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## Description

### RELATED APPLICATION

**[0001]** This application claims priority to USSN 62/291,217, filed February 4, 2016.

### FIELD OF THE INVENTION

**[0002]** This invention relates to production of immunoglobulin molecules, including methods for rapid screening of antigen-specific antibody-secreting cells for the generation of monoclonal antibodies.

### BACKGROUND OF THE INVENTION

**[0003]** In the following discussion certain articles and methods are described for background and introductory purposes. Nothing contained herein is to be construed as an "admission" of prior art. Applicant expressly reserves the right to demonstrate, where appropriate, that the articles and methods referenced herein do not constitute prior art under the applicable statutory provisions.

**[0004]** Monoclonal antibodies are important biologics that have been widely employed in biomedical research, in clinical diagnostics, and as therapeutic agents because of their exquisite ability to bind antigens of diverse molecular forms. In drug development, monoclonal antibodies are often the molecules of choice because they exhibit desirable pharmacokinetics that are associated with powerful immunological functions normally involved in fending off infectious agents. Furthermore, laboratory animals can readily mount a specific antibody response against any target molecule that is not present natively in the body, making antibody generation a relatively low-risk and cost-efficient approach when compared to alternative strategies.

**[0005]** Although hybridoma technology was developed more than four decades ago, today it is still the most widely employed technique to generate antigen-specific monoclonal antibodies. In this approach, an animal (typically, a rodent or a rabbit) is first immunized with an antigen of interest. B lymphocytes in the immunized animal that have the receptor specificity for the antigen then become activated, clonally expand, and differentiate into antibody-secreting cells (ASCs). The immunized animal is then sacrificed, and because the ASCs isolated from these animals cannot survive indefinitely in culture, these cells are often immortalized by fusion with malignant plasma cells (such as myeloma or plasmacytoma cells) to generate hybrid cells called hybridomas. The hybridoma cells are then screened and selected for their ability to secrete antibodies with reactivity to the antigen of interest, often involving multiple rounds of limiting dilution and propagation in culture.

**[0006]** Alternatively, the ASCs can be individually sorted, and the genes encoding the heavy chain and light chain variable domains ($V_H$ and $V_L$, respectively) directly cloned without the need to propagate the ASCs *in vitro.* The $V_H$- and $V_L$-encoding DNA fragments are next subcloned into an expression vector containing exon sequences for the desired heavy chain and light chain constant regions, respectively. Each $V_H$ and $V_L$ pair of expression vectors are then transfected into a cell line to express the monoclonal antibodies, which are subsequently screened for their ability to recognize the antigen of interest.

**[0007]** Despite the eventual success in producing monoclonal antibodies against the antigen of interest using either hybridoma or single-cell cloning technique, the efficiency of both techniques is hampered by the labor-intensive process of screening and selection. This is because it has not been feasible to pre-select only the antigen-specific ASCs for fusion with myeloma cells or for single-cell cloning. When B lymphocytes differentiate into ASCs in response to an antigenic encounter, the membrane-bound form of antigen receptors is down-regulated in favor of the secreted form. Thus, selection methods based on the cell surface expression of antigen receptors, such as magnetic or flow-cytometric sorting, do not work well as tools to select for antigen-specific ASCs. Due to this lack of ability to pre-select ASCs, only a small fraction of cells screened in both hybridoma and single-cell cloning techniques produces monoclonal antibodies with specificity for the antigen of interest.

**[0008]** US patent No. 7,148,040 B2 provides methods to express the membrane-bound form of antigen receptors on hybridoma cells to improve the efficiency of hybridoma screening by selection techniques based on the cell surface expression of antigen receptors. In this approach, myeloma cells are transfected with expression constructs encoding CD79A and CD79B, also known as Ig$\alpha$ and Ig$\beta$, respectively. CD79A and CD79B are expressed as heterodimers that are necessary for both cell surface expression and signaling functions of the antigen receptors on B cells. As B lymphocytes differentiate into ASCs, they down-regulate CD79A and CD79B expression, thus contributing to the loss of antigen receptor expression on the cell surface. Therefore, re-introducing the expression of CD79A and CD79B allows for increased representation of the membrane-bound form of antigen receptors on the hybridomas. Although this strategy helps reduce the labor of hybridoma screening and selection, the efficiency could be greatly improved if it was feasible to pre-select only the antigen-specific ASCs for fusion with myeloma cells. Moreover, the specified methods do not

provide a strategy to increase the efficiency of monoclonal antibody generation using direct $V_H$ and $V_L$ cloning technique from sorted single cells.

**[0009]** Re-introduction of CD79A and CD79B expression by ASCs *in vivo* may not provide a viable strategy to increase the expression of antigen receptors on the cell surface either. Because CD79A and CD79B expression is tightly regulated during B lymphocyte development, alterations in their expression levels *in vivo* may have profound consequences on B lymphocyte survival, functions, and/or antigen receptor selection. Moreover, the antigen receptors on ASCs are likely to be internalized at the time of ASC isolation due to their active engagement with the immunogen, since the immune response is still ongoing when the mouse is euthanized. If instead, signaling-deficient mutant CD79A and CD79B are expressed on ASCs to prevent antigen receptor internalization, it remains unexplored whether the mutant forms of these molecules exhibit a dominant-negative effect that negatively impacts ASC survival and functions *in vivo.* Finally, expressing CD79A and CD79B on ASCs *ex vivo* to circumvent the aforementioned problems associated with their enforced expression *in vivo* is not a practical strategy because ASCs are not amenable to gene transfer by most methods currently available.

**[0010]** Thus, a method for more efficient screening for antigen-specific ASCs is an important unmet need. The methods and compositions provided by the present specification meet this important need.

## SUMMARY OF THE INVENTION

**[0011]** This Summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This Summary is not intended to identify key or essential features of the claimed subject matter, nor is it intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the following written Detailed Description including those aspects illustrated in the accompanying drawings and defined in the appended claims.

**[0012]** The present invention provides methods and compositions for enhanced production of immunoglobulin molecules. Specifically, the invention provides methods and compositions for the capture of secreted immunoglobulin molecules, including those of IgG, IgA, IgE and IgM isotypes, at the surface of ASCs. The present invention also comprises transgenic rodents comprising engineered ASCs that can capture and display on their cell surface immunoglobulin molecules produced endogenously from within the ASCs.

**[0013]** According to the invention, there is provided a genetically modified rodent comprising antibody-secreting cells comprising an exogenous nucleic acid sequence comprising

i. a promoter, that is expressed in antibody-secreting cells, and
ii. a nucleic acid sequence encoding an immunoglobulin-capturing molecule comprising a cell surface tether portion and an immunoglobulin-binding portion, wherein the promoter is operably linked to said nucleic acid sequence encoding said immunoglobulin-capturing molecule,

wherein the cell surface tether portion comprises a transmembrane peptide, and
wherein the immunoglobulin-binding portion is selected from:

a. one or more bacterial proteins that naturally have affinity for the constant region of a heavy or light chain of an immunoglobulin;
b. one or more variable domains of an immunoglobulin that has affinity for any part of another immunoglobulin; or
c. a single chain variable fragment against a heavy or light chain constant region of an immunoglobulin;

wherein the antibody secreting cells of the genetically modified rodent are capable of producing immunoglobulin-capturing molecules that can bind, retain, and display endogenously produced immunoglobulin molecules at a cell surface of the antibody-secreting cells.

**[0014]** Specifically, the antibody-secreting cells constitutively express the immunoglobulin-capturing molecule or can be induced to express the immunoglobulin-capturing molecule *in vivo, in vitro,* or *ex vivo.*

**[0015]** Specifically, the rodent is a mouse or rat.

**[0016]** Specifically, the bacterial protein is Protein A and/or Protein G.

**[0017]** Specifically, the bacterial protein is selected from Protein A from *Staphylococcus aureus,* Protein G from group C and G *Streptococci,* Protein H from *Streptococcus pyogenes,* and Protein L from *Peptostreptococcus magnus.*

**[0018]** Specifically, the antibody-secreting cell is a cell that has differentiated from an antigen-experienced B cell.

**[0019]** Specifically, the transmembrane peptide is selected from human Lymphocyte Activation Gene 3, human CD58, and human CD7.

**[0020]** According to the invention, there is further provided an antibody-secreting cell expressing a membrane-bound

immunoglobulin-capturing molecule that can bind, retain, and display endogenously produced immunoglobulin molecules at its cell surface, the antibody-secreting cell comprising:

(A) a nucleic acid encoding an endogenously produced immunoglobulin, and
(B) a nucleic acid vector comprising

(i) a promoter that is expressed in antibody-secreting cells, and
(ii) a nucleic acid sequence coding for the immunoglobulin-capturing molecule which comprises an immunoglobulin-binding portion, and a cell surface tether portion,

wherein the promoter is operably linked to said nucleic acid sequence coding for said immunoglobulin-capturing molecule, and
wherein the cell surface tether portion comprises a transmembrane peptide, and
wherein the immunoglobulin-binding portion is selected from:

(a) one or more bacterial proteins that naturally have affinity for the constant region of a heavy or light chain of an immunoglobulin;
(b) one or more variable domains of an immunoglobulin that has affinity for any part of another immunoglobulin; or
(c) a single chain variable fragment against a heavy or light chain constant region of an immunoglobulin.

**[0021]** Specifically, the antibody-secreting cell provided herein comprises a vector for expressing the immunoglobulin-capturing molecule, wherein the vector comprises:

(a) nucleic acid sequences coding for a signal peptide and a stalk structure; or
(b) nucleic acid sequences coding for a signal peptide, a stalk structure, and a reporter peptide linked to an IRES sequence or picornavirus 2A ribosomal skip sequence.

**[0022]** Specifically, the promoter is an inducible or constitutive promoter.

**[0023]** Specifically, the promoter is selected from: (a) a promoter that drives expression of a gene selected from *B Lymphocyte-Induced Maturation Protein 1* (*Blimp1*), *Syndecan 1* (*Sdc1*), *Tumor Necrosis Factor Receptor Superfamily Member 17* (*Tnfrsf17*), and *Fucosyltransferase 1* (*Fut1*); (b) a tetracycline-responsive promoter; or (c) a tamoxifen-responsive promoter.

**[0024]** Specifically, the antibody-secreting cell provided herein displays immunoglobulin molecules bound by the immunoglobulin-capturing molecule on its surface.

**[0025]** Further provided herein is use of the antibody-secreting cell provided herein for large-scale production of antibodies.

**[0026]** Specifically, described herein is restricted constitutive expression of an engineered immunoglobulin-capturing molecule comprising one or more immunoglobulin-binding portions or domains derived from bacterial protein(s) such as Protein A and/or Protein G, using an expression system that expresses the immunoglobulin-capturing molecule preferentially on ASCs with minimal expression at the stages of B cell development prior to antigen-induced differentiation.

**[0027]** Specifically also described herein is restricted constitutive expression of an engineered immunoglobulin-capturing molecule comprising a single-chain antibody with specificity to an immunoglobulin, using an expression system that expresses the immunoglobulin-capturing molecule preferentially on ASCs with minimal expression at the stages ofB cell development prior to antigen-induced differentiation.

**[0028]** The engineered ASCs express immunoglobulin-capturing molecules that are tethered to the cell surface and have the ability to selectively bind immunoglobulin molecules (also, as used herein "immunoglobulins" or "antibodies") with sufficient affinity to immobilize the immunoglobulin molecules at the plasma membrane. Because ASCs secrete thousands of immunoglobulin molecules per second, the immunoglobulin-capturing molecules on a given ASC are saturated primarily with the immunoglobulin molecules secreted by that ASC rather than with immunoglobulins secreted by other ASCs. Expression of genes encoding cell surface immunoglobulin-capturing molecules provides a means for identifying ASCs based on the particular monoclonal immunoglobulin molecules being expressed.

**[0029]** In certain aspects, the immunoglobulin-capturing molecule is tethered to the membrane by a peptide sequence derived from a transmembrane protein such as but not limited to human Lymphocyte-Activation Gene 3 (LAG3). In other aspects, the immunoglobulin-capturing molecule is tethered to the plasma membrane via a post-translational modification with, *e.g.*, glycosylphosphatidylinositol (GPI). In some of these aspects, the immunoglobulin-capturing molecule further comprises a long stalk for support, flexibility, and extended protrusion into the extracellular space.

**[0030]** In certain aspects, expression of the immunoglobulin-capturing molecules is driven by a promoter derived from

a human or mouse gene that is highly expressed in ASCs developed *in vivo* or *in vitro.* In other aspects, the immunoglobulin-capturing molecules are expressed by an inducible system, such as the tetracycline system, *in vivo* or *in vitro.* In some aspects, expression of the immunoglobulin-capturing molecule is coupled to the expression of a reporter gene, such as green fluorescent protein (GFP), via an internal ribosomal entry site sequence (IRES) or a picornavirus 2A ribosomal skip sequence in the expression vector.

**[0031]** Further described herein are methods for generating a non-human transgenic animal expressing immunoglobulin-capturing molecules on ASCs. The methods comprise introducing an immunoglobulin-capturing molecule-encoding gene into the genome of a non-human vertebrate, wherein the introduced gene provides constitutive or inducible expression of the immunoglobulin-capturing molecule on host ASCs. In some aspects the transgenic animal is a rodent, preferably a mouse. In other aspects, the transgenic animal is avian, preferably a chicken. In particularly preferred aspects, the transgenic animal is a mouse that expresses human genes encoding the variable domains of the heavy and light chains and lacks the mouse versions of these genes; for example, as described in US Pub. No. 2013/0219535.

**[0032]** Specifically described herein are processes for isolating genes that encode immunoglobulins of a particular specificity from ASCs that display the specific immunoglobulins captured on the surface of the ASCs.

**[0033]** Further described herein are libraries for identification of antibodies of interest from the engineered cells as described herein. The antibody libraries produced using the methods and compositions described herein provide a facilitated means for the screening and production of antibodies that selectively bind to a target of interest. Such libraries thus enhance the isolation of monoclonal antibodies for use in the clinical, diagnostic, and research settings.

**[0034]** An advantage of the invention is that the determination of immunoglobulin specificity can be made using established techniques such as binding to fluorescently labeled antigen and flow cytometric or microscopic procedures. Such procedures allow for enhanced efficiency in identification and isolation of rare antigen-specific cells and the cloning of the rearranged immunoglobulin genes from the isolated cells.

**[0035]** These and other aspects, objects and features are described in more detail below.

## BRIEF DESCRIPTION OF THE FIGURES

**[0036]**

**Figures 1A, 1B and 1C** are illustrations of a secreting ASC with no immunoglobulin-capturing molecules on the cell surface (**Figure 1A**), an ASC with immunoglobulin-capturing molecules and captured immunoglobulin molecules (*i.e.,* antibodies) on the surface of the ASC (**Figure 1B**), and the binding of labeled antigens to the antibodies retained by the immunoglobulin-capturing molecules expressed on an ASC (**Figure 1C**).

**Figure 2A** is a schematic diagram depicting part of a DNA vector encoding an embodiment of an immunoglobulin-capturing molecule. **Figure 2B** is a simplified illustration of the embodiment of Figure 2A expressed as an immunoglobulin-capturing molecule on an ASC surface.

**Figure 3A** is a schematic diagram depicting part of a DNA vector encoding an alternative embodiment of an immunoglobulin-capturing molecule. **Figure 3B** is a simplified illustration of the embodiment of Figure 3A expressed as an immunoglobulin-capturing molecule on an ASC surface.

**Figure 4A, 4B, and 4C** are illustrations of a secreting ASC with no immunoglobulin-capturing molecules on the cell surface (**Figure 4A**), an ASC with immunoglobulin-capturing molecules and immunoglobulins (*i.e.*, antibodies) on the surface of the ASC (**Figure 4B,** also as depicted in detail in Figure 3B), and labeled antigens bound to the immunoglobulin-capturing molecules expressed on an ASC (**Figure 4C**).

**Figure 5A** is a schematic diagram depicting part of a DNA vector encoding an exemplary embodiment of an immunoglobulin-capturing molecule. **Figure 5B** provides two flow cytometry scatter plots showing the results of retention of secreted immunoglobulin molecules on the cell surface of transfected RPMI 8226 (ATCC® CCL-155™) human cells per the methods of the invention.

## DEFINITIONS

**[0037]** The terms used herein are intended to have the plain and ordinary meaning as understood by those of ordinary skill in the art. The following definitions are intended to aid the reader in understanding the present invention, but are not intended to vary or otherwise limit the meaning of such terms unless specifically indicated.

**[0038]** An "antibody-secreting cell" or "ASC" refers to a cell that has differentiated from an antigen-experienced B cell and acquired the capacity to express as well as secrete large amounts of immunoglobulin molecules. ASCs include plasmablasts and short-lived or long-lived plasma cells in the animal, as well as plasmablasts and plasma cells developed *in vitro* from B cell cultures.

**[0039]** A "capture molecule" is any moiety that contains a region that selectively binds to a part of or a whole molecule of interest.

**[0040]** "Capture" refers to selective binding and immobilization of a molecule at a cell surface due to a durable interaction between that molecule and a membrane-bound capture molecule.

**[0041]** "Cell surface" refers to the plasma membrane of a cell, *i.e.*, that part of a cell most directly exposed to extracellular spaces and available for contact both with cells and proteins in the extracellular (including intercellular) space.

**[0042]** An "immature B cell" refers to a cell at an intermediate phase of B cell differentiation, during which a hematopoietic stem cell undergoes genetic programming to become a mature, yet antigen-inexperienced, B cell. A "mature" B cell refers to an antigen-inexperienced B cell, which is capable of clonal expansion, as well as differentiation into a memory cell or an antibody-secreting cell, upon activation by an antigen.

**[0043]** An "immunoglobulin" refers to an antibody, whether a part of or whole antibody molecule. In most vertebrate animals including humans, antibodies normally exist as dimers of two identical heavy (H) chains that are each paired with an identical light (L) chain. The N-termini of both H and L chains consist of a variable domain ($V_H$ and $V_L$, respectively) that together provide the H-L pair with its unique antigen-binding specificity. The constant region of the H chain consists of 3 to 4 immunoglobulin domains (referred to as $C_H1$ to $C_H4$) with or without a hinge, depending on the isotype (or antibody class). In mice, the isotypes are IgM, IgD, IgG3, IgG1, IgG2b, IgG2a or IgG2c, IgE, and IgA. The light chain constant region consists of either a $\kappa$ or $\lambda$ immunoglobulin domain (referred to as $C\kappa$ or $C\lambda$). In both mice and humans, the presence of $\kappa$ light chains predominates over that of $\lambda$ light chains in the total pool of immunoglobulins within an individual. In certain mammals, such as camelids or animals made deficient in light chain expression, immunoglobulins may consist of heavy chains only. Despite the lack of light chains, these immunoglobulins are also efficiently retained on the cell surface by immunoglobulin-capturing molecules designed to bind to the immunoglobulin heavy chain described herein. Additionally, an immunoglobulin can refer to an unconventional antibody, whether in part or in whole, such as a bispecific antibody that consists two or more $V_H$ and/or $V_L$ domains, for example, as described in USSN 15/246,181, filed 24 August 2016. Finally, an immunoglobulin also refers to a hybrid molecule consisting of part of an antibody, particularly the antibody constant region, and part of another protein. The immunoglobulin-capturing molecules described herein also may be designed and engineered to retain hybrid immunoglobulin molecules for display at the cell surface.

**[0044]** An "immunoglobulin-capturing molecule" refers to a plasma membrane-bound molecule that can bind, retain, and display immunoglobulin molecules (*i.e.*, immunoglobulins or antibodies) at the cell surface.

**[0045]** An "immunoglobulin superfamily" or "IgSF" molecule refers to a molecule that possesses immunoglobulin folds (Ig folds) that are structurally similar to the immunoglobulin domains found in antibody molecules.

**[0046]** The term "transgene" is used herein to describe genetic material which has been or is about to be artificially inserted into the genome of a cell.

**[0047]** "Transgenic animal" refers to a non-human animal, usually a mammal such as a rodent, particularly a mouse or rat although other animals are envisioned, having an exogenous nucleic acid sequence present as a chromosomal or extrachromosomal element in a portion of its cells or stably integrated into its germ-line DNA (*i.e.*, in the genomic sequence of most or all of its cells).

**[0048]** A "vector" or "expression construct" includes plasmids and viruses and any DNA or RNA molecule, whether self-replicating or not, which can be used to transform, transduce, or transfect a cell.

## DETAILED DESCRIPTION OF THE INVENTION

**[0049]** The practice of the techniques described herein may employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology, which are within the skill of those who practice in the art. Such conventional techniques include polymer array synthesis, hybridization and ligation of polynucleotides, and detection of hybridization using a label. Specific illustrations of suitable techniques can be had by reference to the examples herein. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Green, et al., Eds. (1999) Genome Analysis: A Laboratory Manual Series (Vols. I-IV); Weiner, Gabriel, Stephens, Eds. (2007), Genetic Variation: A Laboratory Manual; Dieffenbach, Dveksler, Eds. (2007), PCR Primer: A Laboratory Manual; Sambrook and Russell (2006), Condensed Protocols from Molecular Cloning: A Laboratory Manual; and Green and Sambrook (2012), Molecular Cloning: A Laboratory Manual (all from Cold Spring Harbor Laboratory Press); Stryer, L. (1995) Biochemistry (4th Ed.) W.H. Freeman, New York N.Y.; Lehninger, Principles of Biochemistry 3rd Ed., W. H. Freeman Pub., New York, N.Y.; and Berg et al. (2002) Biochemistry, 5th Ed., W.H. Freeman Pub., New York, N.Y.; Nagy, et al., Eds. (2003) Manipulating the Mouse Embryo: A Laboratory Manual (3rd Ed.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., Immunology Methods Manual (Lefkovits ed., Academic Press 1997); Gene Therapy Techniques, Applications and Regulations From Laboratory to Clinic (Meager, ed., John Wiley & Sons 1999); M. Giacca, Gene Therapy (Springer 2010); Gene Therapy Protocols (LeDoux, ed., Springer 2008); Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, eds., John Wiley & Sons 1998); and Mammalian Chromosome Engineering - Methods and Protocols (G. Hadlaczky, ed., Humana Press 2011).

**[0050]** Note that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an immunoglobulin" refers to one or more such immunoglobulins, and reference to "the method" includes reference to equivalent steps and methods known to those skilled in the art, and so forth.

**[0051]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

**[0052]** Where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

**[0053]** In the following description, numerous specific details are set forth to provide a more thorough understanding of the present invention. However, it will be apparent to one of skill in the art that the present invention may be practiced without one or more of these specific details. In other instances, well-known features and procedures well known to those skilled in the art have not been described in order to avoid obscuring the invention.

***The Invention in General***

**[0054]** Antibody-secreting cells (ASCs) normally do not display the immunoglobulins they express and secrete on their plasma membrane, making highly advanced techniques based on cell surface labeling, such as magnetic and flow-cytometric sorting, inapplicable as methods to select for antigen-specific ASCs. The present invention was born out of the need for a system that allows for efficient screening of ASCs based on cell surface presentation of secreted immunoglobulin molecules (also as used herein "immunoglobulins" or "antibodies"). Specifically, provided herein is a means for expressing immunoglobulin-capturing molecules that can retain and immobilize immunoglobulins at the surface of the secreting cells, such as ASCs or hybridomas, which do not normally express high levels of membrane-bound immunoglobulins or naturally have the ability to retain immunoglobulins on their cell surface. ASCs express and release large amounts of immunoglobulins (thousands of molecules per second) (see, *e.g.*, Mitchell, Advances in Immunology 28:451-511 (1979)). Therefore, the immunoglobulin-capturing molecules expressed on these cells are saturated primarily with immunoglobulins produced from within, rather than with the immunoglobulins secreted by other cells. Thus, the immunoglobulin-capturing molecules must possess a high affinity and a low dissociation rate for the immunoglobulin molecules they capture. Described herein are methods and compositions for expression of such high-affinity immunoglobulin-capturing molecules with low dissociation rates.

**[0055]** Engineering ASCs to capture endogenously produced immunoglobulins on their cell surface provides a facile means for discriminating the antigen specificity of the antibodies that each ASC produces, and for separating ASCs secreting desired immunoglobulins from those ASCs that do not. Discrimination can be accomplished by, *e.g.,* using antigens labeled with substances that facilitate identification and purification of cells (*e.g.*, magnetic, biotinylated, fluorescent, radioactive, or enzymatic molecules) by well-established procedures known in the art.

**[0056]** Figures 1A, 1B and 1C illustrate the principles of the present disclosure in one exemplary embodiment. As depicted in Figure 1A, an antibody-secreting cell (ASC) (101) does not normally express the membrane-bound form of the immunoglobulin molecule, nor does it retain the secreted form of the immunoglobulin molecule (*i.e.*, antibody) (102) on its cell surface. Provided herein are methods and compositions for the expression of immunoglobulin-capturing molecules (103) on the cell surface of ASCs. Specifically, during the synthesis of the immunoglobulin molecules in the endoplasmic reticulum and subsequent packaging in the vesicles for secretion-or soon after their secretion-some immunoglobulin molecules (102) are retained on the cell surface by immunoglobulin-capturing molecules (103) as depicted in Figure 1B. Labeled antigens (104) (*e.g.*, fluorescently-labeled antigens) are then allowed to bind to the immunoglobulin molecules (antibodies) (102) that have bound to an immunoglobulin-binding portion of the immunoglobulin-capturing molecules (103) on the cell surface of the ASC, as depicted in Figure 1C. Antigen-specific ASCs are then identified by the labeled antigens (104) bound to the immunoglobulin-binding portion of the immunoglobulin-capturing molecules (102) that have been captured on the cell surface. Detection of antigen binding on the ASCs is accomplished by, *e.g.,* using antigens that are directly labeled with a fluorophore or other reporter molecule. The ASCs that bind labeled antigens are then purified by, *e.g.,* cell-sorting techniques known in the art.

**[0057]** The purified ASCs expressing antibodies specific for a particular antigen may then be immortalized by fusion with myeloma or plasmacytoma cells, or directly used as a source of nucleic acids (DNA or mRNA) for the creation of libraries of sequences encoding immunoglobulins.

**[0058]** Libraries from purified ASCs contain rearranged immunoglobulin genes encoding antibodies of defined specificity (*i.e.*, specificity for the antigens used in the purification process). The $V_H$ and $V_L$ genes can be identified from the antigen-specific ASCs by deep sequencing coupled with bioinformatics data mining (see, *e.g.,* Haessler and Reddy, Methods in Molecular Biology 1131:191-203 (2014)). Alternatively, the antigen-specific ASCs can be individually sorted.

The $V_H$ and $V_L$ domains unique to each ASC are then cloned via established RT-PCR or 5' Rapid Amplification of cDNA Ends (5' RACE) techniques adapted to single-cell cloning (for review, see, *e.g.,* Tiller, et al., New Biotechnology 5:453-7 (2011)). In yet another alternative, the $V_H$ and $V_L$ sequences can be identified using the methods and materials described in US Pat. Nos. 9,328,172; 8,309,035; and 8,309,317.

***The Immunoglobulin-capturing molecule***

**[0059]** The immunoglobulin-capturing molecules described herein that are expressed at the cell surface comprise at least two components, and in preferred embodiments may comprise additional components, as described in detail below. In a simple form, the immunoglobulin-capturing molecules comprise a cell surface tether component, and an immunoglobulin-binding component. The cell surface tether component may comprise a transmembrane peptide domain that tethers or anchors the expressed immunoglobulin-binding component in the cell surface membrane, or the cell surface tether component may comprise a chemical moiety (for example, glycosylphosphatidylinositol) that allows for the immunoglobulin-binding component to be tethered to the cell surface membrane via a chemical bond. In addition to these components, the immunoglobulin-capturing molecules described herein may comprise a stalk component, one or more linker components, and/or a reporter peptide.

**[0060]** In one embodiment, the immunoglobulin-capturing molecule consists of one or more immunoglobulin-binding domains or portions derived from one or more bacterial proteins that naturally have affinity for the constant region of the heavy or light chain of immunoglobulins. Such immunoglobulin-binding proteins include but are not limited to Protein A from *Staphylococcus aureus,* Protein G from group C and G *Streptococci,* Protein H from *Streptococcus pyogenes,* or Protein L from *Peptostreptococcus magnus.* In some embodiments, the immunoglobulin-capturing molecule is expressed as a hybrid molecule comprising two or more immunoglobulin-binding domains derived from two or more different bacterial proteins. As an example, the capture molecule may be expressed as a fusion protein, which contains two immunoglobulin-binding domains from Protein G and two immunoglobulin-binding domains from Protein A. In some aspects of this embodiment, one or more of the bacterial immunoglobulin-binding protein domains are modified to, *e.g.,* remove potential sites for glycosylation or other post-translational modifications in eukaryotic cells, improve affinity for certain immunoglobulin isotypes, or improve translation efficiency in mammalian cells by codon optimization.

**[0061]** In another embodiment, the immunoglobulin-capturing molecules consist of single-chain variable fragments (scFv). The scFv is expressed as a fusion protein of the $V_H$ and $V_L$ domains derived from a hybridoma cell line that produces monoclonal antibodies against the heavy chain or light chain constant region of another immunoglobulin molecule (*e.g.*, a common epitope present in all murine IgG isotypes). In some aspects, the scFv capture molecule comprises the $V_H$ domain connected in tandem to the $V_L$ domain by a glycine/serine-rich linker sequence in either order. The glycine/serine-rich linker sequence includes but is not limited to repetitions of $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_n$ [as in SEQ ID No. 29] or $(Gly\text{-}Ser)_n$ [as in SEQ ID No. 28].

**[0062]** In some embodiments, a polypeptide sequence encoding a transmembrane domain is fused to the immunoglobulin-binding domain in order to tether the immunoglobulin-capturing molecule on the cell surface. Preferably in this embodiment, the transmembrane domain is inert (lacking cell signaling functions) and not prone to internalization. Such a transmembrane domain could be an artificial sequence, or a motif derived from Major Histocompatibility Class I (MHC I), an IgSF molecule such as Lymphocyte-Activation Gene 3 (LAG3 or CD223), or any other transmembrane protein of any species-that is naturally inserted into the plasma membrane upon protein translation.

**[0063]** In other embodiments, the immunoglobulin-capturing molecule contains-in addition to the immunoglobulin-binding domain-a C-terminal peptide sequence for post-translational modification with, *e.g.*, glycosylphosphatidylinositol (GPI), where GPI acts as a tether portion of the immunoglobulin-capturing molecule. GPI is a normal post-translational moiety that comprises a phosphoethanolamine group, a trimannosyl-nonacetylated glucosamine (Mans-GlcN) core, and a phosphatidylinositol group that tethers the protein to the plasma membrane. The phosphoethanolamine group of GPI is linked to a protein C-terminus via a phosphodiester bond. The GPI tether sequences may consist of the C-termini of proteins that are naturally anchored to the ASC plasma membrane by this post-translational process. Table 3 lists exemplary GPI tether or anchor sequences that may be used to construct the immunoglobulin-capturing molecule.

**[0064]** In certain embodiments, the immunoglobulin-capturing molecule contains a "stalk" structure for structural flexibility and support, as well as for increased exposure to the extracellular space. Since the cell surface is ubiquitously crowded with various molecules, the immunoglobulins captured on the immunoglobulin-capturing molecules may be occluded from access to their cognate antigen in the extracellular space by other molecules on the ASC surface. Thus, inclusion of a long stalk in the immunoglobulin-capturing molecule can alleviate any steric hindrance that compromises antigen binding by the displayed immunoglobulins. In preferred aspects, the stalk of the immunoglobulin-capturing molecule comprises one or more immunoglobulin domains derived from one or more IgSF proteins. Examples of these domains include but are not limited to the immunoglobulin domains of CD2, CD4, or CD22. Additionally, the stalk of the immunoglobulin-capturing molecule may be expressed as a macromolecular complex of two or more subunits. For example, the stalk of the ScFv-containing capture molecule may consist of $C_H2$ and $C_H3$ domains as well as the hinge

region of an IgG molecule; thus, the immunoglobulin-capturing molecule is expressed as a homodimer.

***Expression of the immunoglobulin-capturing molecule***

**[0065]** In certain aspects, expression of the immunoglobulin-capturing molecules is driven by a promoter derived from a gene that is highly expressed in ASCs but not in immature B cells or antigen-inexperienced mature B cells. These genes include but are not limited to *B Lymphocyte-Induced Maturation Protein 1* (*Blimp1*), *Syndecan 1* (*Sdc1*), *Tumor Necrosis Factor Receptor Superfamily Member 17* (*Tnfrsf17*), and *Fucosyltransferase 1* (*Fut1*). The gene chosen for ASC expression may be of mouse origin, or it may be from another species in which the gene shows an appropriately conserved expression pattern.

**[0066]** In certain other aspects, expression of the immunoglobulin-capturing molecules is driven by an inducible promoter, such as the tetracycline- or tamoxifen-inducible system. The inducible promoter is used to drive the expression of the immunoglobulin-capturing molecule either directly or indirectly via expression of a recombinase such as Cre (see, *e.g.,* Albanese, et al., Seminars in Cell & Developmental Biology, 13:129-141 (2002); Sakai, Methods in Molecular Biology, 1142:33-40 (2014)). Such inducible expression in ASCs is accomplished either in the transgenic animal or *in vitro* during culture of ASCs as well as at the stage of hybridoma culture.

**[0067]** In order to express the immunoglobulin-capturing molecule on the cell surface, a signal peptide is included for protein translation in the endoplasmic reticulum. The signal peptide may be a consensus sequence or one that naturally exists as part of cell surface or secreted protein. In preferred aspects, the signal peptide is derived from that of an immunoglobulin heavy chain [as in SEQ ID Nos. 5-7] or light chain protein [as in SEQ ID Nos 1-3].

**[0068]** In some aspects, in addition to the immunoglobulin-capturing molecule, the expression vector may include an open-reading frame for a reporter protein such as GFP, red fluorescent protein (RFP), or the like. The reporter gene in the expression construct is linked to the immunoglobulin-capturing molecule via, *e.g.,* an IRES sequence or a picornavirus 2A ribosomal skip sequence. Expression of the reporter gene allows for improved purity when used in combination with antigen selection to sort for antigen-specific ASCs.

**[0069]** Transgenes providing for expression of the immunoglobulin-capturing molecules are generated by inserting the coding sequences for the immunoglobulin-capturing molecules into a large piece of genomic DNA containing the gene that is highly expressed in ASCs (*e.g, Blimp1* or *Tnfrsf17*). The insertion can be accomplished by homologous recombination mediated by sequences appended to the ends of the coding fragments, or by other standard molecular biology approaches. The large pieces of genomic DNA may be contained within bacterial artificial chromosome vectors, *e.g.*, such as the pieces of DNA in these vectors that can be obtained from commercially or publicly available genomic DNA libraries.

**[0070]** Transgenic mice (or other animals) expressing the immunoglobulin-capturing molecules may be generated by any facility with the requisite skills using known techniques, as will be understood by one skilled in the art upon reading the present disclosure. Analysis of the animals carrying the transgene is performed using standard methodology such as immunofluorescence microscopy, flow cytometry and/or immunoblotting.

**[0071]** Illustrated in Figures 2A and 2B are the transgene (201) and expressed structure (202) of an immunoglobulin-capturing molecule according to one embodiment. The transgene (201) comprises two exons with an intervening intron (203) [SEQ ID Nos. 5-7]. The first exon and the beginning of the second exon encode a leader peptide (*e.g.*, $V_H$ leader peptide). Contiguous with the leader peptide-encoding sequence are sequences encoding the following components: one or more immunoglobulin-binding domains (204) derived from one or more bacterial proteins [*e.g.*, a sequence chosen from SEQ ID Nos. 8-11], a glycine/serine-rich linker (205) [*e.g.*, a sequence chosen from SEQ ID Nos. 12 or 13], a "stalk" structure or region (206) [*e.g.*, a sequence chosen from SEQ ID Nos. 14-16], and a transmembrane domain (207) [*e.g.*, a sequence chosen from SEQ ID No. 17-20]. Following protein translation, the leader peptide is excised from the immunoglobulin-capturing molecule (202), which is expressed as a cell surface protein tethered to the plasma membrane (212). The respective components (208-211) of the immunoglobulin-capturing molecule (202) shown are immunoglobulin-binding domain(s) (208) [*e.g.*, a sequence chosen from SEQ ID Nos. 24-27], glycine/serine-rich linker (209) [*e.g.*, a sequence chosen from SEQ ID Nos. 28 or 29], stalk (210) [*e.g.*, a sequence chosen from SEQ ID Nos. 30-32], and transmembrane domain (211) [*e.g.*, a sequence chosen from SEQ ID Nos. 33-36].

**[0072]** Exemplary nucleic acid sequences for components of the immunoglobulin-capturing molecule illustrated in Figure 2A (with the expressed structure illustrated in Figure 2B) are listed in Table 1. The immunoglobulin-capturing molecule may be assembled by combining together one sequence of the several possible options for each component from Table 1 in the order depicted in Figure 2A (*i.e.,* from N-terminus to C-terminus). For example, a small immunoglobulin-capturing molecule may consist of only two immunoglobulin-binding domains of Protein G, a $(glycine\text{-}serine)_3$ linker, and a transmembrane domain without a stalk; while a larger one may contain five Protein A immunoglobulin-binding domains as well as four Protein G immunoglobulin-binding domains, a $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$ linker, a human CD22 stalk composed of six immunoglobulin folds, and a long human CD7 transmembrane domain.

**[0073]** An expression construct and the molecular structure of an alternative embodiment of an immunoglobulin-

capturing molecule is illustrated in Figures 3A and 3B. In this aspect, the transgene (301) similarly comprises a leader sequence encoded by two exons with an intervening intron (303), followed by sequences encoding the components of a scFv with specificity for a part of an immunoglobulin molecule (*e.g.,* a conserved part of the heavy or light chain constant region): $V_H$ (304), glycine/serine-rich linker (305), and $V_L$ (306). For extended protrusion of the immunoglobulin-capturing molecule into the extracellular space, a sequence encoding a stalk comprising a hinge (307) as well as Fc fragment (308) of an immunoglobulin molecule is appended to the scFv-encoding sequence. Finally, one or more exons (309) encoding a transmembrane domain is also included in the expression construct (301). Shown in Figure 3B is the immunoglobulin-capturing molecule (302) expressed as a homodimer of two subunits, each consisting of a $V_L$ (310) domain, glycine/serine-rich linker (311), and $V_H$ (312) domain of scFv connected to a hinge (313) and Fc (314) of an immunoglobulin molecule. The two subunits of the immunoglobulin-binding portion of the immunoglobulin-capturing molecule are covalently linked via disulfide bonds in the hinge region (313) of each chain. The expressed immunoglobulin-capturing molecule is tethered or anchored into the plasma membrane (316) by a transmembrane domain (315).

**[0074]** Figure 4 illustrates the presentation of immunoglobulin-capturing molecules on a cell surface by an scFV embodiment of the immunoglobulin-capturing molecule. As demonstrated previously, an antibody-secreting cell (401) normally does not express the membrane-bound form of antigen receptors and lacks the ability to display on the cell surface the immunoglobulin molecules (402) they secrete. Expression of the scFv version of the immunoglobulin-capturing molecule (403) allows some of the immunoglobulin molecules (402) to be retained on the cell surface as they are being synthesized in the endoplasmic reticulum and subsequently packaged in the vesicles for secretion, or soon after their secretion. Antigen-specific ASCs are then identified by the binding of antigens (404) to the captured immunoglobulin molecules (402) on the cell surface. Detection of antigen binding on the ASCs is accomplished by using antigens that are directly labeled with a fluorophore or any other reporter molecule.

***Transgenic Cell Libraries***

**[0075]** The transgenic cells described herein also are used to produce expression libraries, preferably low complexity libraries, for identification of antibodies of interest on the surface of ASCs. The present description thus also includes antibody libraries produced using the cell technologies of the invention for identification of antigen-specific antibodies expressed on ASCs.

***Transgenic Animals***

**[0076]** Also described herein are transgenic animals that have been modified to express immunoglobulin-capturing molecules on the cell surface of ASCs.

**[0077]** In preferred aspects, the transgenic animals described herein further comprise human immunoglobulin regions. Numerous methods have been developed for replacing endogenous mouse immunoglobulin regions with human immunoglobulin sequences to create partially- or fully-human antibodies for drug discovery purposes. Examples of such mice include those described in, for example, U.S. Pat Nos. 7,145,056; 7,064,244; 7,041,871; 6,673,986; 6,596,541; 6,570,061; 6,162,963; 6,130,364; 6,091,001; 6,023,010; 5,593,598; 5,877,397; 5,874,299; 5,814,318; 5,789,650; 5,661,016; 5,612,205; and 5,591,669.

**[0078]** The exons that encode the antibody $V_H$ and $V_L$ domains do not exist in the germ-line DNA. Instead, each $V_H$ or $V_L$ exon is generated, respectively, by the recombination of randomly selected V, D, and J genes present in the H chain locus, or of randomly selected V and J genes in the light chain locus. There are multiple V, D, and J genes in the H chain locus as well as multiple V and J genes in each L chain locus, thus allowing for the generation of a vast antibody diversity repertoire per individual when the permutations of H chain VDJ rearrangements are combined with the permutations of L chain VJ gene rearrangements.

**[0079]** In particularly preferred aspects, the transgenic animals are as described in copending application US Pub. No. 2013/0219535. Such transgenic animals have a genome comprising an introduced partially human immunoglobulin region, wherein the endogenous non-human V, D, and J gene coding sequences have been replaced with those of human origin without altering the endogenous noncoding sequences. Preferably, the transgenic cells and animals described herein have genomes in which part or all of the endogenous immunoglobulin genes are removed.

**[0080]** In other aspects, the transgenic animals described herein are avian, preferably chickens.

***Use in Antibody Production***

**[0081]** Culturing cells *in vitro* has been the basis of the production of numerous therapeutic biotechnology products, and involves the production of protein products in cells and release into the support medium. The quantity and quality of protein production over time from the cells growing in culture depends on a number of factors, such as, for example, cell density, cell cycle phase, cellular biosynthesis rates of the proteins, condition of the medium used to support cell

viability and growth, and the longevity of the cells in culture. (See, for example, Fresney, Culture of Animal Cells, Wiley, Blackwell (2010); and Cell Culture Technology for Pharmaceutical and Cell-Based Therapies, Ozturk and Ha, Eds., CRC Press, (2006).)

**[0082]** For certain products, such as monoclonal antibodies, enhancing the presence and protein-expression efficiency of the cells that are actually producing the product is a key aspect of efficient protein production. Capturing antibodies on the surface of ASCs secreting them provides opportunities for discriminating ASCs on the basis of their immunoglobulin specificities, and this in turn provides opportunities for optimizing and enhancing the production of antibodies for various uses.

## EXAMPLES

**[0083]** The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention, nor are they intended to represent or imply that the experiments below are all of or the only experiments performed. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

**[0084]** Efforts have been made to ensure accuracy with respect to terms and numbers used (*e.g.*, vectors, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees centigrade, and pressure is at or near atmospheric.

### Example 1: Expression of a minimal Protein G-containing membrane-bound immunoglobulin-capturing molecule.

**[0085]** An expression vector encoding a small membrane-bound form of the immunoglobulin-capturing molecule without a stalk is generated by direct DNA synthesis or standard molecular cloning techniques. A diagram of the protein-coding part of this vector (501) is shown in Figure 5A. The expression vector encodes two immunoglobulin-binding domains of streptococcal Protein G (504) [SEQ ID No. 8] that are tethered to the cell surface by means of a membrane-spanning domain derived the human LAG3 (or CD223) protein (506) [SEQ ID No. 17]. A fragment of DNA encoding a short linker consisting of Gly-Ser-Gly-Ser-Gly-Ser sequence (505) [SEQ ID No. 28] is placed between the DNA fragments encoding the Protein G immunoglobulin-binding domains (504) and the transmembrane domain (506) to provide structural flexibility to the expressed protein. Finally, a sequence encoding a signal peptide (leader peptide) (503) is included in the construct to allow for extrusion of the immunoglobulin-capturing molecule into the lumen of the endoplasmic reticulum during its biosynthesis. The signal peptide sequence in this example is derived from an immunoglobulin light chain variable ($V_L$) gene segment which includes its native intron (503) [SEQ ID Nos. 1-3]. The promoter is indicated at (502). The nucleotide and amino acid sequences of various components comprising the immunoglobulin-capturing molecule in this example are specified in Table 1 and Table 2, respectively.

**[0086]** The expression vector is transfected into various myeloma, hybridoma or other cell lines using commonly accessible methodology such as electroporation. The transfected cells are then examined for surface expression of the immunoglobulin-capturing molecule using procedures such as immunofluorescence microscopy, flow cytometry, and/or immunoblotting of the membrane protein fractions. The cells are further analyzed using a subset of these procedures for the capacity of the cell surface immunoglobulin-capturing molecules to retain immunoglobulins produced by the transfected cells or added to them.

**[0087]** Figure 5B illustrates the expression of this small immunoglobulin-capturing molecule in a plasmacytoma cell line and its ability to retain immunoglobulin molecules on the cell surface. Human RPMI 8226 (ATCC® CCL-155™) cells were transfected with DNA plasmids encoding the immunoglobulin-capturing molecule (501) under control of the *Blimp1* promoter (502). The cells were also co-transfected with a plasmid encoding mouse IgG. Compared to untransfected cells (top, 507), the transfected cells (bottom, 508) exhibit captured immunoglobulins on the cell surface.

**[0088]** Transgenic animals are then generated to express the membrane-bound immunoglobulin-capturing molecules containing Protein G on the ASCs, and the capacity of the transgene-encoded molecules to capture immunoglobulins on ASCs is determined directly on the ASCs taken from the transgenic mice by standard flow cytometry.

### Example 2: Expression of a Protein G-containing membrane-bound immunoglobulin-capturing molecule containing a stalk.

**[0089]** An expression vector encoding a membrane-bound form of the immunoglobulin-capturing molecule containing a long stalk is generated by direct DNA synthesis or standard molecular cloning techniques. The expression vector encodes three immunoglobulin-binding domains derived from the C-terminal half of streptococcal Protein G [SEQ ID

No. 9]. DNA fragments encoding a short linker consisting of Gly-Ser-Gly-Ser-Gly-Ser [SEQ ID No. 28] sequence, a stalk consisting of six immunoglobulin domains derived from human CD22 protein [SEQ ID No. 16], and a transmembrane domain derived from human CD58 [SEQ ID No. 18] are appended to the immunoglobulin-binding domain-encoding DNA fragment of the vector. Finally, a sequence encoding a signal peptide (leader peptide) is placed preceding the entire open-reading frame of the immunoglobulin-capturing molecule to allow for extrusion of the translated protein into the lumen of the endoplasmic reticulum during its biosynthesis. The sequences encoding the signal peptide in this example are derived from an immunoglobulin heavy chain variable ($V_H$) gene segment and include its native intron [SEQ ID Nos. 5-7]. The nucleotide and amino acid sequences of components comprising the immunoglobulin-capturing molecule in this example are specified in Table 1 and Table 2, respectively.

**[0090]** The expression vector is transfected into various myeloma, hybridoma or other cell lines using commonly accessible methodology such as electroporation. The transfected cells are then examined for surface expression of the Protein G molecule using procedures such as immunofluorescence microscopy, flow cytometry and immunoblotting of the cell membrane protein fractions. The cells are further analyzed using a subset of these procedures for the capacity of the cell surface Protein G to capture immunoglobulins produced by the transfected cells or added to them.

**[0091]** Transgenic animals are then generated to express the membrane-bound immunoglobulin-capturing molecules consisting of Protein G, CD22 and CD58 fusion in the ASCs, and the capacity of the transgene-encoded molecules to capture immunoglobulins on ASCs is determined directly on the ASCs taken from the transgenic mice by standard flow cytometry.

**Example 3: Expression of a Protein G-containing immunoglobulin-capturing molecule anchored to the membrane by a GPI post-translational modification**

**[0092]** An expression vector encoding two immunoglobulin-binding domains derived from the streptococcal Protein G is synthesized. Included in this expression vector downstream of the Protein G-encoding sequence are DNA fragments that encode the following: a Gly/Ser-rich linker sequence, a stalk consisting of two immunoglobulin domains of human CD4, and a GPI anchor sequence. Finally, a signal peptide sequence (leader sequence) is included in the construct to allow for extrusion of the translated protein into the lumen of the endoplasmic reticulum during its biosynthesis. The sequences encoding the signal peptide in this example are derived from an immunoglobulin light chain variable ($V_L$) gene segment and include its native intron. The nucleotide and amino acid sequences of components comprising the immunoglobulin-capturing molecule in this example are specified in Table 1 and Table 2, respectively. The GPI anchor sequences are specified in Table 3.

**[0093]** The expression vector is transfected into various myeloma, hybridoma or other cell lines using commonly accessible methodology such as electroporation. The transfected cells are then examined for surface expression of the Protein G molecule using procedures such as immunofluorescence microscopy, flow cytometry and immunoblotting of the cell membrane protein fractions. The cells are further analyzed using a subset of these procedures for the capacity of the cell surface Protein G to capture immunoglobulins produced by the transfected cells or added to them.

**[0094]** Transgenic animals are then generated to express the GPI-anchored immunoglobulin-capturing molecules in ASCs, and the capacity of the transgene-encoded molecules to capture immunoglobulins on the surface of ASCs is determined directly on the ASCs taken from the transgenic mice by standard flow cytometry.

**Example 4: Expression of a membrane-bound scFv derived from an antibody specific for an immunoglobulin constant region.**

**[0095]** An expression vector encoding a scFv specific for the constant domain of an immunoglobulin is generated by standard molecular cloning or direct DNA synthesis. In this example, the single-chain antibody is specific for the constant domain of the mouse kappa light chain, which is present in more than 90% of antibodies found in normal mice. The exon encoding the scFv comprises $V_L$, linker, and $V_H$ sequences specified at [SEQ ID Nos. 43-48, respectively]. Included in this expression vector downstream of the ScFv-encoding sequence is a contiguous sequence that encodes the Fc part of rat IgG1 consisting of the following: a hinge region, $C_H2$ domain, and $C_H3$ domain of the secreted form. The rat IgG1 Fc-encoding sequence is specified at [SEQ ID Nos. 49]. The vector also includes sequences encoding the transmembrane domain of a mouse Major Histocompatibility Complex Class I protein (the mouse K molecule from the b haplotype), specified at [SEQ ID Nos. 50-54].

**[0096]** The expression vector is transfected into various myeloma, hybridoma and other cell lines using commonly accessible methodology such as electroporation. The transfected cells are examined for surface expression of the single chain antibody molecule using procedures such as immunofluorescence microscopy, flow cytometry and immunoblotting of the cell membrane protein fractions. The cells are further analyzed using a subset of these procedures for the capacity of the cell surface single chain antibody molecule to capture immunoglobulins produced by the transfected cells or added to them.

**[0097]** Transgenic animals are generated to express the scFv-containing immunoglobulin-capturing molecules on ASCs, and the capacity of the transgene-encoded molecules to capture immunoglobulins on the surface of ASCs is determined by standard flow cytometry directly on the ASCs taken from the mice.

**Example 5: Use of transgenic animal expressing immunoglobulin-capturing molecules to isolate ASCs producing monoclonal antibodies against antigen of interest.**

**[0098]** Transgenic mice are generated using a bacterial artificial chromosome vector containing the promoter of human *TNFRSF17* gene, the coding sequence of an immunoglobulin-capturing molecule, for example as in Examples 1-4, an IRES sequence, and GFP. Spleen, lymph nodes, and bone marrow from several transgenic founder lines are harvested, processed, and analyzed for the expression of GFP as well as the immunoglobulin-capturing molecule by standard flow cytometry. GFP-positive cells from the transgenic mice are then pooled, sorted, and verified by enzyme-linked immunospot (ELISPOT) for their ability to secrete immunoglobulins. A transgenic line that stably expresses detectable levels of GFP and the immunoglobulin-capturing molecule is selected for propagation.

**[0099]** Adult transgenic mice are immunized with an antigen of interest. Spleens as well as the relevant lymph nodes are isolated from the immunized mice, processed, and stained for flow cytometric analyses. Additionally, the isolated cells are subjected to antigen binding during the flow cytometric staining. The antigen is either directly labeled with a fluorophore or with biotin for use with a labeled avidin, streptavidin, or similar system. ASCs are sorted on the basis of GFP-positive staining as well as antigen-positive staining.

**[0100]** The purified ASCs are then fused to myeloma cells to generate hybridoma cells using established methodologies familiar to those with ordinary skill in the art. In this invention, the provided methods to express the immunoglobulin-capturing molecules also allow for the screening of hybridoma cells based on GFP expression as well as positive staining of antigens captured on the cell surfaces.

**[0101]** Alternatively, the purified ASCs are individually sorted, and genes encoding their $V_H$ and $V_L$ domains are cloned via RT-PCR or 5' RACE techniques adapted for single cells. The cloned $V_H$- and $V_L$- coding sequences are subcloned into an expression vector containing a sequence encoding the desired constant regions of heavy chain and light chain, respectively. The $V_H$- and $V_L$-expression vectors are transfected into a HEK-293T or CHO cell lines, and the secreted monoclonal antibodies are further tested for antigen binding and other functions.

Table 1 - exemplary nucleic acid sequences

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 1 | Leader Exon1/Intron/ Exon 2/Tags $V_L$ Leader Exon 1 | Figure 2A, 203 Figure 3A, 303 Figure 5A, 503 | ATGGACATCAGGGCTCCTGCTCAGTTTCTTG GCATCTTGTTGCTCTGGTTTCCAG |
| 2 | Leader Exon1/Intron/ Exon 2/Tags $V_L$ intron | Figure 2A, 203 Figure 3A, 303 Figure 5A, 503 | GTAAAATGAACTAAAATGGGAATTTCACTGT AAGTGTTGACAGGCATTTGGGGACTGTGTTC TTTTATCATGCTTACCTTTGTAGATATTCATT ATGTCTCCACTCCTAG |
| 3 | Leader Exon1/Intron/ Exon 2/Tags $V_L$ Leader Exon 2 | Figure 2A, 203 Figure 3A, 303 Figure 5A, 503 | GTGCCAGATGTGACATCCAGATG |
| 4 | Leader Exon1/Intron/ Exon 2/Tags FLAG+Myc Tags | Figure 2A, 203 Figure 3A, 303 Figure 5A, 503 | GACTACAAGGATGACGACGACAAGGGCAGC GGCGAACAGAAGCTGATTTCGGAGGAGGAC CTG |
| 5 | Leader Exon1/Intron/ Exon 2/Tags $V_H$ Leader Exon 1 | Figure 2A, 203 Figure 3A, 303 Figure 5A, 503 | ATGGGATGGAGCTGTATCATGCTCTTCTTGG CAGCAACAGCTACAG |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 6 | Leader Exon1/Intron/ Exon 2/Tags $V_H$ Intron | Figure 2A, 203<br>Figure 3A, 303<br>Figure 5A, 503 | GTAAGGGGCTCACAGTAGCAGGCTTGAGGTC TGGACATATACATGGGTGACAATGACATCCA CTTTGCCTTTCTCTCCACAG |
| 7 | Leader Exon1/Intron/ Exon 2/Tags $V_H$ | Figure 2A, 203<br>Figure 3A, 303<br>Figure 5A, 503 | GTGTCCACTCCCAGGTCCAACTG |
| 8 | Ig-Binding Domain<br><br>2-Domain Protein G | Figure 2A, 204<br>Figure 5A, 504 | GGTACCCCAGCCGTGACCACCTACAAGCTCG TCATCAACGGAAAGACGCTCAAGGGCGAAA CCACTACCAAGGCGGTGGATGCCGAAACCGC CGAAAAGGCCTTCAAGCAGTACGCTAACGAC AATGGGGTGGACGGAGTCTGGACGTACGAT GATGCCACCAAGACTTTCACCGTGACCGAAG TGAACACTCCGGCCGTCACCACTTATAAGCT CGTGATCAACGGGAAAACCCTGAAGGGAGA GACTACCACAAAGGCCGTGGATGCTGAGACT GCAGAGAAGGCGTTCAAACAGTACGCCAAC GACAACGGCGTGGACGGCGTCTGGACCTACG ATGACGCCACTAAGACCTTCACTGTGACCGA A |
| 9 | Ig-Binding Domain<br><br>3-Domain Protein G | Figure 2A, 204<br>Figure 5A, 504 | ATAGATGAAATTTTAGCTGCATTACCTAAGA CTGACACTTACAAATTAATCCTTAATGGTAA AACATTGAAAGGCGAAACAACTACTGAAGC TGTTGATGCTGCTACTGCAGAAAAAGTCTTC AAACAATACGCTAACGACAACGGTGTTGACG GTGAATGGACTTACGACGATGCGACTAAGAC CTTTACAGTTACTGAAAAACCAGAAGTGATC GATGCGTCTGAATTAACACCAGCCGTGACAA CTTACAAACTTGTTATTAATGGTAAAACATT GAAAGGCGAAACAACTACTGAAGCTGTTGAT GCTGCTACTGCAGAAAAAGTCTTCAAACAAT ACGCTAACGACAACGGTGTTGACGGTGAATG GACTTACGACGATGCGACTAAGACCTTTACA GTTACTGAAAAACCAGAAGTGATCGATGCGT CTGAATTAACACCAGCCGTGACAACTTACAA ACTTGTTATTAATGGTAAAACATTGAAAGGC GAAACAACTACTAAAGCAGTAGACGCAGAA ACTGCAGAAAAAGCCTTCAAACAATACGCTA ACGACAACGGTGTTGATGGTGTTTGGACTTA TGATGATGCGACTAAGACCTTTACGGTAACT GAA |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 10 | Ig-Binding Domain<br><br>2-Domain Protein A + 2-Domain Protein G | Figure 2A, 204<br>Figure 5A, 504 | GTGGATAACAAGTTCAACAAGGAACAGCAG<br>AACGCCTTTTACGAGATTCTGCATCTGCCCA<br>ACCTGAATGAGGAACAGCGGAACGCATTCAT<br>TCAGTCTCTGAAGGATGATCCTAGCCAGTCG<br>GCCAACCTCCTGGCTGAAGCAAAGAAGCTGA<br>ACGATGCCCAAGCGCCCAAAGTGGACAACA<br>AGTTTAACAAGGAGCAGCAGAATGCTTTCTA<br>CGAGATCCTGCACCTCCCGAATCTGAACGAG<br>GAGCAGAGAAACGCCTTCATCCAATCACTGA<br>AGGACGACCCGTCACAGTCCGCCAACCTTCT<br>GGCGGAAGCCAAGAAACTGAACGACGCCCA<br>GGCGCCAAAGGTGGACGGATCCGGGTCCGG<br>CAGCGGTACCCCAGCCGTGACCACCTACAAG<br>CTCGTCATCAACGGAAAGACGCTCAAGGGCG<br>AAACCACTACCAAGGCGGTGGATGCCGAAA<br>CCGCCGAAAAGGCCTTCAAGCAGTACGCTAA<br>CGACAATGGGGTGGACGGAGTCTGGACGTA<br>CGATGATGCCACCAAGACTTTCACCGTGACC<br>GAAGTGAACACTCCGGCCGTCACCACTTATA<br>AGCTCGTGATCAACGGGAAAACCCTGAAGG<br>GAGAGACTACCACAAAGGCCGTGGATGCTG<br>AGACTGCAGAGAAGGCGTTCAAACAGTACG<br>CCAACGACAACGGCGTGGACGGCGTCTGGA<br>CCTACGATGACGCCACTAAGACCTTCACTGT<br>GACCGAA |
| 11 | Ig-Binding Domain | Figure 2A, 204<br>Figure 5A, 504 | GCCAATGCCGCCCAGCACGACGAGGCTCAGC<br>AGAACGCATTCTACCAGGTGCTGAACATGCC |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| | 5-Domain Protein A + 4-Domain Protein G | | AAACCTCAACGCCGATCAGCGCAATGGTTTC<br>ATTCAGTCCCTGAAGGACGATCCGAGCCAGT<br>CAGCTAACGTGCTCGGGGAGGCCCAAAAGCT<br>GAATGACTCCCAGGCGCCGAAGGCCGACGC<br>CCAGCAAAACAACTTCAACAAGGATCAGCA<br>ATCCGCCTTCTATGAAATCCTGAATATGCCT<br>AACCTGAACGAAGCTCAGCGGAACGGGTTC<br>ATCCAGAGCCTTAAGGACGACCCTAGCCAGT<br>CCACCAACGTGCTGGGGGAGGCCAAGAAAC<br>TTAACGAATCCCAGGCCCCGAAGGCGGACA<br>ACAACTTTAACAAGGAACAGCAGAACGCCTT<br>TTACGAGATCCTCAACATGCCGAACCTCAAC<br>GAGGAACAGCGCAACGGTTTCATCCAGTCCC<br>TGAAGGACGATCCATCCCAGTCCGCCAACCT<br>GTTGAGCGAGGCGAAGAAGCTGAATGAGTC<br>CCAAGCCCCCAAGGCTGACAACAAGTTCAAT<br>AAGGAACAACAGAATGCCTTCTACGAAATTC<br>TGCACTTGCCCAATCTGAACGAGGAGCAGCG<br>CAACGGCTTCATCCAATCTCTGAAAGACGAC<br>CCGTCGCAGTCGGCCAACTTGCTGGCCGAAG<br>CCAAGAAGCTCAACGACGCTCAGGCCCCTAA<br>GGCCGACAACAAGTTCAACAAAGAGCAACA<br>GAACGCGTTCTACGAGATTCTCCACTTGCCG<br>AACCTGACCGAAGAACAACGGAACGGATTC<br>ATTCAGAGCCTGAAGGATGACCCTTCGGTGT<br>CAAAGGAGATCCTGGCAGAAGCCAAAAAGC<br>TGAACGATGCCCAGGCACCAAAGGAAGAGG<br>ACAACAACAAGCCGGGCGACCCGAGGATCT<br>CCGAAGCCACTGATGGGCTGTCCGATTTTCT<br>GAAGTCACAGACTCCTGCTGAGGACACCGTG<br>AAGTCCATCGAGCTCGCCGAGGCCAAGGTGC<br>TGGCCAACCGGGAGCTGGATAAGTACGGAG<br>TGTCCGACTACTACAAAAACCTGATTAACAA<br>CGCCAAGACTGTGGAAGGAGTGAAGGCATT<br>GATCGATGAAATCCTGGCGGCGCTCCCAAAA<br>ACCGACACCTACAAACTGATTCTCAACGGAA<br>AGACGCTGAAGGGGGAAACTACCACCGAAG<br>CGGTGGACGCCGCCACCGCCGAAAAGGTGTT<br>TAAGCAGTATGCTAACGACAACGGTGTCGAC<br>GGAGAGTGGACCTACGACGACGCCACTAAG<br>ACTTTCACCGTGACCGAGAAGCCCGAGGTCA<br>TCGACGCGAGCGAGCTCACTCCCGCCGTGAC<br>CACCTACAAGCTGGTCATCAATGGAAAGACT<br>CTGAAGGGCGAAACTACTACTGAAGCCGTGG<br>ATGCGGCAACCGCCGAGAAAGTGTTCAAGC<br>AATACGCAAACGATAACGGGGTGGACGGAG<br>AGTGGACCTACGACGATGCCACAAAGACCTT<br>CACCGTCACCGAAAAGCCCGAAGTGATCGAC<br>GCTTCCGAACTGACGCCGGCCGTGACAACTT |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| | | | ACAAGCTCGTCATTAACGGAAAGACCCTTAA GGGCGAAACCACGACCAAGGCAGTGGACGC CGAAACTGCCGAGAAGGCGTTCAAGCAGTA CGCCAACGACAACGGCGTGGACGGAGTGTG GACTTACGATGATGCGACCAAGACGTTCACT GTGACCGAGATGGTCACCGAAGTGCCG |
| 12 | Gly/Ser Linker | Figure 2A, 205<br>Figure 3A, 305<br>Figure 5A, 505 | GGATCCGGCTCCGGATCC |
| 13 | Gly/Ser Linker | Figure 2A, 205<br>Figure 3A, 305<br>Figure 5A, 505 | GGAGGCGGAGGCAGCGGAGGCGGTGGCTCG GGAGGCGGAGGCTCG |
| 14 | Stalk<br>Rat CD2 | Figure 2A, 206<br>Figure 5A, 506 | GAGATGGTGTCCAAGCCGATGATCTACTGGG AGTGTTCCAACGCGACTCTGACCTGTGAAGT GCTGGAGGGAACCGACGTGGAACTGAAGCT GTACCAGGGTAAAGAACATCTGCGGTCGTTG CGCCAAAAGACCATGAGCTACCAGTGGACC AACTTGCGGGCGCCTTTCAAGTGCAAAGCCG TCAATAGAGTGTCCCAGGAGAGCGAAATGG AGGTCGTGAACTGCCCCGAAAAGGGACTG |
| 15 | Stalk<br>Rat CD4 | Figure 2A, 206<br>Figure 5A, 506 | TCAACTTCCATCACCGCCTACAAGAGCGAGG GAGAGAGCGCCGAGTTTTCCTTCCCCCTGAA CCTGGGCGAAGAAAGCCTCCAGGGAGAACT GCGCTGGAAGGCAGAAAAGGCCCCAAGCTC TCAGTCCTGGATCACCTTCAGCCTGAAGAAC CAGAAGGTGTCCGTGCAGAAGTCCACTTCAA ACCCGAAGTTCCAGCTCTCCGAAACCCTCCC TCTGACCCTGCAAATCCCTCAAGTGTCGCTG CAATTCGCGGGGAGCGGAAATCTGACTCTGA CTCTTGACCGGGGCATCTTGTACCAGGAGGT GAACCTGGTGGTCATGAAGGTGACCCAGCCC GATAGCAACACCCTGACCTGTGAAGTGATGG GACCCACGTCCCCGAAGATGCGGCTCATTCT GAAGCAGGAGAACCAGGAGGCTCGGGTGTC CAGACAGGAAAAGGTCATCCAAGTGCAGGC CCCGGAAGCCGGCGTGTGGCAGTGCCTGCTG TCCGAGGGAGAGGAAGTCAAGATGGACTCG AAAATCCAGGTGCTGTCCAAAGGGCTGAACC AGACTATG |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 16 | Stalk<br>Human CD22 | Figure 2A, 206<br>Figure 5A, 506 | GAAAGGCCTTTTCCACCTCATATCCAGCTCC<br>CTCCAGAAATTCAAGAGTCCCAGGAAGTCAC<br>TCTGACCTGCTTGCTGAATTTCTCCTGCTATG<br>GGTATCCGATCCAATTGCAGTGGCTCCTAGA<br>GGGGGTTCCAATGAGGCAGGCTGCTGTCACC<br>TCGACCTCCTTGACCATCAAGTCTGTCTTCAC<br>CCGGAGCGAGCTCAAGTTCTCCCCACAGTGG<br>AGTCACCATGGGAAGATTGTGACCTGCCAGC<br>TTCAGGATGCAGATGGGAAGTTCCTCTCCAA |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| | | | TGACACGGTGCAGCTGAACGTGAAGCACACC<br>CCGAAGTTGGAGATCAAGGTCACTCCCAGTG<br>ATGCCATAGTGAGGGAGGGGGACTCTGTGAC<br>CATGACCTGCGAGGTCAGCAGCAGCAACCCG<br>GAGTACACGACGGTATCCTGGCTCAAGGATG<br>GGACCTCGCTGAAGAAGCAGAATACATTCAC<br>GCTAAACCTGCGCGAAGTGACCAAGGACCA<br>GAGTGGGAAGTACTGCTGTCAGGTCTCCAAT<br>GACGTGGGCCCGGGAAGGTCGGAAGAAGTG<br>TTCCTGCAAGTGCAGTATGCCCCGGAACCTT<br>CCACGGTTCAGATCCTCCACTCACCGGCTGT<br>GGAGGGAAGTCAAGTCGAGTTTCTTTGCATG<br>TCACTGGCCAATCCTCTTCCAACAAATTACA<br>CGTGGTACCACAATGGGAAAGAAATGCAGG<br>GAAGGACAGAGGAGAAAGTCCACATCCCAA<br>AGATCCTCCCCTGGCACGCTGGGACTTATTC<br>CTGTGTGGCAGAAAACATTCTTGGTACTGGA<br>CAGAGGGGCCCGGGAGCTGAGCTGGATGTC<br>CAGTATCCTCCCAAGAAGGTGACCACAGTGA<br>TTCAAAACCCCATGCCGATTCGAGAAGGAGA<br>CACAGTGACCCTTTCCTGTAACTACAATTCC<br>AGTAACCCCAGTGTTACCCGGTATGAATGGA<br>AACCCCATGGCGCCTGGGAGGAGCCATCGCT<br>TGGGGTGCTGAAGATCCAAAACGTTGGCTGG<br>GACAACACAACCATCGCCTGCGCAGCTTGTA<br>ATAGTTGGTGCTCGTGGGCCTCCCCTGTCGC<br>CCTGAATGTCCAGTATGCCCCCCGAGACGTG<br>AGGGTCCGGAAAATCAAGCCCCTTTCCGAGA<br>TTCACTCTGGAAACTCGGTCAGCCTCCAATG<br>TGACTTCTCAAGCAGCCACCCCAAAGAAGTC<br>CAGTTCTTCTGGGAGAAAAATGGCAGGCTTC<br>TGGGGAAAGAAAGCCAGCTGAATTTTGACTC<br>CATCTCCCCAGAAGATGCTGGGAGTTACAGC<br>TGCTGGGTGAACAACTCCATAGGACAGACAG<br>CGTCCAAGGCCTGGACACTTGAAGTGCTGTA<br>TGCACCCAGGAGGCTGCGTGTGTCCATGAGC<br>CCGGGGGACCAAGTGATGGAGGGGAAGAGT<br>GCAACCCTGACCTGTGAGAGCGACGCCAACC<br>CTCCCGTCTCCCACTACACCTGGTTTGACTGG<br>AATAACCAAAGCCTCCCCTACCACAGCCAGA<br>AGCTGAGATTGGAGCCGGTGAAGGTCCAGC<br>ACTCGGGTGCCTACTGGTGCCAGGGGACCAA<br>CAGTGTGGGCAAGGGCCGTTCGCCTCTCAGC<br>ACCCTCACCGTCTACTATAGCCCGGAGACC |
| 17 | Transmembrane Domain<br>Human LAG3 | Figure 2A, 207<br>Figure 5A, 507 | GCGCCTGGAGCGCTGCCGGCCGGTCATCTGT<br>TGTTGTTCCTGACCCTGGGGGTGCTGTCACTG<br>CTGCTGCTCGTGACCGGGGCATTCGGTTTCC<br>ACCTGTGGAGAAGGCAGTGGCGGTAG |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 18 | Transmembrane Domain Human CD58 | Figure 2A, 207 Figure 5A, 507 | CATTCCCGGCACCGCTACGCGCTGATTCCGA TTCCTCTGGCCGTGATCACCACCTGTATCGTG CTCTACATGAACGGTATCCTGAAATGCGACA GAAAGCCCGACAGGACTAACAGCAATTAG |
| 19 | Transmembrane Domain Rat CD2 | Figure 2A, 207 Figure 5A, 507 | CCGCTGTACCTGATCGTGGGGGTGTCAGCCG GCGGTCTGCTGCTCGTGTTCTTCGGGGCACT GTTCATCTTCTGCATTTGCAAGAGGAAGAAG CGGTAG |
| 20 | Transmembrane Domain Human CD7 | Figure 2A, 207 Figure 5A, 507 | CCACCCCGGGCGTCCGCACTGCCGGCGCCCC CTACCGGAAGCGCGCTGCCCGATCCGCAAAC CGCCAGCGCCCTGCCTGACCCGCCCGCGGCT AGCGCCTTGCCTGCCGCACTGGCCGTGATTT CATTCCTGCTGGGTCTGGGGCTCGGGGTGGC CTGCGTGTTGGCACGGACTCAGATCAAGAAG CTGTGCTCCTGGAGAGACAAAAACTCCGCCG CCTGTGTGGTGTACGAGGACATGTCACACTC GAGGTGCAATACCCTGTCCTCGCCGAACCAG TACCAGTAG |

**Table 2 exemplary peptide sequences**

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 21 | Leader Exon1/Intron/ Exon 2/Tags $V_L$ Leader Exon | | MGWSCIMLFLAATATGVHSQVQL |
| 22 | Leader Exon1/Intron/ Exon 2/Tags FLAG+Myc Tags | | DYKDDDDKGSGEQKLISEEDL |
| 23 | Leader Exon1/Intron/ Exon 2/Tags $V_H$ Leader Exon | | MDIRAPAQFLGILLLWFPGARCDIQM |
| 24 | Ig-Binding Domain 2-Domain Protein G | Figure 2B, 208 | GTPAVTTYKLVINGKTLKGETTTKAVDAETAE KAFKQYANDNGVDGVWTYDDATKTFTVTEV NTPAVTTYKLVINGKTLKGETTTKAVDAETAE KAFKQYANDNGVDGVWTYDDATKTFTVTE |
| 25 | Ig-Binding Domain 3-Domain Protein G | Figure 2B, 208 | IDEILAALPKTDTYKLILNGKTLKGETTTEAVD AATAEKVFKQYANDNGVDGEWTYDDATKTF TVTEKPEVIDASELTPAVTTYKLVINGKTLKGE TTTEAVDAATAEKVFKQYANDNGVDGEWTY DDATKTFTVTEKPEVIDASELTPAVTTYKLVIN GKTLKGETTTKAVDAETAEKAFKQYANDNGV DGVWTYDDATKTFTVTE |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 26 | Ig-Binding Domain 2-Domain Protein A + 2-Domain Protein G | Figure 2B, 208 | VDNKFNKEQQNAFYEILHLPNLNEEQRNAFIQS LKDDPSQSANLLAEAKKLNDAQAPKVDNKFN KEQQNAFYEILHLPNLNEEQRNAFIQSLKDDPS QSANLLAEAKKLNDAQAPKVDGSGSGSGTPA VTTYKLVINGKTLKGETTTKAVDAETAEKAFK QYANDNGVDGVWTYDDATKTFTVTEVNTPA VTTYKLVINGKTLKGETTTKAVDAETAEKAFK QYANDNGVDGVWTYDDATKTFTVTE |
| 27 | Ig-Binding Domain 5-Domain Protein A + 4-Domain Protein G | Figure 2B, 208 | ANAAQHDEAQQNAFYQVLNMPNLNADQRNG FIQSLKDDPSQSANVLGEAQKLNDSQAPKADA QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQ SLKDDPSQSTNVLGEAKKLNESQAPKADNNFN KEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPS QSANLLSEAKKLNESQAPKADNKFNKEQQNAF YEILHLPNLNEEQRNGFIQSLKDDPSQSANLLA EAKKLNDAQAPKADNKFNKEQQNAFYEILHLP NLTEEQRNGFIQSLKDDPSVSKEILAEAKKLND AQAPKEEDNNKPGDPRISEATDGLSDFLKSQTP AEDTVKSIELAEAKVLANRELDKYGVSDYYKN LINNAKTVEGVKALIDEILAALPKTDTYKLILN GKTLKGETTTEAVDAATAEKVFKQYANDNGV DGEWTYDDATKTFTVTEKPEVIDASELTPAVT |
| | | | TYKLVINGKTLKGETTTEAVDAATAEKVFKQY ANDNGVDGEWTYDDATKTFTVTEKPEVIDASE LTPAVTTYKLVINGKTLKGETTTKAVDAETAE KAFKQYANDNGVDGVWTYDDATKTFTVTEM VTEVP |
| 28 | Gly/Ser Linker | Figure 2B, 209 | GSGSGS |
| 29 | Gly/Ser Linker | Figure 2B, 209 | GGGGSGGGGSGGGGS |
| 30 | Stalk Rat CD2 | Figure 2B, 210 | EMVSKPMIYWECSNATLTCEVLEGTDVELKLY QGKEHLRSLRQKTMSYQWTNLRAPFKCKAVN RVSQESEMEVVNCPEKGL |
| 31 | Stalk Rat CD4 | Figure 2B, 210 | STSITAYKSEGESAEFSFPLNLGEESLQGELRW KAEKAPSSQSWITFSLKNQKVSVQKSTSNPKFQ LSETLPLTLQIPQVSLQFAGSGNLTLTLDRGILY QEVNLVVMKVTQPDSNTLTCEVMGPTSPKMR LILKQENQEARVSRQEKVIQVQAPEAGVWQCL LSEGEEVKMDSKIQVLSKGLNQTM |
| 32 | Stalk Human CD22 | Figure 2B, 210 | MKVTQPDSNTLTCEVMGPTSPKMRLILKQENQ EARVSRQEKVIQVQAPEAGVWQCLLSEGEEVK MDSKIQVLSKGLNQTM |
| 33 | Transmembrane Domain Human LAG3 | Figure 2B, 211 | APGALPAGHLLLFLTLGVLSLLLLVTGAFGFHL WRRQWR |
| 34 | Transmembrane Domain Human CD58 | Figure 2B, 211 | HSRHRYALIPIPLAVITTCIVLYMNGILKCDRKP DRTNSN |

(continued)

| SEQ ID No. | Description | Corresponding Structure | Sequence |
|---|---|---|---|
| 35 | Transmembrane Domain Rat CD2 | Figure 2B, 211 | PLYLIVGVSAGGLLLVFFGALFIFCICKRKKR |
| 36 | Transmembrane Domain Human CD7 | Figure 2B, 211 | PPRASALPAPPTGSALPDPQTASALPDPPAASA LPAALAVISFLLGLGLGVACVLARTQIKKLCSW RDKNSAACVVYEDMSHSRCNTLSSPNQYQ |

**Table 3-GPI anchor sequences**

| SEQ ID No. | Description | Sequence |
|---|---|---|
| 37 | Human CD59 | GAATTCCTTGAAAATGGTGGGACATCCTTATCAGAGAAAACAG TTCTTCTGCTGGTGACTCCATTTCTGGCAGCAGCCTGGAGCCTTC ATCCC |
| 38 | Human CD59 | EFLENGGTSLSEKTVLLLVTPFLAAAWSLHP |
| 39 | Human CD24 | ACCAATGCCACAACAAAGGCAGCAGGGGGAGCACTCCAGTCAA CAGCAAGTTTGTTTGTCGTGTCACTGAGTCTCTTGCATCTTTATT CA |
| 40 | Human CD24 | TNATTKAAGGALQSTASLFVVSLSLLHLYS |
| 41 | Human CNTN1 (Contactin 1) | GTCTCCCAGGTGAAAATTTCAGGAGCCCCTACCCTCTCCCCATC CCTCCTGGGTTTGCTGCTGCCCGCCTTTGGCATTCTCGTGTATCT GGAGTTC |
| 42 | Human CNTN1 (Contactin 1) | VSQVKISGAPTLSPSLLGLLLPAFGILVYLEF |

**Table 4-Example 4 sequences**

| SEQ ID No. | Description | Sequence |
|---|---|---|
| 43 | $V_L$ Leader exon 1 | |
| 44 | $V_L$ intron | ATGGAATCACAGACCCAGGTCCTCATGTTTCTTCTGCTCTGGGT ATCTG GTAAGAAATTTAAAGTATTAAAACCTTTTCAAAGTTTCATCTTT GTGGTAAGAAATTTGCAATATGTGCCAGTGTGTAATATTTCTTA CATAATAAATTTGTGACAGTATGATAAGGACATTTAAATGAAA AATTTCGACTGTTGTTATAATCTATGTCTGTGTATCTATGAATTT TCACTGCCTATTAATTATTACAG |
| 45 | $V_L$ exon 2 end of VL leader sequence | GTGCCTGTGCA |

(continued)

| SEQ ID No. | Description | Sequence |
|---|---|---|
| 46 | $V_L$ exon 2 | GACATTCAGATGACCCAGTCTCCATCCTCCATGTCTGTGTCTCT GGGAGACACAGTCACTATTACTTGCCGGGCAAGTCAGGACGTT GGGATTTATGTAAACTGGTTCCAGCAGAAACCAGGGAAATCTC CTAGGCGTATGATTTATCGTGCAACGAACTTGGCAGATGGGGTC CCATCAAGGTTCAGCGGCAGTAGGTCTGGATCAGATTATTCTCT CACCATCAGCAGCCTGGAGTCTGAAGATGTGGCAGACTATCAC TGTCTACAGTATGATGAGTATCCATTCACGTTCGGATCCGGGAC GAAGTTGGAAATAAAACGG |
| 47 | $V_L$ exon 2 linker | GGAGGCGGAGGCAGCGGAGGCGGTGGCTCGGGAGGCGGAGGC TCG |
| 48 | $V_H$ exon 2 | CAGGTACAGCTGAAAGAGTCAGGACCTGGTCTGGTGCAGCCCT CACAGACCCTGTCTCTCACCTGCACTGTCTCTGGACTCTCATTA ATCAGTTATGGTGTAAGTTGGGCTCGCCAGCCTCCAGGGAAGG GTCTGGAGTGGATTGCAGCAATATCAAGTGGTGGAAGCACATA TTATAATTCAGTTCTCACATCTCGACTGAGCATCAGCAGGGACA |
| | | CCTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCAAACT GAAGACACAGCCATTTACTTCTGTACCAGAGAACTCTGGGACTA CTATGATTACTGGGGCCAAGGAGTCATGGTCACAGTCTCCTCA |
| 49 | Exon 2-Rat IgG1 Fc | GCTGAAACAACAGCCCCCAGAAACCCGGGAGGTGATTGCAAGC CTTGTATATGTACAGGCTCAGAAGTATCATCTGTCTTCATCTTCC CCCCAAAGCCCAAAGATGTGCTCACCATCACTCTGACTCCTAAG GTCACGTGTGTTGTGGTAGACATTAGCCAGGACGATCCCGAGGT CCATTTCAGCTGGTTTGTAGATGACGTGGAAGTCCACACAGCTC AGACTCGACCACCAGAGGAGCAGTTCAACAGCACTTTCCGCTC AGTCAGTGAACTCCCCATCCTGCACCAGGACTGGCTCAATGGCA GGACGTTCAGATGCAAGGTCACCAGTGCAGCTTTCCCATCCCCC ATCGAGAAAACCATCTCCAAACCCGAAGGCAGAACACAAGTTC CGCATGTATACACCATGTCACCTACCAAGGAAGAGATGACCCA GAATGAAGTCAGTATCACCTGCATGGTAAAAGGCTTCTATCCCC CAGACATTTATGTGGAGTGGCAGATGAACGGGCAGCCACAGGA AAACTACAAGAACACTCCACCTACGATGGACACAGATGGGAGT TACTTCCTCTACAGCAAGCTCAATGTGAAGAAGGAAAAATGGC AGCAGGGAAACACGTTCACGTGTTCTGTGCTGCATGAAGGCCT GCACAACCACCATACTGAGAAGAGTCTCTCCCACTCCCCCGGT |
| 50 | Exon 2 part of mouse MHC I ($H2K^b$) transmembrane domain | AAAGAGCCTCCTCCATCCACTGTCTCCAACATGGCGACCGTTGC TGTTCTGGTTGTCCTTGGAGCTGCAATAGTCACTGGAGCTGTGG TGGCTTTTGTGATGAAGATGAGAAGGAGAAACACAG |
| 51 | Intron | GTAGGAAAGGGCAGAGTCTGAGTTTTCTCTCAGCCTCCTTTAGA GTGTGCTCTGCTCATCAATGGGGAACACAGGCACACCCCACATT GCTACTGTCTCTAACTGGGTCTGCTGTCAGTTCTGGGAACTTCCT AGTGTCAAGATCTTCCTGGAACTCTCACAGCTTTTCTTCTCACA G |

(continued)

| SEQ ID No. | Description | Sequence |
|---|---|---|
| 52 | Exon 3-part of mouse MHC I ($H2K^b$) transmembrane domain | GTGGAAAAGGAGGGGACTATGCTCTGGCTCCAG |
| 53 | Intron | GTTAGTGTGGGGACAGAGTTGTCCTGGGGACATTGGAGTGAAG TTGGAGATGATGGGAGCTCTGGGAATCCATAATAGCTCCTCCAG AGAAATCTTCTAGGTGCCTGAGTTGTGCCATGAAATGAATATGT ACATGTACATATGCATATACATTTGTTTTGTTTTACCCTAG |
| 54 | Exon 4 - end of mouse MHC I ($H2K^b$) transmembrane domain | GCTCCCAGACCTCTGATCTGTCTCTCCCAGATTGTAAAGGTGAC ACTCTAGGGTCTGATTGGGGAGGGGCAATGTGGACATGA |
| 55 | $V_L$ leader | MESQTQVLMFLLLWVSGACA |
| 56 | $V_L$ | DIQMTQSPSSMSVSLGDTVTITCRASQDVGIYVNWFQQKPGKSPRR MIYRATNLADGVPSRFSGSRSGSDYSLTISSLESEDVADYHCLQYD EYPFTFGSGTKLEIKR |
| 29 | Linker | GGGGSGGGGSGGGGS |
| 57 | $V_H$ | QVQLKESGPGLVQPSQTLSLTCTVSGLSLISYGVSWARQPPGKGLE WIAAISSGGSTYYNSVLTSRLSISRDTSKSQVFLKMNSLQTEDTAIY FCTRELWDYYDYWGQGVMVTVSS |
| 58 | Rat IgG1 Fc | AETTAPRNPGGDCKPCICTGSEVSSVFIFPPKPKDVLTITLTPKVTCV VVDISQDDPEVHFSWFVDDVEVHTAQTRPPEEQFNSTFRSVSELPIL HQDWLNGRTFRCKVTSAAFPSPIEKTISKPEGRTQVPHVYTMSPTK EEMTQNEVSITCMVKGFYPPDIYVEWQMNGQPQENYKNTPPTMD TDGSYFLYSKLNVKKEKWQQGNTFTCSVLHEGLHNHHTEKSLSHS PG |
| 59 | Mouse MHC I ($H2K^b$) transmembrane domain | KEPPPSTVSNMATVAVLVVLGAAIVTGAVVAFVMKMRRRNTGGK GGDYALAPGSQTSDLSLPDCKGDTLGSDWGGAMWT |

**[0102]** All examples recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art.

## REFERENCES CITED IN THE DESCRIPTION

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PATENTKRAV

1. Genmodificeret gnaver, som omfatter antistofsecernerende celler, der omfatter en exogen nukleinsyresekvens, som omfatter
   (i) en promotor, der udtrykkes i antistofsecernerende celler, og
   (ii) en nukleinsyresekvens, der koder for et immunoglobulin-indfangningsmolekyle, der omfatter en celleoverfladeforankringsdel og en immunoglobulinbindingsdel,
   hvor promotoren er operabelt koblet til nukleinsyresekvensen, der koder for immunoglobulin-indfangningsmolekylet,
   hvor celleoverfladeforankringsdelen omfatter et transmembranpeptid, og hvor immunoglobulinbindingsdelen er valgt blandt:
   a) et eller flere bakterielle proteiner, der naturligt har affinitet til den konstante region af en tung eller let kæde af et immunoglobulin;
   (b) et eller flere variable domæner af et immunoglobulin, der har affinitet til en hvilken som helst del af et andet immunoglobulin; eller
   (c) et enkeltkædet variabelt fragment mod en konstant region af en tung eller let kæde af et immunoglobulin;
   hvor de antistofsecernerende celler af den genmodificerede gnaver danner immunoglobulin-indfangningsmolekylerne, der kan binde, fastholde og fremvise endogent dannede immunoglobulinmolekyler på en celleoverflade af de antistofsecernerende celler.

2. Genmodificeret gnaver ifølge krav 1, hvor de antistofsecernerende celler udtrykker immunoglobulin-indfangningsmolekylet konstitutivt eller kan induceres til at udtrykke immunoglobulin-indfangningsmolekylet *in vivo*, *in vitro* eller *ex vivo*.

3. Genmodificeret gnaver ifølge krav 1, hvor gnaveren er en mus eller en rotte.

4. Genmodificeret gnaver ifølge krav 1, hvor det bakterielle protein er protein A og/eller protein G.

5. Genmodificeret gnaver ifølge krav 1, hvor det bakterielle protein er valgt blandt protein A fra *Staphylococcus aureus*, protein G fra gruppe C og G *Streptococci*, protein H fra *Streptococcus pyogenes* og protein L fra *Peptostreptococcus magnus*.

6. Genmodificeret gnaver ifølge krav 1, hvor den antistofsecernerende celle er en celle, der er differentieret fra en antigen-erfaren B-celle.

7. Genmodificeret gnaver ifølge krav 1, hvor transmembranpeptidet er valgt blandt human lymfocytaktiveringsgen 3, human CD58 og human CD7.

8. Antistofsecernerende celle, der udtrykker et membranbundet immunoglobulin-indfangningsmolekyle, der kan binde, fasthold og fremvise endogent dannede immunoglobulinmolekyler på dens celleoverflade, hvilken antistofsecernerende celle omfatter:

(A) en nukleinsyre, der koder for et endogent dannet immunoglobulin, og

(B) en nukleinsyrevektor, der omfatter:

(i) en promotor, der udtrykkes i antistofsecernerende celler, og

(ii) en nukleinsyresekvens, der koder for immunoglobulin-indfangningsmolekylet, der omfatter en immunoglobulinbindingsdel og en celleoverfladeforankringsdel,

hvor promotoren er operabelt koblet til nukleinsyresekvensen, der koder for immunoglobulin-indfangningsmolekylet, og

hvor celleoverfladeforankringsdelen omfatter et transmembranpeptid, og

hvor immunoglobulinbindingsdelen er valgt blandt:

(a) et eller flere bakterielle proteiner, der naturligt har affinitet til den konstante region af en tung eller let kæde af et immunoglobulin;

(b) et eller flere variable domæner af et immunoglobulin, der har affinitet til en hvilken som helst del af et andet immunoglobulin; eller

(c) et enkeltkædet variabelt fragment mod en konstant region af en tung eller let kæde af et immunoglobulin.

9. Antistofsecernerende celle ifølge krav 8, som omfatter en vektor til ekspression af immunoglobulin-indfangningsmolekylet, hvor vektoren omfatter:

a) nukleinsyresekvenser, der koder for et signalpeptid og en stængelstruktur; eller

b) nukleinsyresekvenser, der koder for et signalpeptid, en stængelstruktur og et reporterpeptid koblet til en IRES-sekvens eller picornavirus 2A ribosomal skip-sekvens.

10. Antistofsecernerende celle ifølge krav 8, hvor promotoren er en inducerbar eller konstitutiv promotor.

11. Antistofsecernerende celle ifølge krav 10, hvor promotoren er valgt blandt: (a) en promotor, der driver ekspression af et gen valgt blandt B-lymfocytinduceret modningsprotein 1 (Blimp1), syndecan 1 (Sdc1), tumornekrosefaktorreceptor-

superfamiliemedlem 17 (Tnfrsf17) og fucosyltransferase 1 (Fut1), (b) en tetracyklin-inducerbar promotor; eller (c) en tamoxifen-inducerbar promotor.

12. Antistofsecernerende celle ifølge krav 8, hvor cellen fremviser endogent dannede immunoglobulinmolekyler, der er bundet af immunoglobulin-indfangningsmolekylet på dens overflade.

13. Anvendelse af den antistofsecernerende celle ifølge et hvilket som helst af kravene 8 til 11 til storskalaproduktion af antistoffer.

# DRAWINGS

Drawing

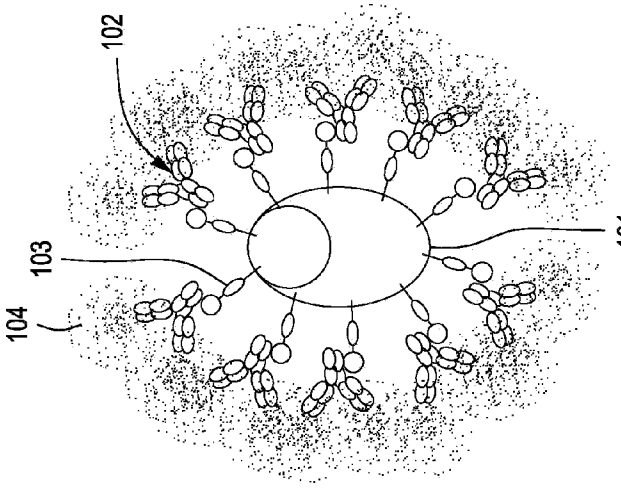


FIG. 1C

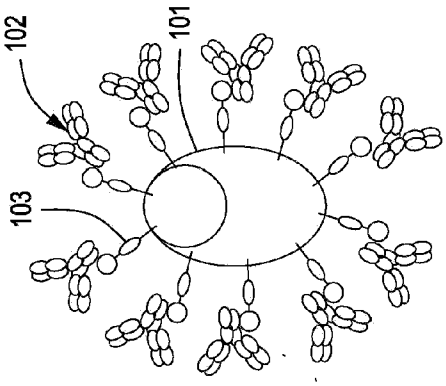


FIG. 1B

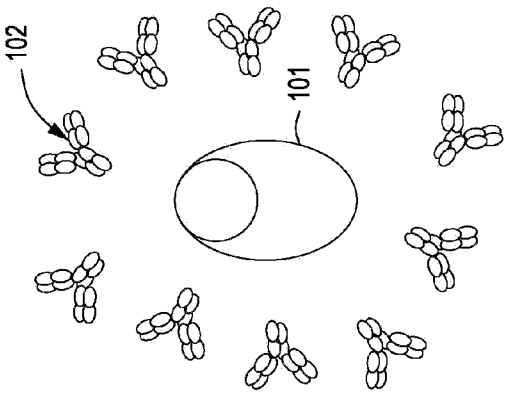


FIG. 1A

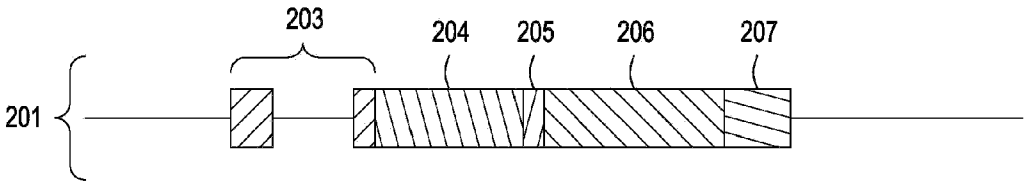


FIG. 2A

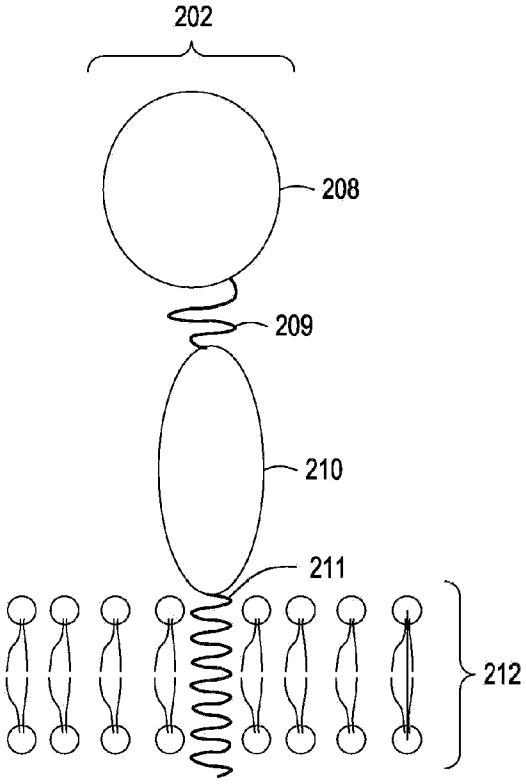


FIG. 2B

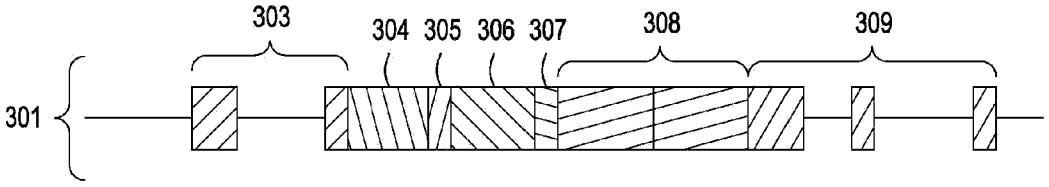
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FIG. 3A

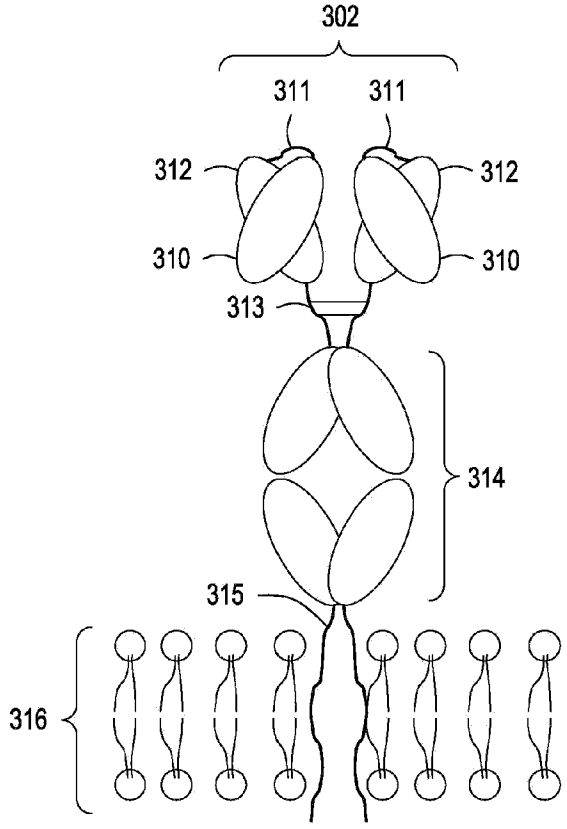
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FIG. 3B

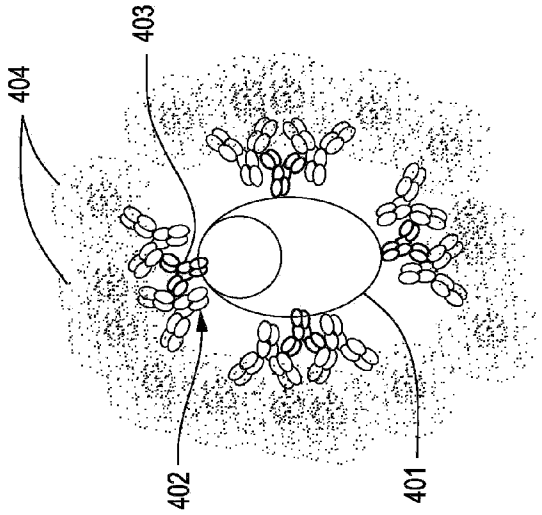


FIG. 4C

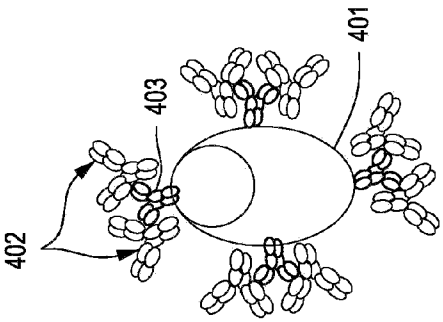


FIG. 4B

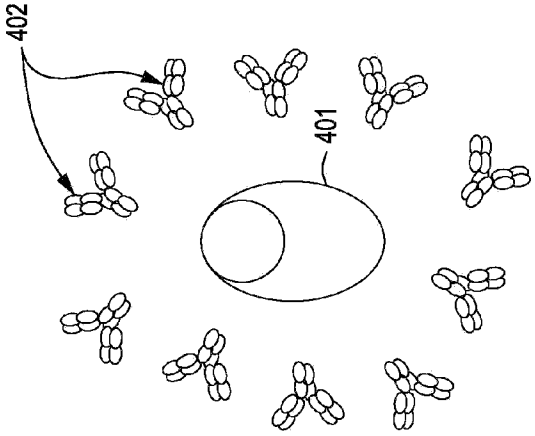


FIG. 4A

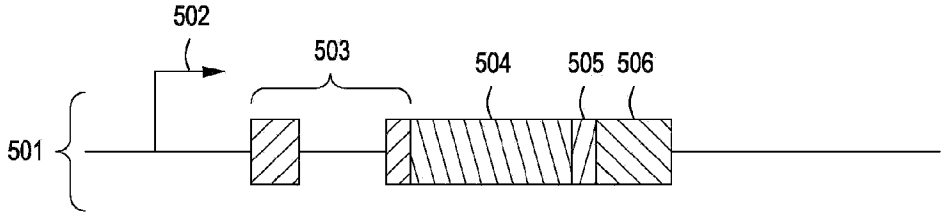
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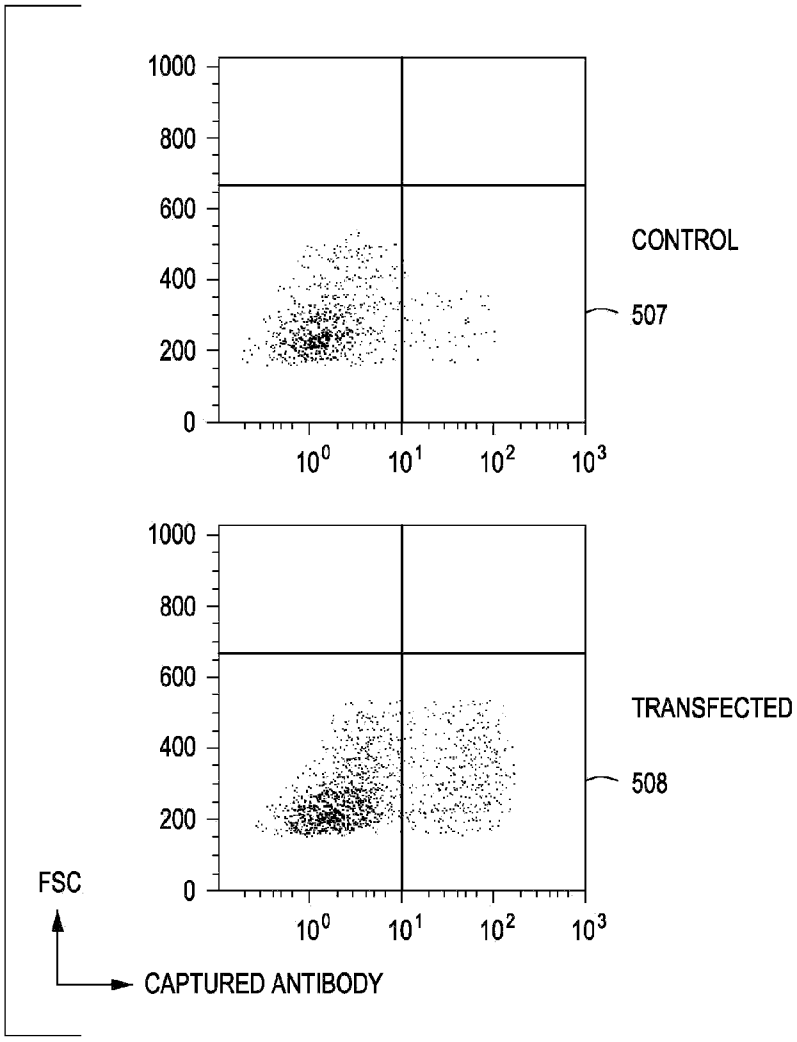
FIG. 5A

FIG. 5B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TRANSFECTED
508
FSC
CAPTURED ANTIBODY

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The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.