithelium and cancer
prostate	EPSA	17	enhanced prostate-specific antigen promoter
testes	ATC	25	AlphaT-catenin specific for testes, skeletal, brain cardiomyocytes
prostate	PB	67	probasin promoter
Vision			
rod/cone	mCAR	3	cone photoreceptors and pinealocytes
retina	ATH5	15	functions in retinal ganglia and precursors
eye, brain	rhodopsin	27	
kertocytes	keratocan	42	specific to the corneal stroma
retina	RPE65	59	
Muscle			
vascular smooth muscle	TFPI	13	Tissue Factor Pathway Inhibitor – low level expression in endothelial and smooth muscle cells of vascular system
cardiac specific	MLC2v	14, 26	ventricular myosin light chain
cardiac	CAR3	18	BMP response element that directs cardiac specific expression
skeletal	C5-12	22	high level, muscle spec expression to drive target gene
skeletal	AdmDys,		
smooth muscle	AdmCTLA4Ig	32	muscle creatine kinase promoter
	PDE5A	41	chromosome 4q26, phosphodiesterase use intronic splicing elements to restrict expression to smooth muscle vs skeletal
smooth muscle	AlphaTM	45	
skeletal	myostatin	48	fiber type-specific expression of myostatin
		34	

Reproductive tissue	Promoter	Ref.	Function/comments
Endocrine/nervous glucocorticoid neuroblastoma	GR 1B-1E M2-2	4, 12 8, 36	glucocorticoid receptor promoter/ all cells M2 muscarinic receptor
brain	Abeta	16	amyloid beta-protein; 30 bp fragment needed for PC12 and glial cell expression
brain	enolase	21	neuron-specific; high in hippocampus, intermediate in cortex, low in cerebellum
synapses	rapsyn	29	clusters acetylcholine receptors at neuromuscular junction
neuropeptide precursor	VGF	39	express limited to neurons in central and peripheral nervous system and specific endocrine cells in adenohypophysis,
mammalian nervous system central and peripheral noradrenergic neurons	BMP/RA	46	adrenal medulla, GI tract and pancreas use of methylation to control tissue specificity in neural cells.
brain	Phox2a/Phox2b BAI1-AP4	47 55	regulation of neuron differentiation spec to cerebral cortex and hippocampus
Gastrointestinal UDP glucoronsyltransferase	UGT1A7 UGT1A8 UGT1A10	11 11 11	gastric mucosa small intestine and colon small intestine and colon
colon cancer	PKCbetaII	20	Protein kinase C betaII (PKCbetaII); express in colon cancer to selectively kill it.
Cancer tumor suppressor 4.1B nestin cancer spec promoter	4.1B nestin hTRT/hSPA1	5 63 68	2 isoforms, 1 spec to brain, 1 in kidney second intron regulates tissue specificity dual promoter system for cancer specificity
Blood/lymph system Thyroid Thyroid Thyroid	thyroglobulin calcitonin GR 1A	10 10 12	Thyroid spec. -- express to kill thyroid tumors medullary thyroid tumors
thyroid arterial endothelial cells	thyroglobulin ALK1	50 60	regulation controlled by DREAM transcriptional repressor activin receptor-like kinase
Nonspecific RNA polymerase II gene silencing beta-globin	Gnasx1, Nespas beta globin	7 31 53	
Cardiac	M2-1	8	M2 muscarinic receptor
Lung	hBD-2	19 35	IL-17 induced transcription in airway

Reproductive tissue	Promoter	Ref.	Function/comments epithelium
pulmonary surfactant protein	SP-C	62	Alveolar type II cells
ciliated cell-specific prom surfactant protein expression	FOZJ1	70	use in ciliated epithelial cells for CF treatment
Clara cell secretory protein	SPA-D	73	Possible treatment in premature babies
Dental	CCSP	75	
teeth/bone	DSPP	28	extracellular matrix protein dentin sialophosphoprotein
Adipose			
adipogenesis	EPAS1	33	endothelial PAS domain -- role in adipocyte differentiation
Epidermal differentiated epidermis	involucrin	38	
desmosomal protein	CDSN	58	stratum granulosum and stratum corneum of epidermis
Liver			
liver spec albumin	Albumin	49	
serum alpha-fetoprotein	AFP	56	liver spec regulation

References

1. Biol Pharm Bull. 2004 Nov;27(11):1867-70
- 5 2. J Virol. 2004 Nov;78(22):12157-68
3. Invest Ophthalmol Vis Sci. 2004 Nov;45(11):3877-84
4. Biochim Biophys Acta. 2004 Oct 21;1680(2):114-28
5. Biochim Biophys Acta. 2004 Oct 21;1680(2):71-82
6. Curr Cancer Drug Targets. 2004 Sep;4(6):531-42
- 10 7. Biotechnol Bioeng. 2004 Nov 20;88(4):417-25
8. J Neurochem. 2004 Oct;91(1):88-98
10. Curr Drug Targets Immune Endocr Metabol Disord. 2004 Sep;4(3):235-44
11. Toxicol Appl Pharmacol. 2004 Sep 15;199(3):354-63
12. J Immunol. 2004 Sep 15;173(6):3816-24
- 15 13. Thromb Haemost. 2004 Sep;92(3):495-502
14. Acad Radiol. 2004 Sep;11(9):1022-8
15. Development. 2004 Sep;131(18):4447-54
16. J Neurochem. 2004 Sep;90(6):1432-44

17. Mol Ther. 2004 Sep;10(3):545-52
18. Development. 2004 Oct;131(19):4709-23. Epub 2004 Aug 25
19. J Immunol. 2004 Sep 1;173(5):3482-91
20. J Biol Chem. 2004 Oct 29;279(44):45556-63. Epub 2004 Aug 20
- 5 21. J Biol Chem. 2004 Oct 22;279(43):44795-801. Epub 2004 Aug 20
22. Hum Gene Ther. 2004 Aug;15(8):783-92
25. Nucleic Acids Res. 2004 Aug 09;32(14):4155-65. Print 2004
26. Mol Imaging. 2004 Apr;3(2):69-75
27. J Gene Med. 2004 Aug;6(8):906-12
- 10 28. J Biol Chem. 2004 Oct 1;279(40):42182-91. Epub 2004 Jul 28
29. Mol Cell Biol. 2004 Aug;24(16):7188-96
31. Nat Genet. 2004 Aug;36(8):894-9. Epub 2004 Jul 25
32. Gene Ther. 2004 Oct;11(19):1453-61
33. J Biol Chem. 2004 Sep 24;279(39):40946-53. Epub 2004 Jul 15
- 15 36. Brain Res Mol Brain Res. 2004 Jul 26;126(2):173-80
38. J Invest Dermatol. 2004 Aug;123(2):313-8
39. Cell Mol Neurobiol. 2004 Aug;24(4):517-33
41. Int J Impot Res. 2004 Jun;16 Suppl 1:S8-S10
42. Invest Ophthalmol Vis Sci. 2004 Jul;45(7):2194-200
- 20 45. J Biol Chem. 2004 Aug 27;279(35):36660-9. Epub 2004 Jun 11
46. Brain Res Mol Brain Res. 2004 Jun 18;125(1-2):47-59
47. Brain Res Mol Brain Res. 2004 Jun 18;125(1-2):29-39
48. Am J Physiol Cell Physiol. 2004 Oct;287(4):C1031-40. Epub 2004 Jun 09
49. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2003 Nov;19(6):601-3
- 25 50. J Biol Chem. 2004 Aug 6;279(32):33114-22. Epub 2004 Jun 04
53. Brief Funct Genomic Proteomic. 2004 Feb;2(4):344-54
55. FEBS Lett. 2004 May 21;566(1-3):87-94
56. Biochem Biophys Res Commun. 2004 Jun 4;318(3):773-85
58. J Invest Dermatol. 2004 Mar;122(3):730-8
- 30 59. Mol Vis. 2004 Mar 26;10:208-14
60. Circ Res. 2004 Apr 30;94(8):e72-7. Epub 2004 Apr 01
62. Am J Physiol Lung Cell Mol Physiol. 2004 Dec 3; [Epub ahead of print]
63. Lab Invest. 2004 Dec;84(12):1581-92
67. Prostate. 2004 Jun 1;59(4):370-82

68. Cancer Res. 2004 Jan 1;64(1):363-9
 70. Mol Ther. 2003 Oct;8(4):637-45
 73. Front Biosci. 2003 May 01;8:d751-64
 75. Am J Respir Cell Mol Biol. 2002 Aug;27(2):186-93

5

B. Methods of Transfecting Cells

1. *Transfection of LMH or LMH2A Cells in vitro*

DNA

Antibody expression vector DNA (*e.g.*, any one of SEQ ID NOs:17-27) DNA was prepared in either methylating or non-methylating bacteria, and was endotoxin-free. Agarose gels showed a single plasmid of the appropriate size. DNA was resuspended in molecular biology grade, sterile water at a concentration of at least 0.5 µg/µl. The concentration was verified by spectrophotometry, and the 260/280 ratio was 1.8 or greater. A stock of each DNA sample, diluted to 0.5 µg/µl in sterile, molecular biology grade water, was prepared in the cell culture lab, and this stock used for all transfections. When not in use, the DNA stocks were kept frozen at -30°C in small aliquots to avoid repeated freezing and thawing.

Transfection

The transfection reagent used for LMH2A cells was FuGENE 6 (Roche Applied Science). This reagent was used at a 1:6 ratio (µg of DNA: µl of transfection reagent) for all transfections in LMH2A cells. The chart below shows the amount of DNA and FuGENE 6 used for typical cell culture formats (T25 and T75 tissue culture flasks). If it is necessary to perform transfections in other formats, the amounts of serum free medium (SFM), FuGENE 6 and DNA are scaled appropriately based on the surface area of the flask or well used. The diluent (SFM) is any serum-free cell culture media appropriate for the cells, and it did not contain any antibiotics or fungicides.

Table 2

DNA:FuGENE = 1:6

[DNA]=0.5µg/µl

	T25	T75
SFM	250 µl	800 µl
FuGENE 6	12 µl	48 µl
DNA	4 µl	16 µl

Protocol

1. Cells used for transfection were split 24-48 hours prior to the experiment, so that they were actively growing and 50-80% confluent at the time of transfection.
2. FuGENE was warmed to room temperature before use. Because FuGENE is sensitive to prolonged exposure to air, the vial was kept tightly closed when not in use. The vial of FuGENE was returned to the refrigerator as soon as possible.
3. The required amount of FuGENE was pipetted into the SFM in a sterile microcentrifuge tube. The fluid was mixed gently but thoroughly, by tapping or flicking the tube, and incubated for 5 minutes at room temperature.
4. The required amount of DNA was added to the diluted FuGENE and mixed by vortexing for one second.
5. The mixture was incubated at room temperature for 1 hour.
6. During the incubation period, media on cells was replaced with fresh growth media. This media optionally contained serum, if needed, but did not contain antibiotics or fungicides unless absolutely required, as this can reduce the transfection efficiency.
7. The entire volume of the transfection complex was added to the cells. The flask was rocked to mix thoroughly.
8. The flasks were incubated at 37°C and 5% CO₂.
9. Cells were fed, and samples obtained as required. After the first 24 hours, cells were optionally fed with media containing antibiotics and/or fungicides, if desired.

2. Transfection of LMH Cells and Tubular Gland Cells

The same methods described above for LMH and LMH2A cells are used for transfection of chicken tubular gland cells or other cell types such as Chinese hamster ovary (CHO) cells, CHO-K1 cells, chicken embryonic fibroblasts, HeLa cells, Vero cells, FAO (liver cells), human 3T3 cells, A20 cells, EL4 cells, HepG2 cells, J744A cells, Jurkat cells, P388D1 cells, RC-4B/c cells, SK-N-SH cells, Sp2/mIL-6 cells, SW480 cells, 3T6 Swiss cells, and human ARPT-19 cells.

C. Purification of Monoclonal Antibodies

Media preparation

Media containing recombinant monoclonal antibodies produced by transfected cells was harvested and immediately frozen. Later the medium was thawed, filtered through a 0.45 micron cellulose acetate bottle-top filter to ensure that all particulate was removed prior to being loaded on the column.

Purification Procedure

Human monoclonal antibodies (hIgG) were purified using the EGGstract® IgY Purification System (cat.# G2610, Promega, USA) as follows.

1. Stirred the 30 mls cell culture media at room temperature and slowly added 10 mls of Precipitation Solution B (EGGstract® IgY Purification System).
2. Continued stirring the mixture for 5 minutes to precipitate the IgG.
3. Centrifuged the mixture at $10,000 \times g$ for 15 minutes at 4°C . (Increased time for lower centrifugal forces.)
4. Poured off the supernatant and discard.
5. Resuspended the IgG pellet in a 1 ml of 1x PBS by capping the 50-ml conical tube and rocking at room temperature for up to 2 hours.

The suspended monoclonal antibodies were concentrated and cleaned using PIERCE PAGEprep® Advance kit (Cat. 89888, Pierce, USA) as follows.

1. Vortexed the PAGE-prep Protein Binding Resin to evenly disperse the resin into a slurry.
2. Used a cut or large-orifice pipette tip to transfer 20 μl of resin slurry into the center of a spin cup. Note: Resin particles may clog ordinary pipette tips.
3. Added 300 μl of suspended IgG in 1x PBS to the resin. Capped tube and briefly vortexed.
4. Added 300 μl of 100% DMSO to the suspended hIgG added in Step 3. Capped tube and briefly vortexed.
5. Incubated sample for 4 minutes at room temperature with occasional mixing to ensure maximum protein adsorption to the resin.
6. Centrifuged sample at $2,000 \times g$ for 2 minutes. Discarded flow-through and blotted collection tube on a paper towel.
7. Reinserted spin cup into the same collection tube.
8. Prepared Wash Solution by mixing 3 mls of 100% DMSO with 3 mls of water.
9. Stored Wash Solution at room temperature.
10. Added 300 μl of Wash Solution to the resin. Capped tube and vortexed until a homogeneous suspension was obtained
11. Centrifuged sample at $2,000 \times g$ for 2 minutes. Discarded flow-through and blotted collection tube on a paper towel.
12. Reinserted spin cup into the same collection tube.
13. Repeated wash one additional time for a total of two washes.
14. Transferred the spin cup to a new collection tube and add 100 μl of Elution Buffer to the resin. Capped tube and briefly vortexed to obtain a homogeneous suspension.

15. Incubated sample at 60°C for 5 minutes. After incubation, briefly vortexed the tube.
16. Centrifuged sample at 2,000 × g for 2 minutes. Discarded spin cup and resin. Retained the collection tube containing human IgG.

The purification procedure was evaluated at various stages using a sandwich ELISA assay (See section D.1. below). SDS-PAGE analysis with subsequent Coomassie blue staining or Western blotting was done to indicate both molecular weight and purity of the purified monoclonal antibodies (See section D.2. below).

D. Monoclonal Antibody Detection

1. *Monoclonal Antibody Measurement with ELISA*

Monoclonal antibody was measured using the following sandwich ELISA protocol:

Heavy Chain Detection:

1. Diluted monoclonal anti-human Ig-Fc antibody (Bethyl Laboratories, Inc., A80-104A) 1:100 in carbonate buffer (Bethyl Laboratories, E101) such that the final working dilution concentration was 10 µg/mL.
2. Add 100 µL of the diluted antibody into to the appropriate wells of the ELISA plate.
3. Allowed 96-well plate to coat for 1 hour at 37°C.
4. Washed the ELISA plate five times with wash buffer (1x TBS/0.05% TWEEN).
5. Transferred 200 µL of blocking buffer (Bethyl Laboratories, E101) to the appropriate wells of the ELISA plate and allowed 96-well plate to block for 30 minutes at room temperature.
6. Diluted human reference serum standard (Bethyl Laboratories, RS10-110) in negative control media (5% FCS/Waymouth, Gibco) such that the final working dilution concentration was 50 ng/mL.
7. Diluted test samples in negative control media (5% FCS/Waymouth, Gibco).
8. Removed the blocking buffer by manually “flicking” the ELISA plate into the sink.
9. Added the diluted samples and fusion protein standards into 96-well plate and incubated the ELISA plate at room temperature for 1 hour.
10. Diluted fresh dilute monoclonal anti-IgG-Fc-HRP antibody (Bethyl Laboratories, A80-104P) 1:100,000 such that the final working dilution concentration was 10 ng/mL.
11. Added 100 µL of the diluted antibody into to the appropriate wells of the ELISA plate.
12. Incubated the ELISA plate at room temperature for 1 hour.
13. Mixed equal volumes of TMB peroxidase substrate (KPL, 50-76-01) and peroxidase solution B (KPL, 50-65-00).
14. Washed the ELISA plate five times with wash buffer (1x TBS/0.05% TWEEN).

15. Added 100 μ L of the mixed substrate reagents solution to the appropriate wells of the ELISA plate

16. Added 100 μ L of 2 M H_2SO_4 to the appropriate wells of the ELISA plate to stop the TMB reaction.

5 17. Using plate reader, took the absorbance readings at 450 nm of the ELISA plate at 20 minutes.

Culture medium was applied to the ELISA either in an undiluted or diluted manner. Monoclonal antibody was detected in this assay. The monoclonal antibody levels were determined by reference to the standard curve and are presented in various figures throughout this application.

10 SDS-PAGE analysis with subsequent Coomassie blue staining or Western blotting was done to indicate both molecular weight and purity of the purified monoclonal antibody (See section D.2. below).

15 Light chain detection:

1. Diluted monoclonal anti-human lambda light chain antibody (Bethyl Laboratories, Inc., A80-116A) 1:100 in carbonate buffer (Bethyl Laboratories, Inc, E101) such that the final working dilution concentration was 10 μ g/mL.

2. Added 100 μ L of the diluted antibody into to the appropriate wells of the ELISA plate.

20 3. Allowed 96-well plate to coat for 1 hour at 37°C.

4. Washed the ELISA plate five times with wash buffer (1x TBS/0.05% TWEEN).

5. Transferred 200 μ L of blocking buffer (Bethyl Laboratories, Inc, E101) to the appropriate wells of the ELISA plate and allowed 96-well plate to block for 30 minutes at room temperature.

6. Diluted the human reference serum standard (Bethyl Laboratories, Inc., human reference serum, RS10-110) in negative control media (5% FCS/Waymouth, Gibco) such that the final working dilution concentration was 100 ng/mL.

7. Diluted test samples in negative control media (5% FCS/Waymouth , Gibco).

8. Removed the blocking buffer by manually "flicking" the ELISA plate into the sink.

9. Added the diluted samples and human reference standards into 96-well plate and incubated the ELISA plate at room temperature for 1 hour.

10. Diluted fresh Dilute anti-human lambda light chain-HRP antibody (Bethyl Laboratories, Inc., A80-116P) 1:100,000 such that the final working dilution concentration was 10 ng/mL.

11. Added 100 μ L of the diluted antibody into to the appropriate wells of the ELISA plate.

12. Incubated the ELISA plate at room temperature for 1 hour.

13. Mixed equal volumes of TMB peroxidase substrate (KPL, 50-76-01) and peroxidase solution B (KPL, 50-65-00).

14. Washed the ELISA plate five times with wash buffer (1x TBS/0.05% TWEEN).

15. Added 100 μ L of the mixed substrate reagents solution to the appropriate wells of the ELISA plate.

16. Added 100 μ L of 2 M H_2SO_4 to the appropriate wells of the ELISA plate to stop the TMB reaction.

17. Using plate reader, took the absorbance readings at 450 nm of the ELISA plate at 20 minute.

10 Culture medium was applied to the ELISA either in an undiluted or diluted manner. Monoclonal antibody was detected in this assay. The monoclonal antibody levels were determined by reference to the standard curve and are presented in various figures throughout this application.

15 SDS-PAGE analysis with subsequent Coomassie blue staining or Western blotting was done to indicate both molecular weight and purity of the purified monoclonal antibody (See section D.2. below).

2. Detection of Monoclonal Antibody Expression with Immunoblotting

SDS-PAGE:

20 Sample mixtures, including negative control media, were heated under reduced (with DTT) and non-reduced (without DTT) conditions for 8 minutes at 100°C and loaded onto a 10-20% Tris-HCl gel. The samples were run at 200 V for 1 hour 15 minutes in Tris-Glycine-SDS buffer.

Heavy Chain Antibody detection:

- 25 1. The finished gel was placed into the Western blot transfer buffer for 2 minutes. This equilibrated the gel in the buffer used for the transfer.
2. The gel was rehydrated for 1 minute in Western blot transfer buffer. A sheet of nitrocellulose paper was cut to the exact size of the gel to be transferred.
3. The electrophoretic transfer was occurred for 50 minutes at 100 V.
- 30 4. The blot was removed from the transfer apparatus and blocked with 5.0% MILK in TBS/TWEEN 20. Blocking was allowed for 1 hour at 37 °C.
5. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.

6. The blot was incubated in Dilute monoclonal anti-IgG-Fc-HRP antibody (Bethyl Laboratories, Inc., A80-104P) conjugated with horseradish peroxidase and diluted appropriately 1:5,000 with 1% gelatin in TBS/TWEEN 20 for 1 hour at room temperature.
7. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.
8. Antibody bound to antigen was detected by using TMB membrane peroxidase Substrate (KPL, 50-77-00) Liquid Substrate System (KPL). The substrate solution was applied until color was detected (5-10 minutes).
9. Color formation (enzyme reaction) was stopped by rinsing blots with dH₂O.
10. The blot was air-dried on paper towel.

Light Chain Antibody detection:

1. The finished gel was placed into the Western blot transfer buffer for 2 minutes. This equilibrated the gel in the buffer used for the transfer.
2. The gel was rehydrated for 1 minute in Western blot transfer buffer. A sheet of nitrocellulose paper was cut to the exact size of the gel to be transferred.
3. The electrophoretic transfer occurred for 50 minutes at 100 V.
4. The blot was removed from the transfer apparatus and blocked with 5.0% MILK in TBS/TWEEN 20. Blocking was allowed for 1 hour at 37 °C.
5. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.
6. The blot was incubated in anti-human lambda light chain-HRP antibody (Bethyl Laboratories, Inc., A80-116P) conjugated with horseradish peroxidase and diluted appropriately 1:5,000 with 1% gelatin in TBS/TWEEN 20 for 1 hour at room temperature.
7. The blot was washed four times for 5 minutes per wash in TBS/TWEEN 20.
8. Antibody bound to antigen was detected by using the TMB membrane peroxidase Substrate (KPL, 50-77-00) Liquid Substrate System (KPL). The substrate solution was applied until color was detected (5-10 minutes).
9. Color formation (enzyme reaction) was stopped by rinsing blots with dH₂O.
10. The blot was air-dried on paper towel.

3. *Vectors for Monoclonal Antibody Production*

The expression vectors of the present invention employ some of the vector components (backbone vectors and promoters) described in the previous section and also include the multiple cloning site (MCS) comprising the gene of interest. In one embodiment, the gene of interest encodes for a human monoclonal antibody. In one embodiment, the gene of interest encodes for

a human RM2 monoclonal antibody. The following vectors, SEQ ID NOS:17-27, all contain a gene of interest encoding a monoclonal antibody:

- (SEQ ID NO:17) #148 HS4 Flanked Backbone Vector (CMVep-Intron A + RM2 mAb)
- 5 (SEQ ID NO:18) #210 pTn10, HS4 Flanked Backbone Vector (CMVep-Intron A + RM2 mAb)
- (SEQ ID NO:19) #212 pTn10, MAR Flanked Backbone Vector (CMVep-Intron A + RM2 mAb)
- (SEQ ID NO:20) #226 pTn10, Puro-MAR Flanked Backbone Vector (CMVep-Intron A +
10 RM2 mAb)
- (SEQ ID NO:21) #275 pTn10, Puro-MAR Flanked Backbone Vector (CMV-Ovalp Vs.1 – RM2 mAb – OPA in pTn10 PURO-MAR Flanked BV)
- (SEQ ID NO: 22) #278 pTn10, PURO-MAR Flanked Backbone Vector (CMV-Ovalp Vs.1 – Δ CH2 mAb – OPA in pTn10)
- 15 (SEQ ID NO: 23) #273 pTn10 PURO-MAR Flanked Backbone Vector (CMV-Ovalp Vs.1 – RM2 mAb)
- (SEQ ID NO: 24) #146/149 Vitellogenin-Intron A RM2 mAb Flanked with HS4 Backbone Vector in pTnMCS modified
- (SEQ ID NO: 25) #267 Codon optimized herceptin (antibody that targets human epidermal
20 growth factor receptor-2) gene, pTn10, Puro-MAR Flanked Backbone Vector (#5021)
- (SEQ ID NO: 26) #279 Codon optimized Δ CH2 (antibody with deletion of constant region 2 of the heavy chain) gene, pTn10, Puro-MAR Flanked Backbone Vector (#5021)
- (SEQ ID NO: 27) #280 Codon optimized RM2, pTn10, Puro-MAR Flanked Backbone Vector (#5021)
- 25

In specific embodiments, the disclosed backbone vectors are defined by the following annotations:

SEQ ID NO:1 (pTnMCS (base vector, without MCS extension) Vector #5001

- Bp 1 – 130 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp1-130
- 30 Bp 133 – 1812 CMV promoter/enhancer taken from vector pGWIZ (Gene Therapy Systems) bp229-1873
- Bp 1813 – 3018 Transposase, modified from Tn10 (GeneBank accession #J01829) Bp 108-1316
- Bp 3019 – 3021 Engineered stop codon

	Bp 3022 – 3374	Non-coding DNA from vector pNK2859
	Bp 3375 – 3417	Lambda DNA from pNK2859
	Bp 3418 – 3487	70bp of IS10 left from Tn10
5	Bp 3494 – 3700	Multiple cloning site from pBluescriptII sk(-), thru the XmaI site Bp 924-718
	Bp 3701 – 3744	Multiple cloning site from pBluescriptII sk(-), from the XmaI site thru the XhoI site. These base pairs are usually lost when cloning into pTnMCS. Bp 717-673
10	Bp 3745 – 4184	Multiple cloning site from pBluescriptII sk(-), from the XhoI site bp 672-235
	Bp 4190 – 4259	70 bp of IS10 from Tn10
	Bp 4260 – 4301	Lambda DNA from pNK2859
	Bp 4302 – 5167	Non-coding DNA from pNK2859
15	Bp 5168 – 7368	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961
	<i>SEQ ID NO: 2</i>	<i>X – MCS (Vector #5005) pTNMCS (base vector) with MCS extension</i>
	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) Bp 4-135
	Bp 133 – 1785	CMV Promoter/Enhancer from vector pGWIZ (Gene Therapy Systems)
20	Bp 1786 – 3018	Transposase, modified from Tn10 (GeneBank accession #J01829) Bp 81-1313
	Bp 3019 – 3021	Engineered stop codon
	Bp 3022 – 3374	Non-coding DNA from vector pNK2859
	Bp 3375 – 3416	Lambda DNA from pNK2859
	Bp 3417 – 3486	70bp of IS10 left from Tn10 (GeneBank accession #J01829 Bp 1-70)
25	Bp 3487 – 3704	Multiple cloning site from pBluescriptII sk(-), thru XmaI
	Bp 3705 – 3749	Multiple cloning site from pBluescriptII sk(-), from XmaI thru XhoI
	Bp 3750 – 3845	Multiple cloning site extension from XhoI thru PspOMI
	BP 3846 - 4275	Multiple cloning site from pBluescriptII sk(-), from PspOMI
	Bp 4276 – 4345	70 bp of IS10 from Tn10 (GeneBank accession #J01829 Bp 70-1)
30	Bp 4346 – 4387	Lambda DNA from pNK2859
	Bp 4388 – 5254	Non-coding DNA from pNK2859
	Bp 5255 – 7455	pBluescriptII sk(-) base vector (Stratagene, INC) Bp 761-2961

SEQ ID NO: 3		HS4 Flanked BV (Vector #5006)
	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) Bp 4-135
	Bp 133 – 1785	CMV Promoter/Enhancer from vector pGWIZ (Gene Therapy Systems) Bp 229-1873, including the combination of 2 NruI cut sites
5	Bp 1786 – 3018	Transposase, modified from Tn10 (GeneBank accession #J01829) Bp 81-1313
	Bp 3019 – 3021	Engineered stop codon
	Bp 3022 – 3374	Non-coding DNA from vector pNK2859
	Bp 3375 – 3416	Lambda DNA from pNK2859
10	Bp 3417 – 3490	70bp of IS10 left from Tn10 (GeneBank accession #J01829 Bp 1-70)
	Bp 3491 – 3680	Multiple cloning site from pBluescriptII sk(-), thru NotI Bp 926-737
	Bp 3681 – 4922	HS4 – Beta-globin Insulator Element from Chicken gDNA
	Bp 4923 – 5018	Multiple cloning site extension XhoI thru MluI
	Bp 5019 – 6272	HS4 – Beta-globin Insulator Element from Chicken gDNA
15	Bp 6273 – 6342	70 bp of IS10 from Tn10 (GeneBank accession #J01829 Bp 70-1)
	Bp 6343 – 6389	Lambda DNA from pNK2859
	Bp 6390 – 8590	pBluescriptII sk(-) base vector (Stratagene, INC) Bp 761-2961
SEQ ID NO: 4		<i>K Tn-10 HS4 Flanked Backbone (Vector #5012)</i>
20	Bp. 1 - 132	Remaining of F1 (-) Ori from pBluescript II sk(-) (Statagene Bp 4-135).
	Bp. 133 - 1806	CMV Promoter / Enhancer from vector pGWIZ (Gene Therapy Systems) Bp. 229-1873.
	Bp. 1807 - 3015	Tn-10 transposase, from pNK2859 (GeneBank accession #J01829 Bp. 81-1313).
25	Bp. 3016 - 3367	Non-coding DNA, possible putative poly A, from vector pNK2859.
	Bp. 3368 - 3410	Lambda DNA from pNK2859.
	Bp. 3411 - 3480	70bp of IS 10 left from Tn10 (GeneBank accession #J01829 bp. 1-70
	Bp. 3481 - 3674	Multiple cloning site from pBluescript II sk(-), thru NotI Bp. 926-737.
	Bp. 3675 - 4916	Chicken Beta Globin HS4 Insulator Element (Genbank Accession #NW_060254.0).
30	Bp. 4917 - 5012	Multiple cloning site extension Xho I thru Mlu I.
	Bp. 5013 - 6266	Chicken Beta Globin HS4 Insulator Element (Genbank Accession #NW_060254.0).

Bp. 6267 - 6337 70bp of IS 10 left from Tn10 (GeneBank accession #J01829 bp. 1-70)
 Bp. 6338 - 6382 Lambda DNA from pNK2859.
 Bp. 6383 - 8584 pBluescript II sk(-) Base Vector (Stratagene, Inc. Bp. 761-2961).

5 *SEQ ID NO: 5 pTn10 MAR Flanked BV (Vector 5018)*

Bp 1 – 132 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
 Bp 133 – 148 pGWIZ base vector (Gene Therapy Systems) bp 229-244
 Bp 149 - 747 CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
 Bp 748 - 822 CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
 10 Bp 823 - 943 CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
 Bp 944 - 1769 CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
 Bp 1770 - 1777 CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems) bp 1866-1873)
 15 Bp 1778 – 1806 TN10 DNA, 3' end from Genbank Accession #J01829 bp 79 - 107
 Bp 1807 – 3015 Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
 Bp 3016 – 3367 Putative PolyA from vector pNK2859
 Bp 3368 – 3410 Lambda DNA from pNK2859
 20 Bp 3411 – 3480 70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
 Bp 3481 – 3651 pBluescriptII sk(-) base vector (Stratagene, INC)
 Bp 3652 - 3674 Multiple cloning site from pBluescriptII sk(-) thru NotI, Bp 759-737
 Bp 3675 - 5367 Lysozyme Matrix Attachment Region (MAR)
 Bp 5368 – 5463 Multiple Cloning Site Extension from pTn X-MCS, XhoI thru MluI
 25 Bp 5464 - 7168 Lysozyme Matrix Attachment Region (MAR)
 Bp 7169 – 7238 70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
 Bp 7239 - 7281 Lambda DNA from pNK2859
 Bp 7282 - 9486 pBluescriptII sk(-) base vector (Stratagene, INC)

30 *SEQ ID NO:6 (Vector 5020 pTn10 PURO – LysRep2 Flanked BV)*

Bp 1 – 132 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
 Bp 133 – 148 pGWIZ base vector (Gene Therapy Systems) bp 229-244
 Bp 149 - 747 CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)

	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
5	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
10	Bp 3016 – 3367	Putative PolyA from vector pNK2859
	Bp 3368 – 3410	Lambda DNA from pNK2859
	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3481 – 3484	Synthetic DNA added during construction
	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
15	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3675 - 4608	Lysozyme Rep2 from gDNA (corresponds to Genbank Accession #NW_060235)
	Bp 4609 – 4686	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
	Bp 4687 - 4999	HSV-TK polyA from pS65TC1 bp 3873-3561
20	Bp 5000 - 5028	Excess DNA from pMOD PURO (invivoGen)
	BP 5029 - 5630	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116
	Bp 5631 - 6016	SV40 promoter from pS65TC1, bp 2232-2617
	Bp 6017 - 6022	MluI RE site
	Bp 6023 - 6956	Lysozyme Rep2 from gDNA (corresponds to Genbank Accession #NW_060235)
25	Bp 6957 – 6968	Synthetic DNA added during construction including a PspOMI RE site
	Bp 6969 – 7038	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 7039 - 7081	Lambda DNA from pNK2859
	Bp 7082 - 7085	Synthetic DNA added during construction
30	Bp 7086 - 9286	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:7 (Vector #5019 pTN-10 PURO – HS4 Flanked BV)

Bp 1 – 132 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135

	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
5	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems) bp 1866-1873)
	Bp 1778 – 1806	TN10 DNA, 3' end from Genbank Accession #J01829 bp 79 - 107
10	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
	Bp 3016 – 3367	Putative PolyA from vector pNK2859
	Bp 3368 – 3410	Lambda DNA from pNK2859
	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
15	Bp 3481 – 3484	Synthetic DNA added during construction
	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-) thru NotI, Bp 759-737
	Bp 3675 - 4916	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
20	Bp 4917 – 4994	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
	Bp 4995 - 5307	HSV-TK polyA from pS65TC1 bp 3873-3561
	Bp 5308 - 5336	Excess DNA from pMOD PURO (invivoGen)
	BP 5337 - 5938	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116
	Bp 5939 - 6324	SV40 promoter from pS65TC1, bp 2232-2617
25	Bp 6325 - 6330	MluI RE site
	Bp 6331 - 7572	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
	Bp 7573 – 7584	Synthetic DNA added during construction including a PspOMI RE site
	Bp 7585 – 7654	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
30	Bp 7655 - 7697	Lambda DNA from pNK2859
	Bp 7698 - 7701	Synthetic DNA added during construction
	Bp 7702 - 9902	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO: 8 Vector #5021 pTn10 PURO - MAR Flanked BV

	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
5	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
10	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
	Bp 3016 – 3367	Putative PolyA from vector pNK2859
15	Bp 3368 – 3410	Lambda DNA from pNK2859
	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3481 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC)
	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3675 - 5367	Lysozyme Matrix Attachment Region (MAR)
20	Bp 5368 – 5445	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
	Bp 5446 - 5758	HSV-TK polyA from pS65TC1 bp 3873-3561
	BP 5759 - 6389	Puromycin resistance gene from pMOD PURO (invivoGen)
	Bp 6390 - 6775	SV40 promoter from pS65TC1, bp 2232-2617
	Bp 6776 - 8486	Lysozyme Matrix Attachment Region (MAR)
25	Bp 8487 – 8556	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 8557 - 8599	Lambda DNA from pNK2859
	Bp 8600 - 10804	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO:9 (Vector #5022; pTn10 Gen – MAR Flanked BV)

30	Bp 1 – 5445	pTn10 MAR Flanked BV, ID #5018
	Bp 5446 - 5900	HSV-TK polyA from Taken from pIRES2-ZsGreen1, bp 4428-3974
	Bp 5901 - 6695	Kanamycin/Neomycin (G418) resistance gene, taken from pIRES2-ZsGreen1, Bp 3973-3179

	Bp 6696 - 7046	SV40 early promoter/enhancer taken from pIRES2-ZsGreen1, bp 3178-2828
	Bp 7047 - 7219	Bacterial promoter for expression of KAN resistance gene, taken from pIRES2-ZsGreen1, bp 2827-2655
5	Bp 7220 - 11248	pTn10 MAR Flanked BV, bp 5458-9486
	<i>SEQ ID NO: 10</i>	<i>pTn10 MAR Flanked BV Vector #5024</i>
	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 154	pGWIZ base vector (Gene Therapy Systems) bp 229-244
10	Bp 155 - 229	CMV promoter (from vector pGWIZ, Gene Therapy Systems bp 844-918
	Bp 230 - 350	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 351 - 1176	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1177 - 1184	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems) bp 1866-1873)
15	Bp 1185 – 1213	TN10 DNA, 3' end from Genbank Accession #J01829 bp 79 - 107
	Bp 1214 – 2422	Transposon, modified from Tn10 GenBank Accession #J01829 bp 108-1316
	Bp 2423 – 2774	Putative PolyA from vector pNK2859
20	Bp 2775 – 2817	Lambda DNA from pNK2859
	Bp 2818 – 2887	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 2888 – 3058	pBluescriptII sk(-) base vector (Stratagene, INC) Bp 3059 - 3081 Multiple cloning site from pBluescriptII sk(-) thru NotI,
	Bp 3082 - 4774	Chicken 5' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408
25	Bp 4775 – 4870	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru MluI
	Bp 4871 - 6575	Chicken 3' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408
	Bp 6576 – 6645	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
30	Bp 6646 - 6688	Lambda DNA from pNK2859
	Bp 6689 - 8893	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO: 11 Vector #5025 pTn10 (-CMV Enh.)PURO - MAR Flanked BV

	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 154	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 155 - 229	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
5	Bp 230 - 350	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 351 - 1176	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1177 - 1184	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
10	Bp 1185 – 1213	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1214 – 2422	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
	Bp 2423 – 2774	Putative PolyA from vector pNK2859
	Bp 2775 – 2817	Lambda DNA from pNK2859
15	Bp 2818 – 2887	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 2888 – 3058	pBluescriptII sk(-) base vector (Stratagene, INC)
	Bp 3059 - 3081	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3082 - 4774	Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408
20	Bp 4775 – 4852	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
	Bp 4853 - 5165	HSV-TK polyA from pS65TC1 bp 3873-3561
	BP 5166 - 5796	Puromycin resistance gene from pMOD PURO (invivoGen)
	Bp 5797 - 6182	SV40 promoter from pS65TC1, bp 2232-2617
	Bp 6183 - 7893	Lysozyme Matrix Attachment Region (MAR)
25	Bp 7894 – 7963	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 7964 - 8010	Lambda DNA from pNK2859
	Bp 8011 - 10211	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO: 12 Vector #5026 pTn10 MAR Flanked BV #5026

30	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 154	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 155 - 540	SV40 promoter from pS65TC1 bp 2232-2617

	Bp 541 - 661	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 662 - 1487	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1488 - 1495	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
5		
	Bp 1496 – 1524	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1525 – 2733	Transposon, modified from Tn10 GenBank Accession #J01829 bp 108-1316
	Bp 2734 – 3085	Putative PolyA from vector pNK2859
10	Bp 3086 – 3128	Lambda DNA from pNK2859
	Bp 3129 – 3198	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3199 – 3369	pBluescriptII sk(-) base vector (Stratagene, INC)
	Bp 3370 - 3392	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3393 - 5085	Chicken 5' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408
15		
	Bp 5086 – 5181	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru MluI
	Bp 5182 - 6886	Chicken 3' Lysozyme Matrix Attachment Region (MAR) from chicken gDNA corresponding to GenBank Accession #X98408
	Bp 6887 – 6956	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
20	Bp 6957 - 6999	Lambda DNA from pNK2859
	Bp 7000 - 9204	pBluescriptII sk(-) base vector (Stratagene, INC)
	<i>SEQ ID NO: 13</i>	<i>pTn10 SV 40 Pr.PURO - MAR Flanked BV Vector #5027</i>
	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene)bp 4-135
25	Bp 133 – 154	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 155 - 540	SV40 Promoter from pS65TC1, Bp 2232-2617
	Bp 541 - 661	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 662 - 1487	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
30	Bp 1488 - 1495	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 1496 – 1524	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107

	Bp 1525 – 2733	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
	Bp 2734 – 3085	Putative PolyA from vector pNK2859
	Bp 3086 – 3128	Lambda DNA from pNK2859
5	Bp 3129 – 3198	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3199 – 3369	pBluescriptII sk(-) base vector (Stratagene, INC)
	Bp 3370 - 3392	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3393 - 5085	Lysozyme Matrix Attachment Region (MAR) from chicken gDNA GenBank Accession #X98408.
10	Bp 5086 – 5163	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru BsiWI
	Bp 5164 - 5476	HSV-TK polyA from pS65TC1 bp 3873-3561
	BP 5477 - 6107	Puromycin resistance gene from pMOD PURO (invivoGen)
	Bp 6108 - 6499	SV40 promoter from pS65TC1, bp 2232-2617
	Bp 6500 - 8204	Lysozyme Matrix Attachment Region (MAR)
15	Bp 8205 – 8274	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 8275 - 8317	Lambda DNA from pNK2859
	Bp 8318 - 10522	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

In specific embodiments, the disclosed hybrid promoters are defined by the following annotations:

SEQ ID NO:14 (CMV/Oval promoter Version 1 = ChOvp/CMVenh/CMVp)

	Bp 1 - 840:	corresponds to bp 421-1260 from the chicken ovalbumin promoter, GenBank accession number
25	Bp 841- 1439:	CMV Enhancer bp 245-843 taken from vector pGWhiz CMV promoter and enhancer bp 844-918 taken from vector pGWhiz (includes the CAAT box at 857-861 and the TATA box at 890-896).
	Bp 1440-1514	CMV promoter

SEQ ID NO:15 (CMV/Oval promoter Version 2 = ChSDRE/CMVenh/ChNRE/CMVp)

30	Bp 1 - 180:	Chicken steroid dependent response element from ovalbumin promoter
	Bp 181 - 779:	CMV Enhancer bp 245-843 taken from vector pGWhiz
	Bp 780 - 1049:	Chicken ovalbumin promoter negative response element

Bp 1050 - 1124: CMV promoter bp 844-918 taken from vector pGWhiz (includes the CAAT box at 857-861 and the TATA box at 890-896. Some references overlap the enhancer to different extents.)

5 In specific embodiments, the disclosed expression vectors are defined by the following annotations:

SEQ ID NO:17 (ID# 148 - CMVep -Intron A RM2 mAb In HS4 Flanked BV)

	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
10	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
15	Bp 770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 1778 - 1785	Synthetic DNA added during construction (combination of two NruI RE sites)
	Bp 1786 – 3021	Transposase modified from Tn10 GenBank Accession #J01829 Bp 81-1316
20	Bp 3022 – 3373	Non-coding DNA from vector pNK2859
	Bp 3374 – 3416	Lambda DNA from pNK2859
	Bp 3417 – 3486	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3487 – 3490	Synthetic DNA added during construction
	Bp 3491 – 3657	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
25	Bp 3658 - 3680	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3681 - 4922	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
	Bp 4923 – 4936	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
	Bp 4937 - 5535	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
30	Bp 5536 - 5610	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 5611 - 5731	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 5732 - 6557	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)

	Bp 6558 - 6565	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 6566 – 6574	Synthetic DNA added to form EcoRV RE site and includes Vitellogenin II Kozak sequence (6572-6577)
5	Bp 6575 - 6622	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession NM_001031276, Bp 1-48)
	Bp 6623 – 7270	Light chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken
	Bp 7271 – 7276	Synthetic DNA added to form AatII RE cut site
10	Bp 7277 – 7745	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# W_060416.1, Bp 5417698 - 5418166)
	BP 7746 – 8269	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2215-2738)
	Bp 8270 – 8277	Synthetic DNA added from combination of two PacI RE cut sites
15	Bp 8278 - 8801	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2738-2215)
	Bp 8802 – 9270	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5418166 – 5417698)
	Bp 9271 – 9273	Synthetic DNA added to form AatII RE cut site
20	Bp 9274 – 10644	Heavy chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken, reverse compliment
	Bp 10645 – 10692	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession #NM_001031276, Bp 48-1)
25	Bp 10693 – 10701	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II Kozak sequence (10690-10695)
	Bp 10702 – 10709	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1873-1866)
	Bp 10710 – 11535	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
30	Bp 11536 - 11656	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
	Bp 11657 – 11731	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 918-844)
	Bp 11732 – 12330	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 843-245)

	BP 12331 – 12354	Multiple Cloning Site Extension from pTn X-MCS, BsiWI thru MluI
	Bp 12355 – 13596	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
	Bp 13597 – 13608	Synthetic DNA added during construction including a PspOMI RE site
5	Bp 13609 – 13678	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 13679 – 13721	Lambda DNA from pNK2859
	Bp 13722 – 13725	Synthetic DNA added during construction
	Bp 13726 – 15926	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961
10	<i>SEQ ID NO:18 (ID# 210 - CMVep -Intron A RM2 mAb in pTn10 HS4 Flanked BV)</i>	
	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
15	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040- 1865)
	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
20	Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
	Bp 3016 – 3367	Putative PolyA from vector pNK2859
	Bp 3368 – 3410	Lambda DNA from pNK2859
25	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3481 – 3484	Synthetic DNA added during construction
	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3675 - 4916	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
30	Bp 4917 – 4930	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
	Bp 4931 - 5529	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 5530 - 5604	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)

	Bp 5605 - 5725	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 5726 - 6551	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 6552 - 6559	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
5		
	Bp 6560 – 6568	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II Kozak sequence (6566-6571)
	Bp 6569 - 6616	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession NM_001031276, Bp 1-48)
10	Bp 6617 – 7264	Light chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken
	Bp 7265 – 7270	Synthetic DNA added to form AatII RE site
	Bp 7271 – 7739	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5417698 - 5418166)
15	BP 7740 – 8263	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2215-2738)
	Bp 8264 – 8271	Synthetic DNA added from combination of two PacI RE sites
	Bp 8272 - 8795	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2738-2215)
20	Bp 8796 – 9264	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5418166 – 5417698)
	Bp 9265 – 9267	Synthetic DNA added to form AatII RE cut site
	Bp 9268 – 10638	Heavy chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken, reverse
25		compliment
	Bp 10639 – 10686	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession #NM_001031276, Bp 48-1)
	Bp 10687 – 10695	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II Kozak sequence (10684-10689)
30	Bp 10696 – 10703	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1873-1866)
	Bp 10704 – 11529	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)

	Bp 11530 - 11650	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
	Bp 11651 – 11725	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 918-844)
	Bp 11726 – 12324	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 843-245)
5	BP 12325 – 12348	Multiple Cloning Site Extension from pTn X-MCS, BsiWI thru MluI
	Bp 12349 – 13590	Chicken HS4-Beta Globin enhancer element from gDNA (corresponds to Genbank Accession #NW_060254 bp 215169-216410)
	Bp 13591 – 13602	Synthetic DNA added during construction including a PspOMI RE site
	Bp 13603 – 13672	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
10	Bp 13673 – 13715	Lambda DNA from pNK2859
	Bp 13716 – 13719	Synthetic DNA added during construction
	Bp 13720 – 15920	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:19 (ID# 212 - CMVep - Intron A RM2 mAb In kTN-10 MAR Flanked BV)

15		
	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
20	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
25	Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
	Bp 3016 – 3367	Putative PolyA from vector pNK2859
	Bp 3368 – 3410	Lambda DNA from pNK2859
30	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3481 – 3484	Synthetic DNA added during construction
	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737

	Bp 3675 - 5367	Lysozyme Matrix Attachment Region (MAR)
	Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
	Bp 5382 - 5980	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 5981 - 6055	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
5	Bp 6056 - 6176	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 6177 - 7002	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 7003 - 7010	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
10	Bp 7011 – 7019	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II kozak sequence
	Bp 7020 – 7067	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession NM_001031276, Bp 1-48)
15	Bp 7068 – 7715	Light chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken
	Bp 7716 – 7721	Synthetic DNA added to form AatII RE site
	Bp 7722 – 8190	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5417698 - 5418166)
20	BP 8191 – 8714	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2215-2738)
	Bp 8715 – 8722	Synthetic DNA added from combination of two PacI RE sites
	Bp 8723 - 9246	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2738-2215)
25	Bp 9247 – 9715	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5418166 – 5417698)
	Bp 9716 – 9718	Synthetic DNA added to form AatII RE site
	Bp 9719 – 11089	Heavy chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken, reverse compliment
30	Bp 11090 – 11137	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession #NM_001031276, Bp 48-1)

- Bp 11138 – 11146 Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II kozak sequence Bp 11147 – 11154 CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1873-1866)
- Bp 11155 – 11980 CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
- 5 Bp 11981 - 12101 CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
- Bp 12102 – 12176 CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 918-844)
- Bp 12177 – 12775 CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 843-245)
- BP 12776 – 12799 Multiple Cloning Site Extension from pTn X-MCS, BsiWI thru MluI
- 10 Bp 12800 – 14492 Lysozyme Matrix Attachment Region (MAR)
- Bp 14493 – 14504 Synthetic DNA added during construction including a PspOMI RE site
- Bp 14505 – 14574 70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
- Bp 14575 – 14617 Lambda DNA from pNK2859
- Bp 14618 – 14621 Synthetic DNA added during construction
- 15 Bp 14622 – 16822 pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:20 (ID# 226 - CMVep-Intron A RM2 mAb in pTn10 PURO-MAR Flanked BV)

- Bp 1 – 132 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
- Bp 133 – 148 pGWIZ base vector (Gene Therapy Systems) bp 229-244
- 20 Bp 149 - 747 CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
- Bp 748 - 822 CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
- Bp 823 - 943 CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
- Bp 944 - 1769 CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
- 25 Bp 1770 - 1777 CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
- Bp 1778 – 1806 TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
- Bp 1807 – 3015 Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
- 30 Bp 3016 – 3367 Putative PolyA from vector pNK2859
- Bp 3368 – 3410 Lambda DNA from pNK2859
- Bp 3411 – 3480 70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
- Bp 3481 – 3484 Synthetic DNA added during construction

	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3675 - 5367	Lysozyme Matrix Attachment Region (MAR)
	Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
5	Bp 5382 - 5980	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 5981 - 6055	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 6056 - 6176	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 6177 - 7002	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
10	Bp 7003 - 7010	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 7011 – 7019	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II Kozak sequence (7017-7022)
	Bp 7020 – 7067	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession NM_001031276, Bp 1-48)
15	Bp 7068 – 7715	Light chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken
	Bp 7716 – 7721	Synthetic DNA added to form AatII RE site
	Bp 7722 – 8190	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5417698 - 5418166)
20	BP 8191 – 8714	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2215-2738)
	Bp 8715 – 8722	Synthetic DNA added from combination of two PacI RE sites
	Bp 8723 - 9246	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2738-2215)
25	Bp 9247 – 9715	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5418166 – 5417698)
	Bp 9716 – 9718	Synthetic DNA added to form AatII RE site
	Bp 9719 – 11089	Heavy chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken, reverse compliment
30	Bp 11090 – 11137	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession #NM_001031276, Bp 48-1)

	Bp 11138 – 11146	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II Kozak sequence (11135-11140)
	Bp 11147 – 11154	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1873-1866)
5	Bp 11155 – 11980	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
	Bp 11981 - 12101	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
	Bp 12102 – 12176	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 918-844)
	Bp 12177 – 12775	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 843-245)
10	Bp 12776 – 12781	BsiWI RE site
	Bp 12782 - 13094	HSV-TK polyA from pS65TC1 bp 3873-3561
	Bp 13095 - 13123	Excess DNA from pMOD PURO (invivoGen)
	BP 13124 - 13725	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116
	Bp 13726 - 14111	SV40 promoter from pS65TC1, bp 2232-2617
15	Bp 14112 - 11417	MluI RE site
	BP 11418 – 15810	Lysozyme Matrix Attachment Region (MAR)
	Bp 15811 – 15822	Synthetic DNA added during construction including a PspOMI RE site
	Bp 15823 – 15892	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 15893 - 15935	Lambda DNA from pNK2859
20	Bp 15936 – 15939	Synthetic DNA added during construction
	Bp 15940 – 18140	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:21

ID# 275 - CMV-Ovalp Vs.1 – RM2 mAb – OPA in pTn10 PURO-MAR Flanked BV

25	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
30	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)

	Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
	Bp 3016 – 3367	Putative PolyA from vector pNK2859
5	Bp 3368 – 3410	Lambda DNA from pNK2859
	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3481 – 3484	Synthetic DNA added during construction
	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
10	Bp 3675 - 5367	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
	Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
	Bp 5382 - 6222	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
	Bp 6223 - 6228	EcoRI RE site
15	BP 6229 - 6827	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 6828 - 6905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
	Bp 6906 - 7026	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
20	BP 7027 - 7852	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 7853 - 7860	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 7861 – 7869	Synthetic DNA added to form EcoRV RE site(includes Vitellogenin II Kozak sequence (7867-7872)
25	Bp 7870 – 7917	Chicken Vitellogenin Signal Sequence (GenBank Accession NM_001031276, Bp 1-48)
	Bp 7918 – 8565	Light chain gene construct from antibody RM2 (provided by Shantha West, Inc.)
	Bp 8566 – 8571	Synthetic DNA added to form AatII RE site
30	Bp 8572 – 9487	Chicken Ovalbumin polyA from gDNA (corresponds to GenBank Accession #J00895 bp 8260-9175)
	Bp 9488 – 9507	Multiple Cloning Site Extension from pTn X-MCS, PacI thru SbfI
	Bp 9508 - 9820	HSV-TK polyA from pS65TC1 bp 3873-3561

	Bp 9821 - 9849	Excess DNA from pMOD PURO (invivoGen)
	BP 9850 - 10451	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116
	Bp 10452 - 10837	SV40 promoter from pS65TC1, bp 2617-2232
	BP 10838 – 10843	BsiWI RE site
5	Bp 10844 - 11759	Chicken Ovalbumin polyA from gDNA (corresponds to GenBank Accession #J00895 bp 9175-8260)
	Bp 11760 – 11762	Synthetic DNA added to form AatII RE site
	Bp 11763 – 13133	Heavy chain gene construct from antibody RM2 (provided by Shantha West, Inc.)
10	Bp 13134 – 13181	Chicken Vitellogenin Signal Sequence (GenBank Accession #NM_001031276, Bp 48-1)
	Bp 13182 – 13190	Synthetic DNA added to form EcoRV RE site (includes Vitellogenin II Kozak sequence (13179-13184))
	Bp 13191 – 13198	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1873-1866)
15	Bp 13199 – 14024	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
	Bp 14025 - 14145	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
	Bp 14146 - 14223	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
20	BP 14224 - 14822	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 14823 - 14828	EcoRI RE site
	Bp 14829 - 15669	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
25	Bp 15670 - 15675	MluI RE site
	Bp 15676 - 17368	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
	Bp 17369 – 17380	Synthetic DNA added during construction including a PspOMI RE site
	Bp 17381 - 17450	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 17451 - 17493	Lambda DNA from pNK2859
30	Bp 17494 - 17497	Synthetic DNA added during construction
	Bp 17498 - 19698	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:22

ID# 278 - CMV-Ovalp Vs.1 – Δ CH2 mAb – OPA in pTn10 PURO-MAR Flanked BV

	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
5	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
10	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
15	Bp 3016 – 3367	Putative PolyA from vector pNK2859
	Bp 3368 – 3410	Lambda DNA from pNK2859
	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3481 – 3484	Synthetic DNA added during construction
	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
20	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3675 - 5367	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
	Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
	Bp 5382 - 6222	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
25	Bp 6223 - 6228	EcoRI RE site
	BP 6229 - 6827	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 6828 - 6905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
	Bp 6906 - 7026	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
30	BP 7027 - 7852	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 7853 - 7860	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)

	Bp 7861 – 7869	Synthetic DNA added to form EcoRV RE site(includes Vitellogenin II Kozak sequence (7867-7872))
	Bp 7870 – 7917	Chicken Vitellogenin Signal Sequence (GenBank Accession NM_001031276, Bp 1-48)
5	Bp 7918 – 8550	Light chain gene construct from antibody CC49ΔCH2V15 (provided by NCI)
	Bp 8551 – 8586	Synthetic DNA added to form AatII RE site
	Bp 8587 – 9502	Chicken Ovalbumin polyA from gDNA (corresponds to GenBank Accession #J00895 bp 8260-9175)
10	Bp 9503 – 9522	Multiple Cloning Site Extension from pTn X-MCS, PacI thru SbfI
	Bp 9523 - 9835	HSV-TK polyA from pS65TC1 bp 3873-3561
	Bp 9836 - 9864	Excess DNA from pMOD PURO (invivoGen)
	BP 9865 - 10466	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116
	Bp 10467 - 10852	SV40 promoter from pS65TC1, bp 2617-2232
15	BP 10853 – 10858	BsiWI RE site
	Bp 10859 - 11774	Chicken Ovalbumin polyA from gDNA (corresponds to GenBank Accession #J00895 bp 9175-8260)
	Bp 11775 – 11780	Synthetic DNA added to form AatII RE site
	Bp 11781 – 12815	Heavy chain gene construct from antibody CC49ΔCH2V15 (provided by NCI)
20	Bp 12816 – 12863	Chicken Vitellogenin Signal Sequence (GenBank Accession #NM_001031276, Bp 48-1)
	Bp 12864 – 12872	Synthetic DNA added to form EcoRV RE site(includes Vitellogenin II Kozak sequence (12861-12866))
25	Bp 12873 – 12880	CMV Immediate Early Gene, Partial Exon 2(pGWIZ, Gene Therapy Systems bp 1873-1866)
	Bp 12881 – 13706	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
	Bp 13707 - 13827	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
30	Bp 13828 - 13905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
	BP 13906 - 14504	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 14505 - 14510	EcoRI RE site

	Bp 14511 - 15351	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
	Bp 15352 - 15357	MluI RE site
	Bp 15358 – 17050	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
5	Bp 17051 – 17062	Synthetic DNA added during construction including a PspOMI RE site
	Bp 17063 - 17132	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 17133 - 17175	Lambda DNA from pNK2859
	Bp 17176 - 17179	Synthetic DNA added during construction
	Bp 17180 - 19380	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961
10	<i>SEQ ID NO:23</i>	
	<i>ID# 273 - CMV-Ovalp Vs.1 – RM2 mAb in pTn10 PURO-MAR Flanked BV</i>	
	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
15	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040- 1865)
20	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
	Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
25	Bp 3016 – 3367	Putative PolyA from vector pNK2859
	Bp 3368 – 3410	Lambda DNA from pNK2859
	Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3481 – 3484	Synthetic DNA added during construction
	Bp 3485 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
30	Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3675 - 5367	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
	Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI

	Bp 5382 - 6222	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
	Bp 6223 - 6228	EcoRI RE site
	BP 6229 - 6827	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
5	Bp 6828 - 6905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
	Bp 6906 - 7026	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	BP 7027 - 7852	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
10	Bp 7853 - 7860	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 7861 – 7869	Synthetic DNA added to form EcoRV RE site(includes Vitellogenin II Kozak sequence (7867-7872)
	Bp 7870 – 7917	Chicken Vitellogenin Signal Sequence (GenBank Accession
15		NM_001031276, Bp 1-48)
	Bp 7918 – 8565	Light chain gene construct from antibody RM2 (provided by Shantha West, Inc.)
	Bp 8566 – 8571	Synthetic DNA added to form AatII RE site
	Bp 8572 – 9040	Chicken Vitellogenin PolyA (corresponds to GenBank Accession#
20		NW_060416.1, Bp 5417698 - 5418166)
	BP 9041 – 9564	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2215-2738)
	Bp 9565 – 9584	Multiple Cloning Site Extension from pTn X-MCS, PacI thru SbfI
	Bp 9585 - 9897	HSV-TK polyA from pS65TC1 bp 3873-3561
25	Bp 9898 - 9926	Excess DNA from pMOD PURO (invivoGen)
	BP 9927 - 10528	Puromycin resistance gene from pMOD PURO (invivoGen) bp 717-116
	Bp 10529 - 10914	SV40 promoter from pS65TC1, bp 2617-2232
	BP 10915 – 10920	BsiWI RE site
	Bp 10921 - 11444	Chicken Vitellogenin 3' Flanking region (GenBank Accession #Y00324,
30		Bp 2738-2215)
	Bp 11445 – 11913	Chicken Vitellogenin PolyA (GenBank Accession# NW_060416.1, Bp 5418166 – 5417698)
	Bp 11914 – 11916	Synthetic DNA added to form AatII RE site

	Bp 11917 – 13287	Heavy chain gene construct from antibody RM2 (provided by Shantha West, Inc.)
	Bp 13288 – 13335	Chicken Vitellogenin Signal Sequence (GenBank Accession #NM_001031276, Bp 48-1)
5	Bp 13336 – 13344	Synthetic DNA added to form EcoRV RE site (includes Vitellogenin II Kozak sequence (13333-13338))
	Bp 13345 – 13352	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1873-1866)
	Bp 13353 – 14178	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
10	Bp 14179 - 14299	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
	Bp 14300 - 14377	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
	BP 14378 - 14976	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
15	Bp 14977 - 14981	EcoRI RE site
	Bp 14982 - 15822	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
	Bp 15823 - 15829	MluI RE site
	Bp 15830 - 17522	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
20	Bp 17523 – 17534	Synthetic DNA added during construction including a PspOMI RE site
	Bp 17535 - 17604	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 17605 - 17647	Lambda DNA from pNK2859
	Bp 17648 - 17651	Synthetic DNA added during construction
	Bp 17652 - 19852	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

25

*SEQ ID NO:24**Vitellogenin-Intron A RM2 mAb Flanked with HS4 in pTnMCS modified BV*

	Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
	Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
30	Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
	Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
	Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)

	Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
	Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1866-1873)
	Bp 1778 – 1785	Synthetic DNA added during construction (combination of 2 NruI sites)
5	Bp 1786 – 3018	Transposase, modified from Tn10 (GenBank accession #J01829 Bp 81-1313)
	Bp 3019 – 3021	Engineered stop codon
	Bp 3022 – 3373	Non-coding DNA from vector pNK2859
	Bp 3374 – 3416	Lambda DNA from pNK2859
10	Bp 3417 – 3486	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
	Bp 3487 – 3490	Synthetic DNA added during construction
	Bp 3491 – 3657	pBluescriptII sk(-) base vector (Stratagene, INC) bp 926-760
	Bp 3658 - 3680	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
	Bp 3681 - 4922	Chicken Beta globin enhancer element (corresponds to GenBank Accession #NW_060254,Bp 215169-216410)
15	Bp 4923 – 4935	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI
	Bp 4936 - 6079	Chicken Vitellogenin Promoter (corresponds to GenBank Accession #X00345,Bp 1-1144)
	Bp 6080 – 6095	pGWIZ base vector (Gene Therapy Systems) bp 903-918
20	Bp 6096 - 6216	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	Bp 6217 – 7042	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1040-1865)
	Bp 7043 – 7050	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1866-1873)
25	Bp 7051 – 7059	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II Kozak sequence (7057-7062)
	Bp 7060 – 7107	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession NM_01031276, Bp 1-48)
	Bp 7108 – 7755	Light chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken
30	Bp 7756 – 7761	Synthetic DNA added to form AatII RE site
	Bp 7762 – 8230	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5417698 - 5418166)

	BP 8231 – 8754	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2215-2738)
	Bp 8755 – 8762	Synthetic DNA added from combination of two PacI RE sites
5	Bp 8763 - 9286	Chicken Vitellogenin 3' Flanking region (corresponds to GenBank Accession #Y00324, Bp 2738-2215)
	Bp 9287 – 9755	Chicken Vitellogenin PolyA (corresponds to GenBank Accession# NW_060416.1, Bp 5418166 – 5417698)
	Bp 9756 – 9758	Synthetic DNA added to form AatII RE site
10	Bp 9759 – 11129	Heavy chain gene construct taken from antibody RM2 provided by Mark Glassy (Shantha West, Inc.) - codon optimized for chicken, reverse compliment
	Bp 11130 – 11177	Chicken Vitellogenin Signal Sequence (corresponds to GenBank Accession #NM_001031276, Bp 48-1)
15	Bp 11178 – 11186	Synthetic DNA added to form EcoRV RE site & includes Vitellogenin II Kozak sequence (11175-11180)
	Bp 11187 – 11194	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems bp 1873-1866)
	Bp 11195 – 12020	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
20	Bp 12021 - 12141	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)
	Bp 12142 – 12157	pGWIZ base vector (Gene Therapy Systems) bp 918-903
	Bp 12158 – 13301	Chicken Vitellogenin Promoter (corresponds to GenBank Accession #X00345, Bp 1144-1)
	BP 13302 – 13324	Multiple Cloning Site Extension from pTn X-MCS, BsiWI thru MluI
25	Bp 13325 – 14566	Chicken Beta globin enhancer element (corresponds to GenBank Accession #NW_060254, Bp 216410-215169)
	Bp 14567 – 14578	Synthetic DNA added during construction including a PspOMI RE site
	Bp 14579 – 14648	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
	Bp 14649 – 14691	Lambda DNA from pNK2859
30	Bp 14692 – 14695	Synthetic DNA added during construction
	Bp 14696 – 16896	pBluescriptII sk(-) base vector (Stratagene, INC) bp 761-2961

SEQ ID NO:25 267-5021 - Puro/Mar (CMV.Ovalp vs.1/Conss(-AA)/Herceptin

HC/OvpyA/OvpyA/Herceptin LC/Conss(-AA)/CMV.Ovalp vs. 1)

	Bp 1 - 5381	Puro/Mar backbone (bp 1-5381)
5	Bp 5382 - 6228	Chicken Ovalbumin Promoter (bp 1090-1929), including synthetic DNA added during vector construction (EcoRI site used for ligation) on 3' end
	Bp 6229 - 6905	CMV enhancer/promoter, bp 245-899 of gWIZ blank Vector, CTC, bp 900-918 of CMVpromoter from gWIZ blank vector
10	Bp 6906 - 7866	CMV intron A (bp 919-1873 of gWIZ; includes CMV immediate-early gene, Exon1; CMV intron A; CMV immediate-early gene, partial Exon 2), including synthetic DNA added during vector construction (SalI site used for ligation) on 3' end
	Bp 7867 - 7926	Chicken Conalbumin Signal Sequence + Kozak sequence (7867-7872) (from GenBank Accession # X02009)
15	Bp 7927 - 8301	Synthetic construct of humAb4D5-8 humanized heavy chain variable region, taken from GenBank Accession #: AY513484 (bp 1-375)
	Bp 8302 - 9285	Human immunoglobulin kappa heavy chain constant region, taken from GenBank Accession #: Y14735 (bp 480-1457), including synthetic DNA added during vector construction (BamHI site used for ligation) on 3' end
20	Bp 9286 - 10202	Chicken Ovalbumin PolyA (taken from GenBank Accession # J00895, bp 8260-9176)
	Bp 10203 - 10236	Puro/Mar backbone (bp 5385-5418); part of the multiple cloning site
25	Bp 10237 - 11159	Chicken Ovalbumin PolyA taken from GenBank Accession # J00895 (bp 9176-8260), including synthetic DNA added during vector construction (BamHI site for ligation)
	Bp 11160 - 11481	Human immunoglobulin kappa light chain constant region, taken from GenBank Accession # Y14736 (bp 725-410)
30	Bp 11482 - 11804	Synthetic construct of humAb4D5-8 humanized antibody light chain variable region, taken from GenBank Accession # AY513485 (bp 323-1)
	Bp 11805 - 11870	Chicken Conalbumin Signal Sequence + Kozak sequence (11859-11864) (from GenBank Accession # X02009); reverse complement,

- including synthetic DNA added during vector construction (Sall site used for ligation) on 3' end
- 5 Bp 11871 – 12825 CMV intron A'(bp 1873-919 of gWiz; includes CMV immediate-early gene, partial Exon2; CMV intron A; CMV immediate-early gene, Exon 1)
- Bp 12826 – 13508 bp 918-900 of CMVpromoter from gWIZ blank vector, GAG, CMV enhancer/promoter (bp 899-245 of gWIZ blank vector), including synthetic DNA added during vector construction (EcoRI site used for ligation) on 3' end
- 10 Bp 13509 – 14349 Chicken Ovalbumin Promoter (bp 1929-1090)
- Bp 14350 – 19714 Puro/Mar backbone (bp 5440-10804)

SEQ ID NO:26

ID# 279

HPvs1/ CMViA/ CAss+koz/ coΔCH2 mAb/ OPA in pTn10 PURO-MAR Flanked BV

- 15 Bp 1 – 132 Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
- Bp 133 – 148 pGWIZ base vector (Gene Therapy Systems) bp 229-244
- Bp 149 - 747 CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
- Bp 748 - 822 CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
- Bp 823 - 943 CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
- 20 Bp 944 - 1769 CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
- Bp 1770 - 1777 CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
- Bp 1778 – 1806 TN10 DNA, 3' end from Genbank Accession #J01829 bp79 - 107
- 25 Bp 1807 – 3015 Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
- Bp 3016 – 3367 Putative PolyA from vector pNK2859
- Bp 3368 – 3410 Lambda DNA from pNK2859
- Bp 3411 – 3480 70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
- 30 Bp 3481 – 3651 pBluescriptII sk(-) base vector (Stratagene, INC)
- Bp 3652 - 3674 Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
- Bp 3675 - 5367 Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
- Bp 5368 – 5381 Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI

	Bp 5382 - 6223	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
	BP 6224 - 6827	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)with 5' EcoRI RE site
5	Bp 6828 - 6905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
	Bp 6906 - 7026	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	BP 7027 - 7852	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
10	Bp 7853 - 7860	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 7861 – 7926	Conalbumin Signal Peptide (Genbank #Y00407 bp 340-385, 1699-1715) with 5'SalI RE site. Kozak sequence (7867-7872)
	Bp 7927 – 8589	Light chain gene construct-codon optimized from antibody
15		CC49ΔCH2V15 (provided by NCI)
	Bp 8590 – 9511	Chicken Ovalbumin polyA from gDNA (GenBank #J00895 bp 8260-9175) with 5'AgeI RE site
	Bp 9512 – 9531	Multiple Cloning Site Extension from pTn X-MCS, PacI thru SbfI
	Bp 9532 - 9844	HSV-TK polyA from pS65TC1 bp 3873-3561
20	BP 9845 - 10475	Puromycin resistance gene from pMOD PURO (invivoGen)
	Bp 10476 - 10867	SV40 promoter from pS65TC1, bp 2617-2232 with 5' BsiWI RE site
	Bp 10868 - 11791	Chicken Ovalbumin polyA from gDNA (GenBank #J00895 bp 9175-8260) with 5'AsiSI RE site
	Bp 11792 – 12826	Heavy chain gene construct-codon optimized from antibody
25		CC49ΔCH2V15 (provided by NCI)
	Bp 12827 – 12892	Conalbumin Signal Peptide (Genbank #Y00407 bp 340-385, 1699-1715) with 5'SalI RE site. Kozak sequence (12881-12886)
	Bp 12893 – 12900	CMV Immediate Early Gene, Partial Exon 2(pGWIZ, Gene Therapy Systems bp 1873-1866)
30	Bp 12901 – 13726	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
	Bp 13727 - 13847	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)

Bp 13848 - 13925	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 918-900, CTC, 899-844)
BP 13926 - 14530	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 843-245) with 5' EcoRI RE site
5 Bp 14531 - 15377	Chicken Ovalbumin promoter from gDNA (Genbank #J00895 bp 1261-421) with 5'MluI RE site
Bp 15378 – 17082	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
Bp 17083 - 17152	70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
Bp 17153 - 17195	Lambda DNA from pNK2859
10 Bp 17196 - 19400	pBluescriptII sk(-) base vector (Stratagene, INC)

SEQ ID NO: 27

VID# 280

Hybrid Promoter Vs.1 – co-RM2 mAb – OPA in pTn10 PURO-MAR Flanked BV

Bp 1 – 132	Remainder of F1 (-) ori of pBluescriptII sk(-) (Stratagene) bp 4-135
15 Bp 133 – 148	pGWIZ base vector (Gene Therapy Systems) bp 229-244
Bp 149 - 747	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)
Bp 748 - 822	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-918)
Bp 823 - 943	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
20 Bp 944 - 1769	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
Bp 1770 - 1777	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
Bp 1778 – 1806	TN10 DNA, 3'end from Genbank Accession #J01829 bp79 - 107
Bp 1807 – 3015	Transposon, modified from Tn10 GenBank Accession #J01829 Bp 108-1316
25 Bp 3016 – 3367	Putative PolyA from vector pNK2859
Bp 3368 – 3410	Lambda DNA from pNK2859
Bp 3411 – 3480	70bp of IS10 left from Tn10 (GenBank Accession #J01829 Bp 1-70)
Bp 3481 – 3651	pBluescriptII sk(-) base vector (Stratagene, INC)
30 Bp 3652 - 3674	Multiple cloning site from pBluescriptII sk(-)thru NotI, Bp 759-737
Bp 3675 - 5367	Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
Bp 5368 – 5381	Multiple Cloning Site Extension from pTn X-MCS, XhoI thru AscI

	Bp 5382 - 6223	Chicken Ovalbumin promoter from gDNA (Genbank Accession #J00895 bp 421-1261)
	BP 6224 - 6827	CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 245-843)with 5' EcoRI RE site
5	Bp 6828 - 6905	CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 844-899, CTC, 900-918)
	Bp 6906 - 7026	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 919-1039)
	BP 7027 - 7852	CMV Intron A (vector pGWIZ, Gene Therapy Systems bp 1040-1865)
10	Bp 7853 - 7860	CMV Immediate Early Gene, Partial Exon 2 (pGWIZ, Gene Therapy Systems)bp 1866-1873)
	Bp 7861 - 7926	Conalbumin Signal Peptide (Genbank #Y00407 bp 340-385, 1699-1715) with 5'SalI RE site. Kozak sequence (7867-7872)
	Bp 7927 - 8574	Light chain gene-codon optimized from antibody RM2 (provided by Shantha West, Inc.)
15	Bp 8575 - 9496	Chicken Ovalbumin polyA from gDNA (GenBank #J00895 bp 8260-9175) with 5' AgeI RE site
	Bp 9497 - 9516	Multiple Cloning Site Extension from pTn X-MCS, PacI thru SbfI
	Bp 9517 - 9829	HSV-TK polyA from pS65TC1 bp 3873-3561
20	BP 9830 - 10460	Puromycin resistance gene from pMOD PURO (invivoGen)
	Bp 10461 - 10852	SV40 promoter from pS65TC1, bp 2617-2232 with 5' BsiWI RE site
	Bp 10853 - 11776	Chicken Ovalbumin polyA from gDNA (GenBank #J00895 bp 9175-8260) with 5' AsiSI RE site
	Bp 11777 - 13144	Heavy chain gene construct from antibody RM2 (provided by Shantha West, Inc.)
25	Bp 13145 - 13210	Conalbumin Signal Peptide (Genbank #Y00407 bp 340-385, 1699-1715) with 5'SalI RE site. Kozak sequence (13199-13204)
	Bp 13211 - 13218	CMV Immediate Early Gene, Partial Exon 2(pGWIZ, Gene Therapy Systems bp 1873-1866)
30	Bp 13219 - 14044	CMV Intron A (vector pGWIZ, Gene Therapy Systems Bp 1865-1040)
	Bp 14045 - 14165	CMV Immediate Early Gene, Exon 1 (vector pGWIZ, Gene Therapy Systems bp 1039-919)

- Bp 14166 - 14243 CMV Promoter (vector pGWIZ, Gene Therapy Systems bp 918-900, CTC, 899-844)
- BP 14244 - 14848 CMV Enhancer (vector pGWIZ, Gene Therapy Systems bp 843-245) with 5' EcoRI RE site
- 5 Bp 14849 - 15695 Chicken Ovalbumin promoter from gDNA (Genbank #J00895 bp 1261-421) with 5'MluI RE site
- Bp 15696 - 17400 Chicken Lysozyme Matrix Attachment region (MAR) from gDNA
- Bp 17401 - 17470 70 bp of IS10 from Tn10 (GenBank Accession #J01829 Bp 70-1)
- Bp 17471 - 17513 Lambda DNA from pNK2859
- 10 Bp 17514 - 19718 pBluescriptII sk(-) base vector (Stratagene, INC)

In one embodiment, the present application provides a novel sequence comprising a promoter, a gene of interest, and a poly A sequence. Each of these novel sequences may be identified from the annotations for each expression vector shown above, and also as sequences within the sequence listing for each expression vector. The specific bases of these novel sequences are provided in Table 3 below for each expression vector SEQ ID NOs: 17 to 27.

Table 3

mAb's		
SEQ ID NO	Light Chain	Heavy Chain
17	4937-8269	8278-12330
18	4931-8263	8272-12324
19	5382-8714	8723-12775
20	5382-8714	8723-12775
21	5382-9487	10844-15669
22	5382-9502	10859-15351
23	5382-9564	10921-15822
24	4936-8754	8763-13301
25	5382-10202	10237-13508
26	5382-9511	10868-15377
27	5382-9496	10853-15695

E. Methods of *In Vivo* Administration

The polynucleotide cassettes may be delivered through the vascular system to be distributed to the cells supplied by that vessel. For example, the compositions may be administered through the cardiovascular system to reach target tissues and cells receiving blood supply. In one embodiment, the compositions may be administered through any chamber of the heart, including the right ventricle, the left ventricle, the right atrium or the left atrium. Administration into the right side of the heart may target the pulmonary circulation and tissues supplied by the pulmonary artery. Administration into the left side of the heart may target the systemic circulation through the aorta and any of its branches, including but not limited to the coronary vessels, the ovarian or testicular arteries, the renal arteries, the arteries supplying the gastrointestinal and pelvic tissues, including the celiac, cranial mesenteric and caudal mesenteric vessels and their branches, the common iliac arteries and their branches to the pelvic organs, the gastrointestinal system and the lower extremity, the carotid, brachiocephalic and subclavian arteries. It is to be understood that the specific names of blood vessels change with the species under consideration and are known to one of ordinary skill in the art. Administration into the left ventricle or ascending or descending aorta supplies any of the tissues receiving blood supply from the aorta and its branches, including but not limited to the testes, ovary, oviduct, and liver. Germline cells and other cells may be transfected in this manner. For example, the compositions may be placed in the left ventricle, the aorta or directly into an artery supplying the ovary or supplying the fallopian tube to transfect cells in those tissues. In this manner, follicles could be transfected to create a germline transgenic animal. Alternatively, supplying the compositions through the artery leading to the oviduct would preferably transfect the tubular gland and epithelial cells. Such transfected cells could manufacture a desired protein or peptide for deposition in the egg white. Administration of the compositions through the left cardiac ventricle, the portal vein or hepatic artery would target uptake and transformation of hepatic cells. Administration may occur through any means, for example by injection into the left ventricle, or by administration through a cannula or needle introduced into the left atrium, left ventricle, aorta or a branch thereof.

Intravascular administration further includes administration in to any vein, including but not limited to veins in the systemic circulation and veins in the hepatic portal circulation. Intravascular administration further includes administration into the cerebrovascular system, including the carotid arteries, the vertebral arteries and branches thereof.

Intravascular administration may be coupled with methods known to influence the permeability of vascular barriers such as the blood brain barrier and the blood testes barrier, in

order to enhance transfection of cells that are difficult to affect through vascular administration. Such methods are known to one of ordinary skill in the art and include use of hyperosmotic agents, mannitol, hypothermia, nitric oxide, alkylglycerols, lipopolysaccharides (Haluska et al., Clin. J. Oncol. Nursing 8(3): 263-267, 2004; Brown et al., Brain Res., 1014: 221-227, 2004; Ikeda et al., Acta Neurochir. Suppl. 86:559-563, 2004; Weyerbrock et al., J. Neurosurg. 99(4):728-737, 2003; Erdlenbruch et al., Br. J. Pharmacol. 139(4):685-694, 2003; Gaillard et al., Microvasc. Res. 65(1):24-31, 2003; Lee et al., Biol. Reprod. 70(2):267-276, 2004)).

Intravascular administration may also be coupled with methods known to influence vascular diameter, such as use of beta blockers, nitric oxide generators, prostaglandins and other reagents that increase vascular diameter and blood flow.

Administration through the urethra and into the bladder would target the transitional epithelium of the bladder. Administration through the vagina and cervix would target the lining of the uterus and the epithelial cells of the fallopian tube.

The polynucleotide cassettes may be administered in a single administration, multiple administrations, continuously, or intermittently. The polynucleotide cassettes may be administered by injection, via a catheter, an osmotic mini-pump or any other method. In some embodiments, a polynucleotide cassette is administered to an animal in multiple administrations, each administration containing the polynucleotide cassette and a different transfecting reagent.

In a preferred embodiment, the animal is an egg-laying animal, and more preferably, an avian, and the transposon-based vectors comprising the polynucleotide cassettes are administered into the vascular system, preferably into the heart. The vector may be injected into the venous system in locations such as the jugular vein and the metatarsal vein. In one embodiment, between approximately 1 and 1000 μ g, 1 and 200 μ g, 5 and 200 μ g, or 5 and 150 μ g of a transposon-based vector containing the polynucleotide cassette is administered to the vascular system, preferably into the heart. In a chicken, it is preferred that between approximately 1 and 300 μ g, or 5 and 200 μ g are administered to the vascular system, preferably into the heart, more preferably into the left ventricle. The total injection volume for administration into the left ventricle of a chicken may range from about 10 μ l to about 5.0 ml, or from about 100 μ l to about 1.5 ml, or from about 200 μ l to about 1.0 ml, or from about 200 μ l to about 800 μ l. It is to be understood that the total injection volume may vary depending on the duration of the injection. Longer injection durations may accommodate higher total volumes. In a quail, it is preferred that between approximately 1 and 200 μ g, or between approximately 5 and 200 μ g are administered to the vascular system, preferably into the heart, more preferably into the left ventricle. The total injection volume for administration into the left ventricle of a quail may range from about 10 μ l to about 1.0 ml, or

from about 100 µl to about 800 µl, or from about 200 µl to about 600 µl. It is to be understood that the total injection volume may vary depending on the duration of the injection. Longer injection durations may accommodate higher total volumes. The microgram quantities represent the total amount of the vector with the transfection reagent.

5 In another embodiment, the animal is an egg-laying animal, and more preferably, an avian. In one embodiment, between approximately 1 and 150 µg, 1 and 100 µg, 1 and 50 µg, preferably between 1 and 20 µg, and more preferably between 5 and 10 µg of a transposon-based vector containing the polynucleotide cassette is administered to the oviduct of a bird. In a chicken, it is preferred that between approximately 1 and 100 µg, or 5 and 50 µg are
10 administered. In a quail, it is preferred that between approximately 5 and 10 µg are administered. Optimal ranges depending upon the type of bird and the bird's stage of sexual maturity. Intraoviduct administration of the transposon-based vectors of the present invention result in a PCR positive signal in the oviduct tissue, whereas intravascular administration results in a PCR positive signal in the liver, ovary and other tissues. In other embodiments, the polynucleotide
15 cassettes is administered to the cardiovascular system, for example the left cardiac ventricle, or directly into an artery that supplies the oviduct or the liver. These methods of administration may also be combined with any methods for facilitating transfection, including without limitation, electroporation, gene guns, injection of naked DNA, and use of dimethyl sulfoxide (DMSO). U.S. Patent No. 7,527,966, U.S. Publication No. 2008/0235815, and PCT Publication No. WO
20 2005/062881 are hereby incorporated by reference in their entirety.

The following examples will serve to further illustrate the present invention without, at the same time, however, constituting any limitation thereof. On the contrary, it is to be clearly understood that resort may be had to various embodiments, modifications and equivalents thereof
25 which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the invention.

EXAMPLE 1

Preparation of Vectors for In Vitro Expression of RM2 Monoclonal Antibody

30 Several expression vectors were constructed to include genes which encode the light and heavy chains of an RM2 monoclonal antibody.

Vector #148 (SEQ ID NO:17) was made by digesting both the pTn10 HS4 flanked backbone (pTn10 HFB #5006) vector (SEQ ID NO:3) and a pTopo vector comprising the RM2 cassette with Asc I and BsiWI. This digestion linearized the pTn10 HFB #5006 vector and

released the RM2 cassette from the pTopo vector. The RM2 cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized pTn10 HFB #5006 vector.

Vector #210 (SEQ ID NO:18) was made by digesting both the pTn10 HS4 flanked backbone (pTn10 HFB) vector (SEQ ID NO:4) and a pTopo vector comprising the RM2 cassette with Asc I and BsiWI. This digestion linearized the pTn10 HFB vector and released the RM2 cassette from the pTopo vector. The RM2 cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized pTn10 HFB vector.

Vector #212 (SEQ ID NO:19) was made by digesting both the pTn10 MAR flanked backbone (pTn10 MAR) vector (SEQ ID NO:5) and a pTopo vector comprising the RM2 cassette with Asc I and BsiWI. This digestion linearized the pTn10 MAR vector and released the RM2 cassette from the pTopo vector. The RM2 cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized pTn10 MAR vector.

Vector #226 (SEQ ID NO:20) was made by digesting both the pTn10 PURO-MAR flanked backbone (pTn10 PURO-MAR) vector (SEQ ID NO:8) and a pTopo vector comprising the RM2 cassette with Asc I and BsiWI. This digestion linearized the pTn10 PURO-MAR vector and released the RM2 cassette from the pTopo vector. The RM2 cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized pTn10 PURO-MAR vector.

Vector #275 (SEQ ID NO:21) was made by digesting both the pTn10 PURO-MAR flanked backbone (pTn10 PURO-MAR) vector (SEQ ID NO:8) and a pTopo vector comprising the RM2 LC cassette with Asc I and Pac I. This digestion linearized the pTn10 PURO-MAR vector and released the RM2 LC cassette from the pTopo vector. The RM2 LC cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized pTn10 PURO-MAR vector. The resulting vector and a pTopo vector comprising the RM2 HC cassette was then digested with Mlu I and BsiW I. This digestion linearized the RM2 LC cassette + pTn10 PURO-MAR vector and released the RM2 HC cassette from the pTopo vector. The RM2 HC cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized RM2 LC cassette + pTn10 PURO-MAR vector.

Vector #278 (SEQ ID NO:22) was made by digesting both the pTn10 PURO-MAR flanked backbone (pTn10 PURO-MAR) vector (SEQ ID NO:8) and a pTopo vector comprising

the Δ CH2 LC cassette with Asc I and Pac I. This digestion linearized the pTn10 PURO-MAR vector and released the Δ CH2 LC cassette from the pTopo vector. The Δ CH2 LC cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized pTn10 PURO-MAR vector. The resulting vector and a pTopo vector comprising the Δ CH2 HC cassette was then digested with Mlu I and BsiW I. This digestion linearized the Δ CH2 LC cassette + pTn10 PURO-MAR vector and released the Δ CH2 HC cassette from the pTopo vector. The Δ CH2 HC cassette was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research) and ligated into the linearized Δ CH2 LC cassette + pTn10 PURO-MAR vector.

Construction of Vectors #273 (SEQ ID NO:23)

The pTopo containing the RM2 monoclonal antibody (mAb) cassettes driven by either the hybrid promoter version 1 (SEQ ID NO:14) or version 2 (SEQ ID NO:15) were digested with restriction enzymes AscI, PacI, BsiWI, and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the RM2 light chain (LC) cassette into the MCS of the p5021 vector (SEQ ID NO:8), the purified LC DNA and the p5021 vector (SEQ ID NO:8) were digested with AscI and PacI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. To insert the RM2 heavy chain (HC) cassette into the MCS of the p5021 vector (SEQ ID NO:8), the purified HC DNA and the p5021 vector (SEQ ID NO:8) were digested with BsiWI and MluI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* XL10-Gold Ultracompetent cells (Stratagene, Inc. La Jolla, CA) using chemical transformation according to Stratagene's protocol. Transformed bacteria were incubated in 0.9 ml of NZY Plus (TEKnova, CAT# N1215) broth for 1 hour at 37°C before being spread to LB (Luria-Bertani media (broth or agar)) plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no

further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System.

Construction of Vectors #146/149 (SEQ ID NO:24)

The pTopo containing the RM2 monoclonal antibody (mAb) cassettes driven by the Vitellogenin II promoter were digested with restriction enzymes AscI, BsiWI, and PacI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified from restriction enzymes using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the RM2 light chain (LC) cassette into the MCS of the p5006 vector (SEQ ID NO:3), the purified LC DNA and the p5006 vector (SEQ ID NO:3) were digested with AscI and PacI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. To insert the RM2 heavy chain (HC) cassette into the MCS of the p5006 vector (SEQ ID NO:3), the purified HC DNA and the p5006 vector (SEQ ID NO:3) were digested with BsiWI and PacI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Ligated product was transformed into *E. coli* XL10-Gold Ultracompetent cells (Stratagene, Inc. La Jolla, CA) using chemical transformation according to Stratagene's protocol. Transformed bacteria were incubated in 0.9 ml of NZY Plus (TEKnova, CAT# N1215) broth for 1 hour at 37°C before being spread to LB (Luria-Bertani media (broth or agar)) plates supplemented with 100 µg/ml ampicillin (LB/amp plates). These plates were incubated overnight at 37°C and resulting colonies picked to LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth and plasmid DNA harvested using a Qiagen Maxi-Prep Kit (column purification) according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained both the Light Chain and Heavy Chain gene, the DNA was isolated by standard procedures. Briefly, *Escherichia coli* containing the plasmid was grown in 500 mL aliquots of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight with shaking. Plasmid DNA was recovered from the bacteria using a Qiagen EndoFree Plasmid Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol.

Plasmid DNA was resuspended in 600µL of Endotoxin free water and stored at -20°C until needed.

Construction of Vector #267 (SEQ ID NO:25)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the Herceptin monoclonal
5 antibody (mAb) cassettes driven by the hybrid promoter version 1 (SEQ ID NO:14) are digested
with restriction enzymes AscI, AsiSI, SbfI, and BsiWI (New England Biolabs, Beverly, MA)
according to the manufacturer's protocol. Digested DNA is purified using a Zymo DNA Clean
and Concentrator kit (Zymo Research, Orange, CA). To insert the Herceptin heavy chain (HHC)
cassette into the MCS of vector p5021 (SEQ ID NO:8), HHC and p5021 vector DNA (SEQ ID
10 NO:8) are digested with AscI and AsiSI, purified as described above, and ligated using a Quick
T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's
protocol. To insert the Herceptin light chain (HLC) cassette into the MCS of p5021 (SEQ ID
NO:5), HLC DNA and vector p5021 (SEQ ID NO:8) containing the Herceptin HC cassette, are
digested with SbfI and BsiWI, purified and ligated as described above. Ligated product is
15 transformed into *E. coli* XL10-Gold Ultracompetent cells (Stratagene, Inc. La Jolla, CA) using
chemical transformation according to the manufacturer's protocol. Transformed bacterial cells
are incubated in 0.9 ml of NZY Plus (TEKnova, CAT# N1215) broth for 1 hour at 37°C then
spread to LB (Luria-Bertani) agar plates supplemented with 100 µg/ml ampicillin (LB/amp
plates). All plates are incubated overnight at 37°C. Resulting colonies are picked into LB/amp
20 broth for overnight growth at 37°C. Plasmid DNA is isolated using a modified alkaline lysis
protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a
U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the
expected size are cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA is harvested
using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc.,
25 Chatsworth, CA). The DNA is then used as a sequencing template to verify that the changes
made in the vector are the desired changes and that no further changes or mutations occurred. All
sequencing is performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone is identified that contains both the Herceptin Heavy Chain and Herceptin
Light Chain gene, the DNA is isolated by standard procedures. Briefly, *E. coli* bacteria
30 containing the plasmid are grown in 250 ml of LB broth (supplemented with an appropriate
antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA is isolated from the bacterial
cells using a Qiagen EndoFree Plasmid Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according
to the manufacturer's protocol. Plasmid DNA is resuspended in 500µL of endotoxin free water
and stored at -20°C until needed.

Construction of Vector #279 (SEQ ID NO:26)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the codon-optimized Δ CH2 monoclonal antibody (mAb) cassettes driven by the hybrid promoter version 1 (SEQ ID NO:14) were digested with restriction enzymes AscI, PacI, BsiWI, and MluI (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the codon-optimized Δ CH2 light chain (LC) cassette into the MCS of vector p5021 (SEQ ID NO:8), LC and p5021 DNA (SEQ ID NO:8) were digested with AscI and PacI, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. To insert the codon-optimized Δ CH2 heavy chain (HC) cassette into the MCS of the p5021 vector (SEQ ID NO:8), HC and the p5021 DNA (SEQ ID NO:8) were digested with BsiWI and MluI, purified and ligated as described above. Ligated product was transformed into *E. coli* XL10-Gold Ultracompetent cells (Stratagene, Inc. La Jolla, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.9 ml of NZY Plus (TEKnova, CAT# N1215) broth for 1 hour at 37°C then spread onto LB (Luria-Bertani) agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C. Resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 0.8% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in a minimum of 250 ml of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify that the changes made in the vector were the desired changes and that no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained both the codon-optimized Light Chain and codon-optimized Heavy Chain cassettes, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest were grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacterial cells using a Qiagen EndoFree Plasmid Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of endotoxin free water and stored at -20°C until needed.

Construction of Vector #280 (SEQ ID NO:27)

Invitrogen's pTopo plasmid (Carlsbad, CA) containing the codon-optimized RM2 monoclonal antibody (mAb) cassettes driven by the hybrid promoter version 1 (SEQ ID NO:14) were digested with restriction enzymes *AscI*, *PacI*, *BsiWI*, and *MluI* (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. Digested DNA was purified using a Zymo DNA Clean and Concentrator kit (Zymo Research). To insert the codon-optimized RM2 light chain (LC) cassette into the MCS of the p5021 vector (SEQ ID NO:8), LC and p5021 DNA (SEQ ID NO:8) were digested with *AscI* and *PacI*, purified as described above, and ligated using a Quick T4 DNA Ligase Kit (New England Biolabs, Beverly, MA) according to the manufacturer's protocol. To insert the codon-optimized RM2 heavy chain (HC) cassette into the MCS of the p5021 vector (SEQ ID NO:8), the HC DNA and the p5021 (SEQ ID NO:8) were digested with *BsiWI* and *MluI*, purified and ligated as described above. Ligated product was transformed into *E. coli* XL10-Gold Ultracompetent cells (Stratagene, Inc. La Jolla, CA) using chemical transformation according to the manufacturer's protocol. Transformed bacterial cells were incubated in 0.9 ml of NZY Plus (TEKnova, CAT# N1215) broth for 1 hour at 37°C then spread to LB (Luria-Bertani) agar plates supplemented with 100 µg/ml ampicillin (LB/amp plates). All plates were incubated overnight at 37°C, and resulting colonies were picked into LB/amp broth for overnight growth at 37°C. Plasmid DNA was isolated using a modified alkaline lysis protocol (Sambrook et al., 1989), electrophoresed on a 1% agarose gel, and visualized on a U.V. transilluminator after ethidium bromide staining. Colonies producing a plasmid of the expected size were cultured in at least 250 ml of LB/amp broth. Plasmid DNA was harvested using a Qiagen Maxi-Prep Kit according to the manufacturer's protocol (Qiagen, Inc., Chatsworth, CA). The DNA was then used as a sequencing template to verify the changes made in the vector were the desired changes and no further changes or mutations occurred. All sequencing was performed using Beckman Coulter's CEQ 8000 Genetic Analysis System.

Once a clone was identified that contained both the codon-optimized Light Chain and codon-optimized Heavy Chain cassettes, the DNA was isolated by standard procedures. Briefly, *E. coli* bacteria containing the plasmid of interest was grown in 250 ml of LB broth (supplemented with an appropriate antibiotic) at 37°C overnight in a shaking incubator. Plasmid DNA was isolated from the bacterial cells using a Qiagen EndoFree Plasmid Maxi-Prep kit (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's protocol. Plasmid DNA was resuspended in 500 µL of endotoxin free water and stored at -20°C until needed.

For each of the vectors, the following steps occurred after ligation of the antibody gene cassette into the appropriate vector for expression:

Column purified DNA was used as template for sequencing to verify the changes made in the vector were the desired changes (*i.e.*, that the RM2 cassette was inserted) and that no further changes or mutations occurred. All sequencing was done on a Beckman Coulter CEQ 8000 Genetic Analysis System.

Table 4 shows some details of four of the vectors used for the *in vitro* production of the RM2 monoclonal antibody, along with the label that corresponds to that vector in the data shown in Example 2 below. All include the same production cassette for the heavy and light chain of the RM2 monoclonal antibody. These vectors differ only in the backbone vector used.

Table 4

Flask	Vector	Backbone Vector	Promoter	SEQ ID NO:
C1	N/A	N/A	N/A	N/A
T1	148	Old HFB	CMV	17
T4	210	K10 HFB	CMV	18
T6	212	K10 MAR HFB	CMV	19
--	226	K10 PURO MAR HFB	CMV	20

Schematics of these vectors are shown in Figures 2-5, respectively. The sequences of the vectors (SEQ ID NO:17, 18, 19, and 20) are shown below in the Appendix.

EXAMPLE 2

In Vitro Expression of Monoclonal Antibody

These experiments were performed to verify that vectors 148 (SEQ ID NO:17), 210 (SEQ ID NO:18), 212 (SEQ ID NO:19), and 226 (SEQ ID NO:20) produced the light and heavy chain polypeptides of RM2 antibody, and to determine whether these antibody products were toxic to the transfected cells.

The graphs in Figure 6 show the ELISA readings for the media samples from one experiment. T1, T4, and T6 represent flasks containing cells transfected with vector #148 (SEQ ID NO:17), 210 (SEQ ID NO:18), and 212 (SEQ ID NO:19), respectively. Vector #212 (SEQ ID NO:19) demonstrated the highest expression of both the heavy and the light chains of the RM2 antibody. Control flasks also were run, but the readings were too low to detect at these dilution

levels (data not shown). Vector #226 (SEQ ID NO:20) also demonstrated expression of the RM2 monoclonal antibody light and heavy chains as is shown in Table 5.

Table 5

Sample	Heavy chain ng/ml	Light chain ng/ml
1159R M1	412.685	337.950
1159 M2	173.698	143.789
1159 M3	48.504	40.781
1159 M4	13.640	11.367

5

There was no indication that the products produced were toxic in any way to the cells. The cells remained alive, healthy, and demonstrated typical morphology throughout the experiment.

10 The remaining expression vectors also have been assayed for their ability to produce heavy chain, light chain, kappa chain, or lambda chain of an antibody as discussed in this example. Typical maximum results for the expression vectors are shown in Table 6.

Table 6

Vector #	SEQ ID NO:	Cell Type	Protein	Max amt of protein (ng/ml)
278	22	LMH	Heavy Chain	14.54
278	22	LMH	Kappa Chain	16.86
278	22	2A	Heavy Chain	6.58
278	22	2A	Kappa Chain	5.02
275	21	LMH	Heavy Chain	494.8
275	21	LMH	Lambda Chain	417.2
275	21	LMH-2A	Heavy Chain	263.9
275	21	LMH-2A	Lambda Chain	219.6
273	23	LMH	Heavy Chain	428.2
273	23	LMH	Light Chain	353.2
273	23	LMH-2A	Heavy Chain	144
273	23	LMH-2A	Light Chain	136.7
226	20	LMH	Heavy Chain	687.5
226	20	LMH	Light Chain	597.5
226	20	LMH-2A	Heavy Chain	239.4
226	20	LMH-2A	Light Chain	201.4
148	17	LMH-2A	Heavy Chain	23.08
148	17	LMH-2A	Light Chain	14.519
149	17	LMH-2A	Heavy Chain	1.02
149	17	LMH-2A	Light Chain	2.204
210	18	LMH-2A	Heavy Chain	16.21
210	18	LMH-2A	Light Chain	12.568
212	18	LMH-2A	Heavy Chain	35.74
212	18	LMH-2A	Light Chain	28.114

EXAMPLE 3

Purification of Monoclonal Antibody from Culture Media

- 5 Monoclonal antibodies were purified from the culture media using the procedure as described above under Section C.

EXAMPLE 4

Transfection efficiency in LMH cells and LMH 2A cells

This experiment compared transfection efficiency and protein production in LMH cells to LMH2A cells. Vectors already proven in cell culture for three of the fusion proteins of interest were used. Transfection was carried out by the standard Fugene 6 protocol using 2 ug DNA/flask and Fugene 6:DNA at 6:1. The cultures were grown on Waymouth's + 10% FCS + G418 until confluent, then fed with Waymouth's + 5% FCS + G418. Samples were taken for M1, 2 days post transfection; M2, 6 days post transfection; M3, 9 days post transfection; and M4, 12 days post transfection. When referring to the data presented in Figure 7, mAb is reported in nanograms/ml. Two graphs are used due to the differences in scale.

Table 7

Flask	Cell line	Vector	Protein
C1 control	LMH	N/A	--
T5	LMH	226	mAb
T6	LMH 2A	226	mAb
C2 control	LMH 2A	N/A	--

Figure 7 shows expression levels of mAb in both LMH cells and LMH2A cells. Based on these data, the vectors are capable of efficient mAb expression in both LMH and LMH2A cells.

EXAMPLE 5

Transfection efficiency in LMH cells and LMH 2A cells

The suspensions of both LMH cells and LMH2A cells were adjusted to deliver 8×10^5 cells / T25 flask. After overnight growth, the confluence was consistent between the two cell lines at 60%. One T75 flask of each cell line was dissociated and brought up to 10 ml with PBS +20% FCS. 500 ul of each suspension was centrifuged in a Volupac tube to determine packed cell volume (PCV) and number of cells/ml. LMH = 3.25×10^6 cells/ml, LMH 2A's = 2.9×10^6 cells/ml. Each cell suspension was diluted to equal 8×10^5 cells/ml and seeded 1 ml per T25 flask in Waymouth's +10% FCS+HEPES (+/-G418). Thirteen T25 flasks of each cell line were prepared for transfection and control.

Table 8

Flask	Cell line	Vector	Protein
C1 control	LMH	N/A	--
T7,8,9	LMH	226	MAb
T19,20,21	2A	226	MAb
C2 control	2A	N/A	--

Transfection was carried out by the standard Fugene 6 protocol using 2 ug DNA/flask and Fugene 6:DNA at 6:1. Complex formation was done in Waymouth's (no additives) and the transfection was done in Waymouth's +10% FBS +HEPES (no antibiotics). After 48 hr the cultures were grown on Waymouth's + 5% FCS +H (+/- G418). Transfection occurred on 11/14/08 with samples taken M1, M2 and M3 at 3, 7 and 10 days later, respectively. mAb results are reported in nanograms/ml (ng/ml).

The LMH line appeared to grow somewhat faster and more evenly across the surface of the flask when split, whereas the LMH2A cells began to grow on top of each other before they were confluent. After 3-4 days, the media from LMH flasks was more yellow than LMH 2A media suggesting increased number of cells or increased metabolic activity. Once confluence was reached, the LMH cells continued to grow and build layers just as the LMH2A cells. During extended growth in flask culture, both cell lines formed 3-dimensional patterns of growth reminiscent of the physical tissue structure of liver. Figure 8 shows that LMH cells appeared to produce more mAb heavy chain than LMH2A cells. Figure 9 shows that LMH cells appear to produce more mAb light chain than LMH2A cells.

EXAMPLE 6

Perfusion of LMH2A Cells in AutoVaxID

The AutoVaxID cultureware (Biovest) is installed, and the Fill-Flush procedure is performed following the procedures in the AutoVaxID Operations Manual. The following day, the Pre-inoculation procedure and the pH calibration are done. The cultureware is seeded with 10^9 AIA cells that are transfected with an expression vector comprising gene(s) encoding the light and/or heavy chains of an antibody. The cells are propagated in Lonza UltraCULTURE media supplemented with cholesterol (Sigma, 50 µg/ml) in 20 gelatin-coated T150 cell culture flasks, and are dissociated with Accutase (Sigma). They are counted, gently pelleted (600xG for 6 minutes), and resuspended in 50 mls of growth media (Lonza UltraCULTURE containing GlutaMax (Invitrogen) and SyntheChol (1:500), Soy Hydrolysate (1:50), and Fatty Acid

Supplement (1:500) (all from Sigma). This is the same media which is included in the “Factor” bags for the AutoVaxID, used for the EC (extra-capillary) media. A 10L bag of Lonza UltraCULTURE media (with GlutaMax) is used initially for the IC (intra-capillary) media. This is designed to give the cells a richer media for the first 7-10 days, to allow them to become established quickly in the hollow fiber system. After this bag is exhausted, the IC media is switched to DMEM/F12 (also including GlutaMax), also purchased from Lonza. This media is purchased in 50L drums, and is removed from the cold room and allowed to warm to room temperature before being connected to the system. The AutoVaxID system is placed under Lactate Control, and pump rates are modified and daily tasks performed, as specified by the AutoVaxID Operating Procedures Manual, provided by the manufacturer (Biovest).

Six days later, cells can be seen growing on the hollow fibers in the bioreactor. Up until this time, there is ample evidence that the cells are growing and metabolizing in the system; the Lactate Controller is increasing the media pump rate regularly in order to keep the lactate levels below the setpoint, and the pH Controller is continually decreasing the percentage of CO₂ in the gas mix, indicating that the cells are producing increasing amounts of acidic metabolic products. After the IC media is changed from the Lonza UltraCULTURE media to the DMEM/F12, however, the metabolic rate of the cells may slow dramatically, to the point where the Lactate Controller slows the media pumps all the way to baseline levels, and the lactate levels may still drop. Samples are taken for protein analysis 4 days later. Samples are taken from the EC (showing current production) from the Harvest Bag (showing accumulated production) and from the IC (showing any protein which crossed the membrane and was lost in the wasted media). By four days later, there may be both visual and metabolic evidence that the cells are growing, so cycling is initiated. For the next week, regular sampling is continued, and cells appear to grow and metabolize normally, although it may become physically difficult to pull samples from the EC sample port. The run is allowed to continue for a couple weeks, although cycling times become greatly extended. Final samples are taken, and the run is ended. All samples are analyzed for proteins to determine if the cells are capable of producing significant amounts of protein in this system.

EXAMPLE 7

mAb production in LMH and LMH2A Cells

This experiment tested 2 vectors, 273 (SEQ ID NO:23) and 275 (SEQ ID NO:21), containing the gene for RM2 mAb. Both vectors were built with the CMV-Oval hybrid promoter,

version 1, in the Puro/Mar backbone. Vector 273 contains the vitellogenin polyA, while vector 275 used the ovalbumin polyA. Both vectors were tested in the LMH and LMH2A cells.

One day prior to transfection the flasks were seeded with equal amounts of cells from each cell type in Waymouth + 10% FCS. The transfection was done with Fugene 6 using 2 ug DNA/ T25 following our standard protocol. The LMH cells were about 90 % confluent and the LMH2A cells were about 60-70% confluent at the time of transfection. Since the LMH seemed to be growing faster, at the time of the final media harvest, 2 random flasks of each type were measured for cell population. The cell count was virtually the same in all flasks. The difference in confluence may be due more to the morphology and growth pattern of the 2 cell types rather than the actual number of cells. The LMH cells seem to spread more while the LMH2A seem to tend to grow in clumps. Media samples were collected and cultures were fed with Waymouth + 5% FCS every 3 days. In both cell lines, vector 275 (SEQ ID NO:21), containing the Oval polyA, gave better expression. M1, M2 and M3 are 3, 6, and 9 days, respectively after transfection.

EXAMPLE 8

Production of transgenic chicken and quail that successfully passed the hmAb transgene through two generations using SEQ ID NO:24

SEQ ID NO:24 (Vector 146/149) has been employed *in vivo* and *in vitro*. Separate *in vivo* experiments in chicken and quail have demonstrated successful passage of the transgene encoding for human monoclonal antibody (hmAb) contained in SEQ ID NO:24 through two generations. Briefly, germ line cells of both chicken and quail were made transgenic following administration of SEQ ID NO:24 containing a gene encoding for hmAb, into the left cardiac ventricle, the source of the aorta which provides an artery leading to the ovary. These birds were mated with naïve males and the resulting eggs hatched. These chicks (G1 birds) contained the transgene encoding hmAb as their blood cells were positive for the transgene encoding hmAb, and 3) These transgenic progeny (G1 birds) were subsequently bred and their progeny (G2 birds) were positive for the transgene encoding hmAb.

Transgenic G1 and G2 quail were generated by injecting females in the left cardiac ventricle. The experiment employed five seven-week old quail hens. The hens were each injected into the left ventricle, allowed to recover, and then mated with naïve males. Isoflurane was used to lightly anesthetize the birds during the injection procedure. Eggs were collected daily for six days and set to hatch on the seventh day. At about 2 weeks of age, the chicks were bled and DNA harvested as described in a kit protocol from Qiagen for isolating genomic DNA from blood and tissue. PCR was conducted using primers specific to the gene of interest. In both

experiments, transgene-positive G1 animals were obtained. These transgene-positive G1 animals were raised to sexual maturity and bred. The G2 animals were screened at 2 weeks of age and transgenic animals were identified in each experiment. A transposon-based vector (SEQ ID NO:24) containing a gene for a hmAb was injected. A total of 85 µg complexed with branched polyethylenimine (BPEI) in a 300 µL total volume was used. G1 and G2 quail were positive for the monoclonal antibody transgene following analysis of blood samples.

Transgenic G1 and G2 chickens were generated by injecting females in the left cardiac ventricle. The experiment was conducted in 20 week old chickens. In the second experiment, the same transposon-based vector (SEQ ID NO:24) containing a gene for a hmAb as described above for quail was injected. DNA (complexed to BPEI) was delivered to the birds at a rate of 1 mg/kg body (up to 3 ml total volume) weight by injection into the left cardiac ventricle. Isoflurane was used to lightly anesthetize the birds during the injection procedure. Once the birds recovered from the anesthesia, they were placed in pens with mature, naïve males. All eggs were collected for 5 days and then incubated. In the first experiment, the eggs were incubated for about 12 days, candled to check for viable embryos; any egg showing a viable embryo was cracked open and tissue samples (liver) taken from the embryo for PCR. The eggs were allowed to hatch, and a blood sample was taken at two days to test the animals for the presence of the transgene using PCR. Approximately 14% of the chicks were positive for the hmAb transgene.

EXAMPLE 9

Production of human monoclonal antibody in vitro using SEQ ID NO:24

LMH2A cells were grown to 50-80% confluency for transfection, the media removed from the cells, and 2 µg of DNA containing SEQ ID NO:24 complexed with Fugene-6 in a final volume of 1 ml media was added to the cells and incubated at 37°C for 1 to 2 hours. After incubation, the DNA/Fugene complex was removed and fresh media added to the flask. Media was harvested every 48 hours (for a total of three harvests designated M1, M2, and M3) and stored at 4°C until the experiment was completed. Once all samples were obtained, an ELISA was conducted using anti-Human antibody. Three were conducted. As an example, in Experiment 1105, vector 149 yielded an absorbance of about 0.3 compared to the negative control absorbance of 0.074 and the positive control absorbance of 1.12 – 0.111 (180 minutes reading at 405 nm) and an average protein concentration of about 4.3 ng/ml. This was the highest absorbance obtained in Experiment 1105. It should be noted that this is a transient transfection, and no selection pressure was applied to these cells.

All patents, publications and abstracts cited above are incorporated herein by reference in their entirety. It should be understood that the foregoing relates only to preferred embodiments of the present invention and that numerous modifications or alterations may be made therein without departing from the spirit and the scope of the present invention as defined in the following claims.

CLAIMS

1. A vector comprising:

a modified transposase gene operably linked to a first promoter, wherein the nucleotide sequence 3' to the first promoter comprises a modified Kozak sequence, and wherein a plurality of the first twenty codons of the transposase gene are modified from the wild-type sequence by changing the nucleotide at the third base position of the codon to an adenine or thymine without modifying the amino acid encoded by the codon;

a multiple cloning site;

transposon insertion sequences recognized by a transposase encoded by the modified transposase gene, wherein the transposon insertion sequences flank the multiple cloning site; and,

one or more insulator elements located between the transposon insertion sequences and the multiple cloning site.

2. The vector of claim 1 comprising any one of SEQ ID NOs: 2 to 13.

3. The vector of claim 1, wherein the vector comprises any one of SEQ ID NOs: 10 to 13.

4. The vector of claim 1, further comprising a second promoter, wherein the second promoter is SEQ ID NO: 14 or SEQ ID NO: 15.

5. The vector of claim 4, further comprising a gene encoding for an antibody inserted into the multiple cloning site.

6. The vector of claim 5, wherein the vector comprises any one of SEQ ID NOs: 17 to 27.

7. A promoter comprising chicken ovalbumin promoter regulatory elements in combination with a cytomegalovirus enhancer and a cytomegalovirus promoter.

8. The promoter of claim 7 comprising SEQ ID NO: 14.

9. A promoter comprising a steroid dependent response element, a cytomegalovirus enhancer, a chicken ovalbumin negative response element and a cytomegalovirus promoter.

10. The promoter of claim 9 comprising SEQ ID NO: 15.

11. A transposon-based vector comprising:

a modified transposase gene operably linked to a first promoter, wherein the nucleotide sequence 3' to the first promoter comprises a modified Kozak sequence, and wherein a plurality of the first twenty codons of the transposase gene are modified from the wild-type sequence by

changing the nucleotide at the third base position of the codon to an adenine or thymine without modifying the amino acid encoded by the codon;

one or more genes of interest encoding an antibody operably-linked to one or more additional promoters, wherein the one or more genes of interest encoding the antibody and their operably-linked promoters are flanked by transposon insertion sequences recognized by a transposase encoded by the modified transposase gene; and,

one or more insulator elements located between the transposon insertion sequences and the one or more genes of interest encoding the antibody.

12. The vector of claim 11, wherein the vector comprises any one of SEQ ID NOs: 17 to 27.

13. A method of producing an antibody comprising:

transfecting a cell with a vector comprising a modified gene encoding for a transposase, a promoter and a gene encoding for an antibody;

culturing the transfected cell in culture medium;

permitting the cell to release the antibody into the culture medium;

collecting the culture medium; and,

isolating the antibody.

14. The method of claim 13 wherein the vector comprises any one of SEQ ID NOs: 17 to 27.

15. The one or more insulator elements of any of the preceding claims comprising an HS4 element, a lysozyme replicator element, a combination of a lysozyme replicator element and an HS4 element, or a matrix attachment region element.

16. The antibody of any of the preceding claims, wherein the antibody is a human antibody.

17. The antibody of any of the preceding claims, wherein the antibody is a monoclonal antibody.

FIG. 1

A.

ChOval Promoter	CMV enhancer + promoter
-----------------	-------------------------

B.

Ch SDRE	CMV enh	Ch NRE	CMV Promoter
---------	---------	--------	--------------

FIG. 2

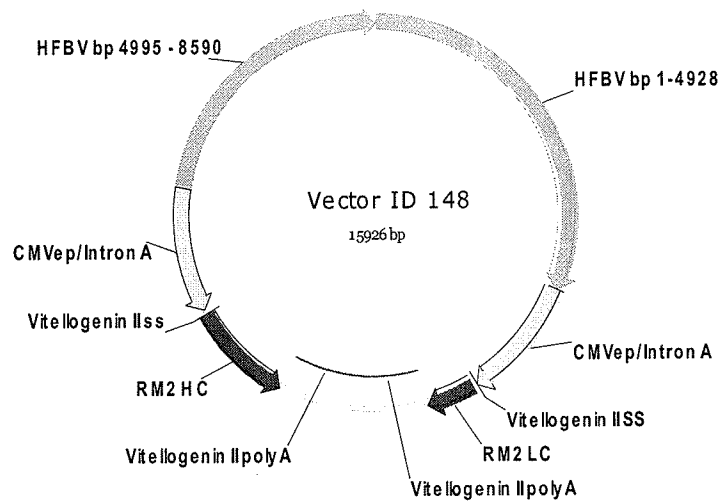


FIG. 3

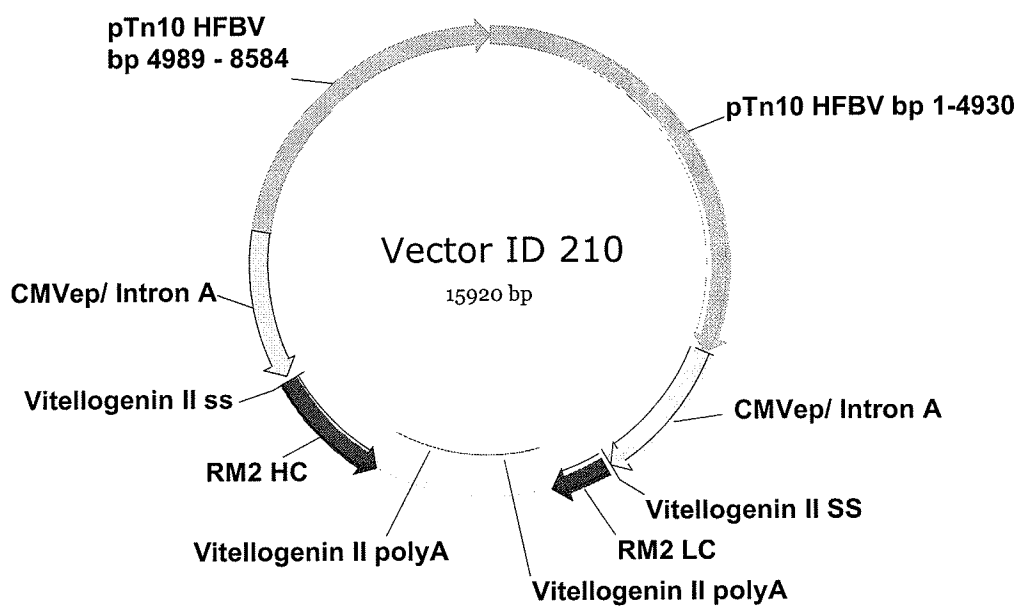


FIG. 4

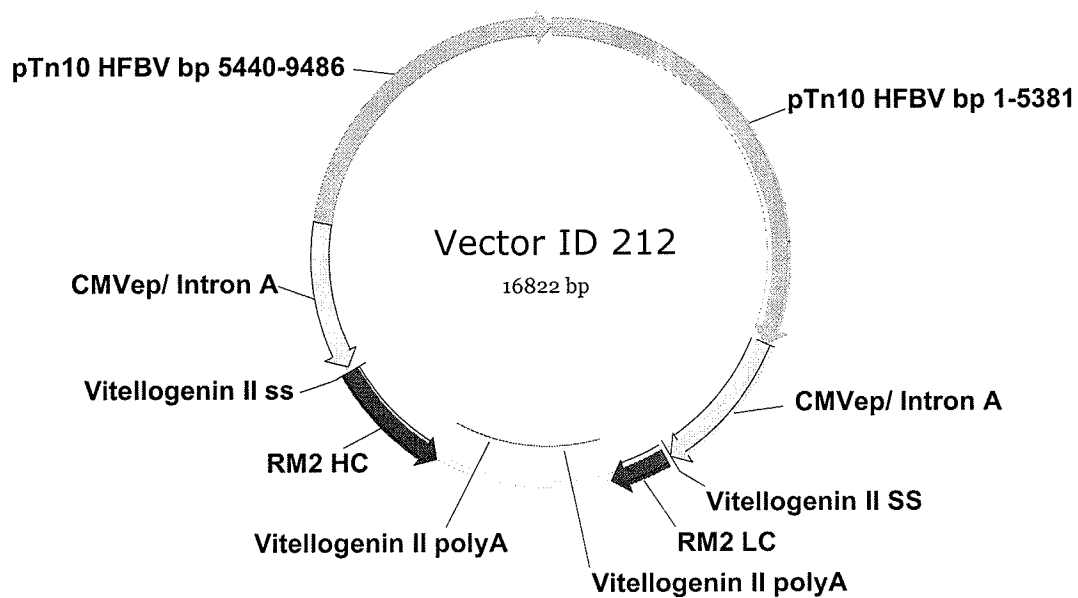


FIG. 5

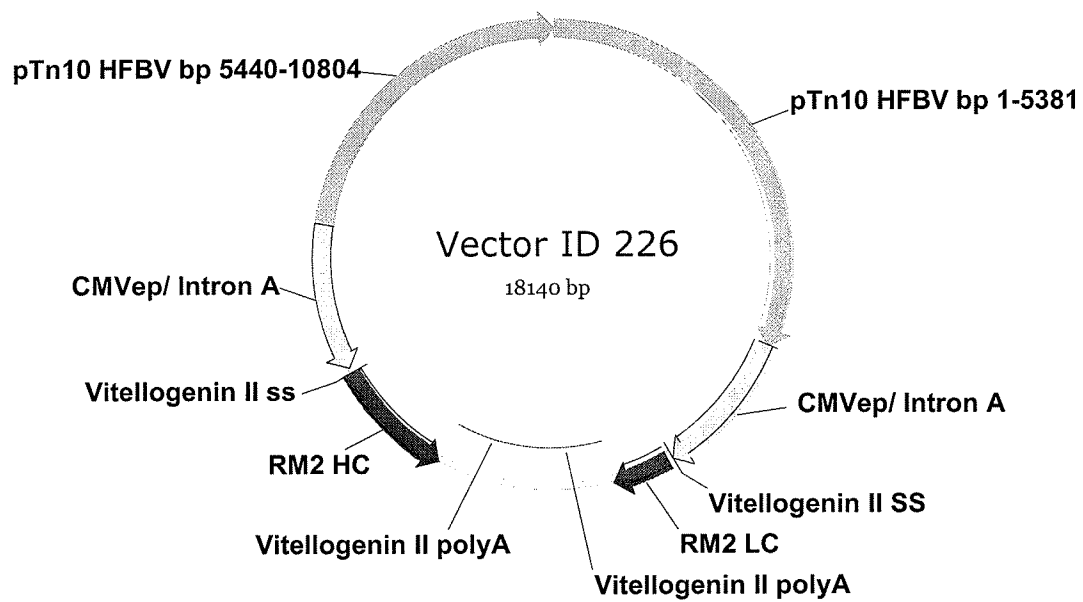
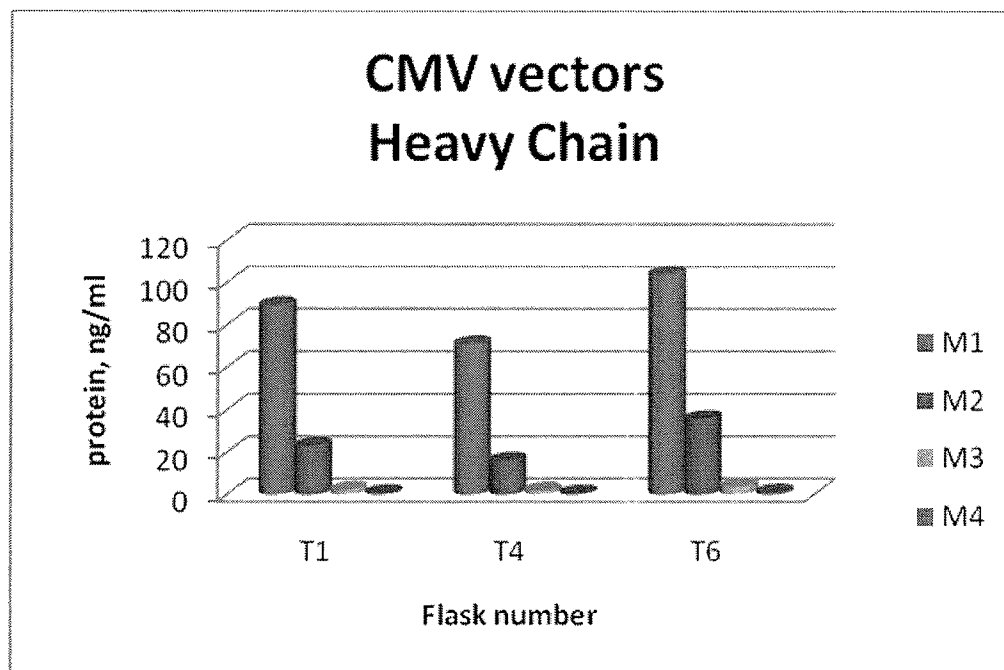


FIG. 6

A.



B.

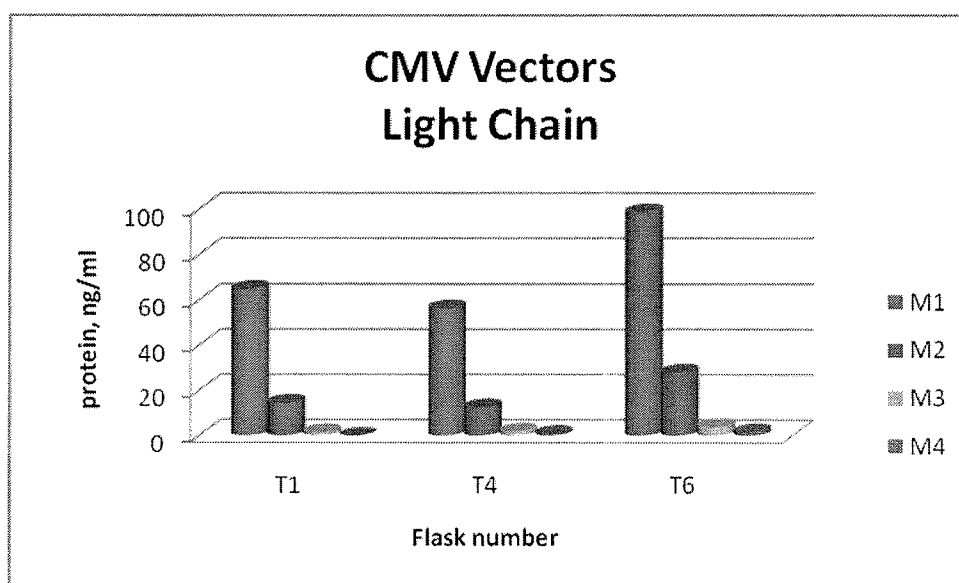
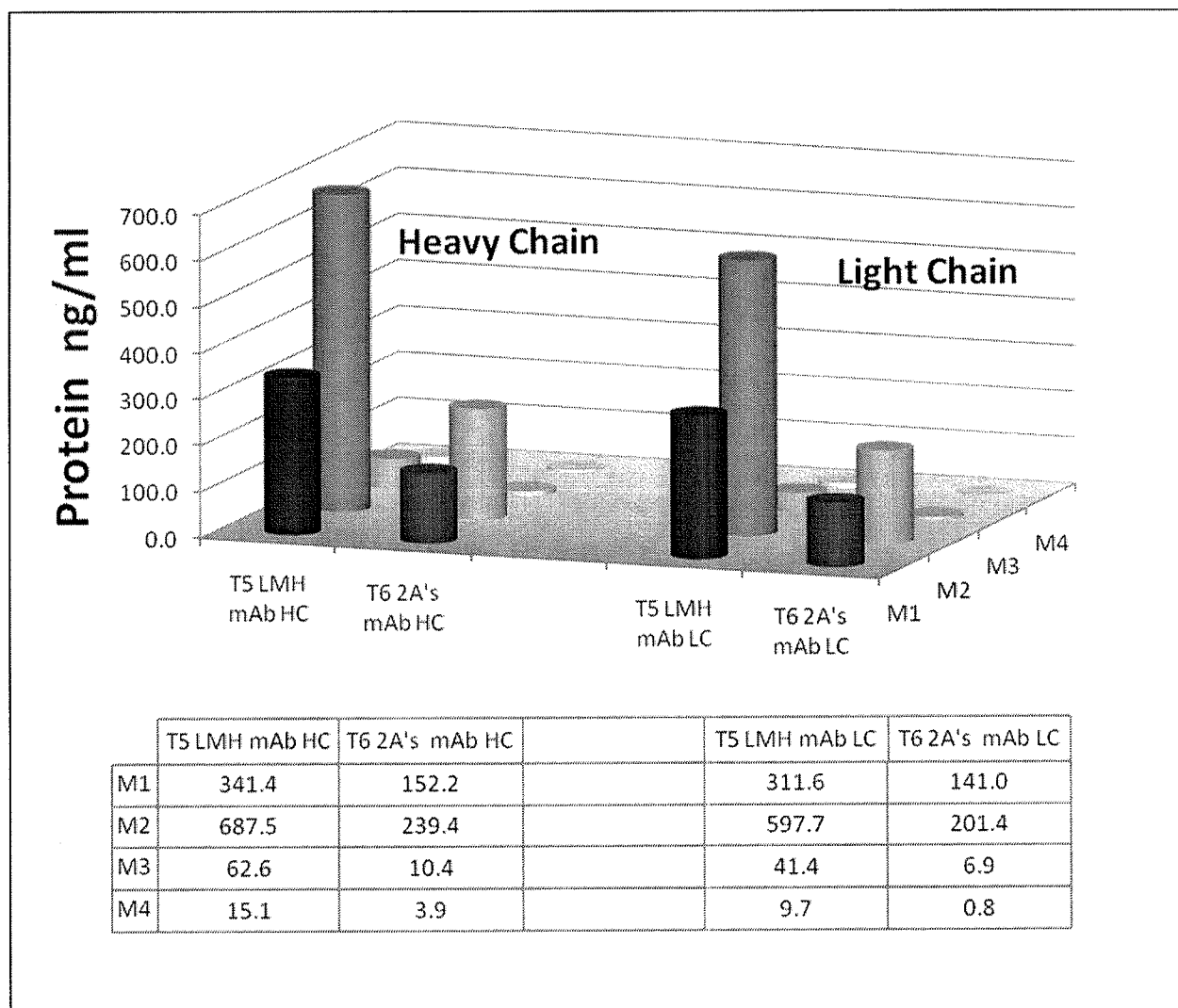


FIG. 7



LMH = First and third rows

LMH 2A's = Second and fourth rows

FIG. 8

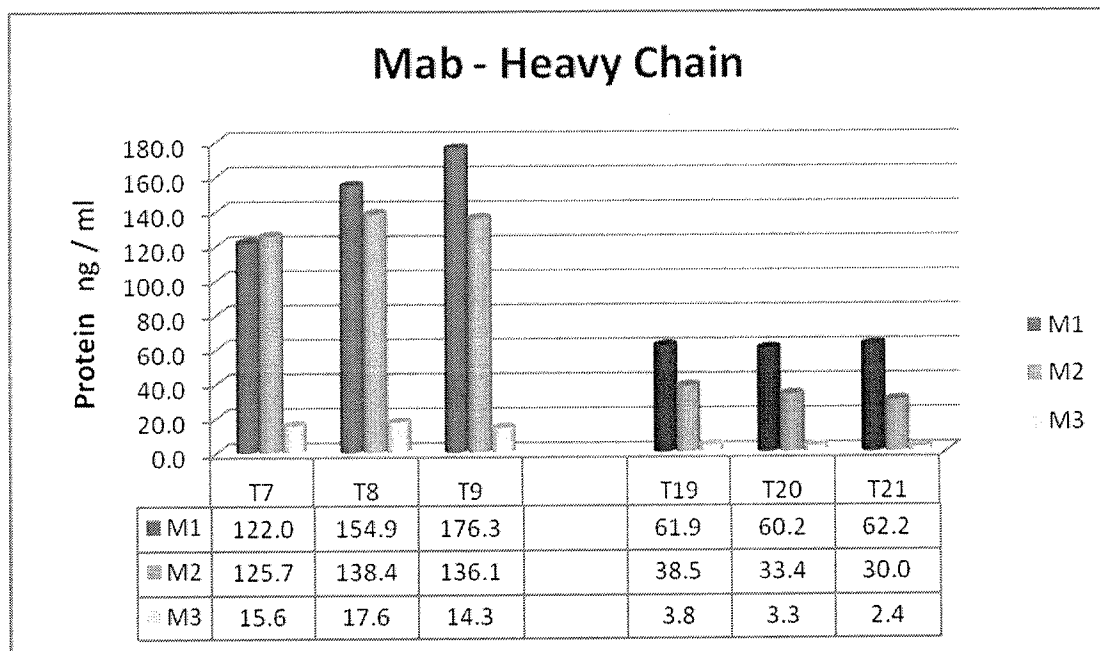


FIG. 9

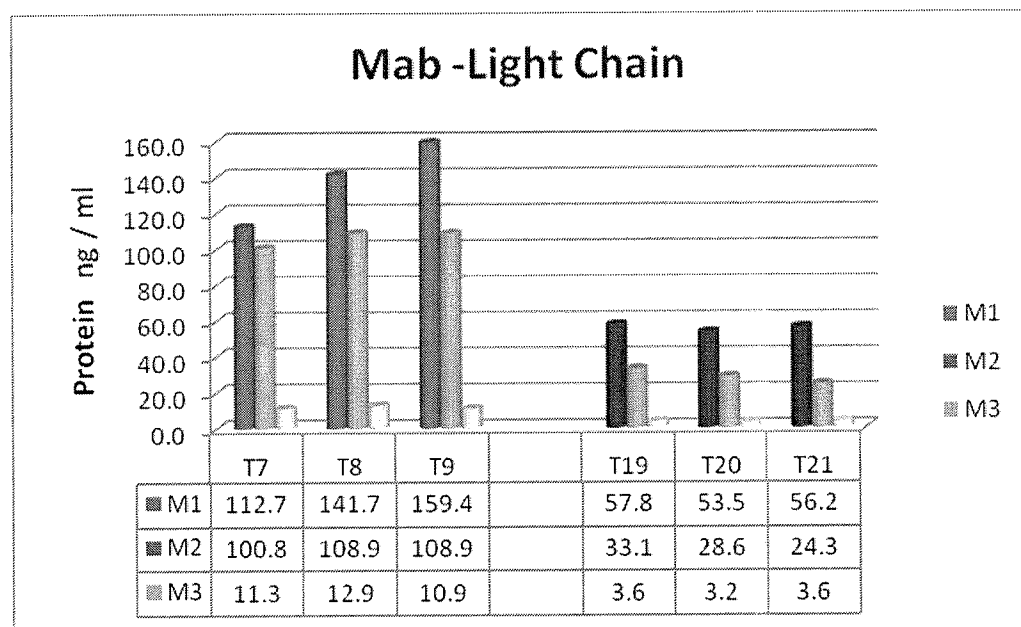


Figure 10

