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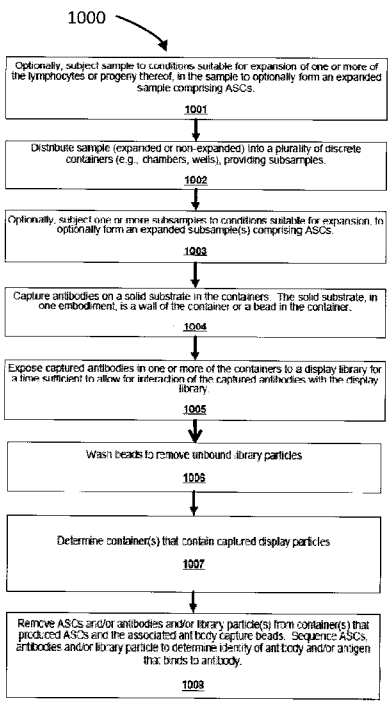
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(54) Title: HIGHLY PARALLEL ASSAYS FOR SIMULTANEOUS IDENTIFICATION OF ANTIBODY SEQUENCES AND BINDING PARTNERS

FIGURE 1



(57) Abstract: The present invention relates to methods for isolating the sequences of an antibody that reacts with a disease related antigen, e.g., an autoantigen, without knowing the identity of the antigen (sequence or structural epitope) a priori. The methods can also be used to identify an antigen that mediates a disease state, e.g., an autoantigen implicated in an autoimmune disorder or a tumor response.

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HIGHLY PARALLEL ASSAYS FOR SIMULTANEOUS IDENTIFICATION OF ANTIBODY SEQUENCES AND BINDING PARTNERS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority from U.S. Provisional Application Serial No. 62/270,094, filed December 21, 2016, the contents of which are incorporated herein in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] The adaptive immune response produces a diversity of different antibodies (on the order of 10^{11} unique sequences in a single individual), each of which is capable of binding to a different target epitope. The isolation of antibodies that bind to a specific target, let alone a specific epitope on a known target, is of high interest for the development of new products in therapeutics, diagnostics, and more generally, for research purposes. Methods for identifying such antibodies have been previously described and include hybridoma approaches, display library approaches, B-cell immortalization, B-cell cloning, and single cell screening methods. For each of these methods, it is required that the target of interest, whether it is a simple target such as a protein or a complex target such as a specific cell type, is identified and has been produced in order to perform the appropriate selections.

[0003] A much more challenging problem in antibody discovery is presented when it is known, or suspected, that an antibody response is present and is biologically relevant, but the identity of the target (and consequently, target epitope) is not known, and could be a single target selected from hundreds to tens of thousands of different targets. For example, the targets of autoantibodies are not all known; and in many instances, only a small number have been identified. In diseases and conditions in which autoantibodies are generated (e.g., autoimmune disease, cancer), the adaptive immune system escapes "tolerance", the internal checks and balances that would normally prevent the generation of antibodies that bind to self-antigens, and results in chronic inflammation, fibrosis, progressive tissue damage or a combination of the foregoing. Examples of such disorders include multiple sclerosis, myasthenia gravis, diabetes, lupus, rheumatoid arthritis, traumatic brain injury, among others. In some of these diseases, autoantigens that are associated with pathology, or which are prognostic of disease occurrence

and/or disease progression have also been discovered. Further, even in the case of two patients that make autoantibodies against the same target, the sequences of these antibodies will be different. In addition, an analogous problem to autoimmune conditions occurs in cases where patients have received tissues or blood from a donor, mount an immune response against this imperfectly matched tissue, but the exact identity of the antigen responsible for the immune response is not known. As such, it is of great interest to identify autoantibodies and their respective antigens.

[0004] The present invention addresses this need, by providing methods for identifying such autoantigens and autoantibodies.

SUMMARY OF THE INVENTION

[0005] In one aspect of the invention, a method is provided for determining the sequence of an antibody and its respective binding partner, e.g., antigen such as an autoantigen. The method entails optionally subjecting a sample to conditions suitable for expansion of one or more of the lymphocytes to form an expanded sample comprising antibody secreting cells (ASCs) and distributing the sample into containers to provide a plurality of subsamples. The containers are, in one embodiment, chambers of a microfluidic device. In another embodiment, the containers are open wells of an array of microfabricated volumes or the wells of a microwell plate or microfuge tubes. Optionally, one or more subsamples are subjected to conditions suitable for expansion of one or more ASCs or ASC precursor cells in the one or more subsamples to form one or more expanded subsamples comprising ASCs. Antibodies from one or more of the subsamples (expanded or non-expanded) are captured on a solid substrate in the respective container(s) to provided captured antibodies. The captured antibodies in one or more of the containers are exposed to an antigen display library comprising a plurality of display particles for a time sufficient to allow for interaction of one or more of the captured antibodies with the one of more of the display particles. Display particles are captured that interact with one or more of the captured antibodies. Unbound display particles are removed via a wash step in some embodiments. However, in another embodiment, a wash step is not employed.

[0006] ASCs, antibodies, display particles or a combination thereof are removed from the container(s) that contain captured display particles, and subjected to sequencing reactions. The

results of the sequencing reactions determine the identity of the antibody and/or its respective antigen binding partner. In one embodiment, prior to sequencing of container contents, a determination is first made as to which containers include captured display particles. For example, in one embodiment, the contents of each of the containers are sequenced followed by a determination of the containers that contain both antibody and antigen, as well as the sequences of the antibody and antigen. In another embodiment, containers are visualized via a detection reagent to determine whether it includes bound display particles. The detection reagent can be an additional reagent, e.g., a fluorescently labeled secondary antibody that binds to a display particle. Alternatively, the display particle serves as the detection reagent. For example, the display particle in one embodiment is a fluorescently labeled display particle.

[0007] In another aspect, a method is provided for determining the sequence of an antibody and its respective binding partner, e.g., antigen such as an autoantigen. The method entails optionally subjecting a sample to conditions suitable for expansion to form an expanded sample comprising antibody secreting cells (ASCs) and distributing the sample into containers to provide a plurality of subsamples. The containers are in one embodiment, chambers of a microfluidic device. In another embodiment, the containers are open wells of a microfabricated array of receptacles or a microwell plate or microfuge tubes. Optionally, one or more subsamples are subjected to conditions suitable for expansion of one or more ASCs or ASC precursor cells in the one or more subsamples to form one or more expanded subsamples comprising ASCs. A functional assay is performed on one or more of the subsamples, wherein the functional assay measures a property of an antibody in the one or more subsamples. Based on the results of the functional assay, one or more functional subsamples that include one or more functional antibodies are identified. Antibodies secreted by ASCs in the one or more functional subsamples are captured on a solid substrate to provide captured antibodies. Antibody capture can be accomplished in the same container as the functional assay, or a different container. For example, a functional subsample can be transferred to a different container to perform antibody capture or alternatively transferred into a plurality of different containers to create multiple subpopulations of the transferred ASCs prior to performing the antibody capture. The captured antibodies in the containers comprising the functional subsamples are exposed to an antigen display library comprising a plurality of display particles for a time sufficient to allow for interaction of one or more of the captured antibodies with the one of more of the display

particles. Display particles are captured that interact with one or more of the captured antibodies. In one embodiment, unbound display particles are removed via a wash step. However, in another embodiment, a wash step is not employed.

[0008] ASCs, antibodies, display particles or a combination thereof are removed from container(s) that contain captured display particles, and subjected to sequencing reactions. The results of the sequencing reactions determine the identity of the antibody and/or its respective antigen binding partner. In one embodiment, prior to sequencing of container contents, a determination is first made as to which containers include captured display particles. In one embodiment, the contents of each of the containers are sequenced followed by a determination of the containers that contain both antibody and antigen, as well as the sequences of the antibody and antigen. In another embodiment, containers are visualized via a detection reagent to determine whether it includes a bound display particle, prior to sequencing. The detection reagent can be an additional reagent, e.g., a fluorescently labeled secondary antibody that binds to a display particle. Alternatively, the display particle serves as the detection reagent. For example, the display particle in one embodiment is a fluorescently labeled display particle. ASCs, antibodies, display particles or a combination thereof are removed from container(s) that contain captured display particles, and subjected to sequencing reactions. The results of the sequencing reactions determine the identity of the antibody and/or its respective antigen binding partner.

[0009] In one embodiment, the sample subjected to the methods provided herein is from a patient with an autoimmune disorder. In a further embodiment, the autoimmune disorder is one of the disorders set forth in Table 1. In a further embodiment, the sample subjected to the methods provided herein is from a patient exhibiting an immune response to a transplanted organ or tissue. In one embodiment, the sample subjected to the methods provided herein is from a patient that has been exposed to an infectious agent. In another embodiment, the sample subjected to the methods provided herein is from a healthy patient. In another embodiment, the sample is derived from an animal that has been exposed to one or more of the antigens represented in the display library, or that is suspected of having mounted an immune response against one or more antigens represented in the display library.

[0010] In one embodiment, the solid substrate used in the methods provided herein is a functionalized bead or a functionalized container surface. In a further embodiment, the functionalized container surface is the surface of a microfluidic chamber or a microwell. The functionalized surface in one embodiment is a protein A or a protein G functionalized surface. In another embodiment, the functionalized surface is a surface functionalized with a polyclonal antibody against human immunoglobulin or against immunoglobulin of an animal being analyzed. In another embodiment, the functionalized surface is a surface functionalized with a monoclonal antibody against human immunoglobulin. In another embodiment, the functionalized surface is a surface functionalized with a moiety that binds to human immunoglobulins of one or more isotype.

[0011] In yet another aspect of the invention, a method is provided to enable the screening of libraries of antibodies against one or more cell-surface antigens without the high background that is obtained when panning antibody display libraries against cells. The method comprises enriching an antibody display library for binding to one or more targets of interest (one or more cell-surface antigens) to provide an enriched antibody display library. The enriched antibody display library is transferred into bacteria, yeast, or a mammalian cell line engineered to express the antibodies present in the enriched antibody display library to produce populations of antibody secreting cells (ASCs) that secrete enriched-for antibodies that are derived from the original display library. The ASCs are distributed into a plurality of containers to provide ASC subsamples. Antibodies secreted by the ASC subsamples are interacted with particles (e.g., cells) that express the one or more targets of interest. A determination is then made as to the nucleic acid sequence of antibody(ies) or antigen binding fragment portions thereof that interacts with the one or more targets of interest. For example, if a secreted antibody interacts with the one or more targets of interest, which can be determined, e.g., by a fluorescence assay, the container contents are harvested for downstream sequencing.

BRIEF DESCRIPTION OF THE FIGURES

[0012] Figure 1 is a flow chart showing one aspect of the present invention (1000), *i.e.*, a method for determining the sequence of an antibody and/or its respective antigen *a priori*.

[0013] Figure 2 is a flow chart showing a second aspect of the present invention (2000), *i.e.*, a method for determining the sequence of an antibody and/or its respective antigen *a priori*.

[0014] Figure 3 is a flow chart of a third aspect of the present invention (3000).

[0015] Figure 4 is an optical micrograph of a microfluidic valve fabricated by multilayer soft lithography.

DETAILED DESCRIPTION OF THE INVENTION

[0016] As used herein, "an antibody" is a polypeptide belonging to the immunoglobulin superfamily. In particular, "an antibody" includes an immunoglobulin molecule or an immunologically active fragment of an immunoglobulin molecule (i.e., a molecule(s) that contains an antigen binding site), an immunoglobulin heavy chain (alpha (α), mu (μ), delta (δ) or epsilon (ε)) or a variable domain thereof (V_H domain), an immunoglobulin light chain (kappa (κ) or lambda (λ)) or a variable domain thereof (V_L domain), or a polynucleotide encoding an immunoglobulin molecule or an immunologically active fragment of the immunoglobulin molecule. The antibody includes a single chain antibody (e.g., an immunoglobulin light chain or an immunoglobulin heavy chain), a single-domain antibody, an antibody variable fragment (F_v), a single-chain variable fragment (scFv), an scFv-zipper, an scFv-Fc, a disulfide-linked Fv (sdFv), a Fab fragment (e.g., C_LV_L or C_HV_H), a F(ab') fragment, an epitope-binding fragment of any of the above (i.e., one or more antigen binding fragments) and a polynucleotide (DNA or RNA) encoding any of the above (including a polynucleotide library encoding a plurality of antibodies). Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclasss. The antibody may be synthetic or from any animal origin including birds and mammals (e.g., human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, llama, horse, or chicken), and/or humanized versions thereof. The antibody includes a polyclonal antibody, a monoclonal antibody, a human antibody, a humanized antibody, a nanobody, a chimeric antibody, a single chain antibody, a heavy chain

antibody (i.e., an antibody consisting of two heavy chains), an anti-idiotypic (anti-Id) antibody, a minibody (mini-antibody), a light chain antibody (i.e., an antibody consisting of two light chains) and a diabody, a fragment of any of the aforementioned or a polynucleotide encoding any of the aforementioned. "Antibody" can be surface bound or non-surface bound, and if surface bound, includes the surface to which it is bound (e.g., a solid support or a cell). The antibody also includes any of the aforementioned immunoglobulin molecules, or fragments thereof, complexed or bound to one or other molecules (e.g., one or antigens). A cell that secretes antibodies is referred to herein as an "antibody secreting cell" (ASC).

[0017] Specifically, an "antibody secreting cell" or "ASC," as used herein, refers to any cell type that produces and secretes an antibody. Plasma cells (also referred to as "plasma B-cells," "plasmocytes" and "effector B-cells") are terminally differentiated, and are one type of ASC. ASCs include, for example, activated memory B-cells, plasmablasts, cells generated through the expansion of memory B-cells, cell lines that express recombinant monoclonal antibodies and hybridoma cell lines. Typically, each ASC has a diameter of from about 7 μm to about 15 μm, depending on source, a diameter approximately $1/10^{th}$ the width of a human hair, and generates only a minute amount of antibody. When analyzed in the volume of conventional formats this small amount of antibody is typically too dilute, making it difficult to analyze its properties by the majority of existing methods. For this reason, antibody discovery typically requires that each ASC be isolated, fused to an immortal cancer cell to create a hybridoma and "grown," ultimately generating many thousands of identical cells that can produce enough antibody to be measured. For example, see McCullough and Spier (1990). Monoclonal Antibodies in Biotechnology: Theoretical and Practical Aspects, Chapter 2, Cambridge University Press, incorporated by reference herein in its entirety). This process is not only inefficient (99% of the starting immune cells are lost) but also very slow and expensive, requiring at minimum 3 months of labor before therapeutic function can be tested.

[0018] In various aspects of the invention, the methods described herein provide a means to simultaneously screen a panel of antibodies in a plurality of different containers (e.g., from about 100 to about 10,000,0000 different containers, each containing from about 0 to about 10,000 unique ASCs), each secreted by a different cell or subset of cells, against a library of possible target epitopes (e.g., a library of from about 10 to about 10^{12} unique target antigen epitopes). The

methods provided herein can be used in various embodiments, (i) to isolate high-value antibodies from one or more libraries, (ii) to understand antibody-antigen interactions, (iii) to optimize antibody sequence, (iv) to evaluate epitope binding properties of antibodies, (v) to screen antibodies expressed and secreted by engineered mammalian cells, (vi) to isolate antibodies from immunized animals or animals exposed to one or more members of the display library, or (vii) a combination thereof. For example, in one embodiment, the methods provided herein are used in the context of infectious diseases to find antibodies against a virus or bacterium without knowing *a priori* the target antigen(s). In another embodiment, the methods provided herein are carried out in the context of autoimmune disease or cancer, to find novel antibody(s) and/or novel antigen(s) associated with the respective autoimmune disease or cancer. In another embodiment, the methods described herein are used to isolate antibodies associated with a functional response that have been produced by an animal immunized with one or more antigens. As there is evidence to suggest that different types of cancer trigger unique autoantibody signatures that reflect the nature of the malignant process in the affected organ, a need exists for methods to identify autoantibody signatures (Casiano et al. (2006). Molecular & Cellular Proteomics 5.10, pp. 1745-1759, incorporated by reference herein in its entirety).

[0019] The value of finding new antigens, e.g., autoantigens is multifold. For example, the methods provided herein have the potential to provide the following benefits: (i) improved understanding of disease progression, e.g., autoimmune disease and cancer (tumor response, metastasis, etc.); (ii) improved methods and tests for diagnosis of autoimmune disease; (iii) identification of therapeutic targets for the treatment of for example, cancer and/or autoimmune disease (iv) informing the design of therapeutics that block the association of autoantibodies by mimicking the epitopes involved; and (v) stratification of patients based on autoimmune reactivity, (vi) the design and evaluation of responses to vaccines in humans and animals. Similarly, newly discovered antibodies that react against self-antigens may be used as reagents in diagnostics, or repurposed as therapeutics to inhibit the destructive effects of autoantibodies.

[0020] In one aspect, provided herein is a high-throughput and sensitive method for (i) isolating the sequences of antibodies that react to antigens, e.g., self-antigens, without knowledge of the identity of the antigen *a priori*, and/or (ii) discovering the identity of antigen(s) that mediate a disease, or whose presence is correlated with a disease state, e.g., an autoantigen(s) that mediates

an autoimmune disorder(s), or whose presence is correlated with the presence of an autoimmune disorder(s), an antigen(s) that mediates a tumor response.

[0021] The methods described herein can be applied to the identification of antibodies that react with particular antigens, either native or mutated. The antigen can be, in various embodiments, an antigen implicated in a tumor or a metastatic response, autoimmune disease, in a response to a bacterium or virus (or any foreign substance), in response to immunization or vaccination, or that are elicited in the context of organ or tissue transplantation. Immune cells (ASCs or ASC precursor cells) can be isolated from cancer patients that have mounted an effective immune response to a cancer, either spontaneously or in conjunction with immune-therapy treatments such as cancer vaccines, oncolytic viruses, immune-modulators and/or checkpoint inhibitors, and subjected to one of the methods described herein.

[0022] The methods provided herein are based in part on the combination of two approaches. The first is the use of a display library that links phenotype (e.g., a peptide or protein antigen) to its genotype (coding sequence) such as is accomplished in formats such as phage display, other cell display methods (bacterial, yeast, etc.), ribosome display, mRNA display, cDNA display, bead display particles or other particles to determine antibody binding to antigen. Here, an isolated protein in a microscale container, e.g., a secreted antibody (or antigen binding fragment thereof) is used to enrich for display particles that present an epitope displayed on a subset of the library particles, followed by sequencing of the DNA or RNA information in the particles to determine the identity of the enriched antigen(s). A related approach has been used in a "library versus library" strategy where two sets (libraries) of display particles presenting different families of molecules, for example, yeast cells expressing antibody fragments on their surface and phage displaying peptide libraries, are mixed together and incubated to promote an association of the subset(s) of particles from each library that interact. Following incubation, sequencing is used to determine identification of the antibodies and antigens that are associated. The present invention differs from the library versus library approach in that it is not limited to the use of fabricated antibody libraries. Rather, it harnesses the capture of secreted antibodies from a clinically or scientifically relevant environment and the ability to isolate the cell which secretes the antibody. Moreover, antibodies used in the present invention are not physically

linked to their respective genetic material, as is done with the library versus library approach (i.e., where antibody display libraries are harnessed).

[0023] A second technology harnessed by the methods provided herein is single cell screening (or in some cases a small population of cells, e.g., from about 2 cells to about 100 cells), based on the analysis of secreted antibodies from a single ASC, using local capture of these antibodies on solid supports in microscale volumes and containers. In this regards, single cell sensitivity in measuring the presence and/or properties of secreted antibodies from an individual ASC is obtainable even when multiple unique ASCs are present in the same microscale volume (in the same container). Stated another way, because each ASC can produce high concentrations of secreted antibody within minutes, the ASC can be present in a population of cells within a container, and its respective secreted antibody can still be captured via an antigen display particle, and such an interaction can be deciphered.

[0024] In one aspect, the present invention provides a method for screening a diverse set of antibodies, or other antigen-binding proteins, against a library of potential antigens. In this regard, the present invention can be used to identify autoantigens implicated in various disease states by screening ASCs (or other ASC precursor lymphocytes that are subsequently differentiated into ASCs) harvested from a patient that has an autoimmune disease against a library of antigens. In one embodiment, the output of the method provided herein is the determination of the nucleic acid or protein sequence of one or more antibodies (or antigen binding fragments thereof) that recognize a member of the library of antigens, and/or the nucleic acid or protein sequence of the antigen that is recognized. As such, the method provided herein can be used to screen lymphocytes (e.g., ASC precursor cells or ASCs such as plasma cells, plasmablasts, etc.) obtained from various tissues of patients that have an autoimmune disorder or cancer, allowing for the discovery, *a priori*, of antibodies that bind to self-antigens (autoantigens) as well as the autoantigens that bind to these antibodies.

[0025] Aspects of the invention, methods **1000** and **2000** are provided at Figures 1 and 2, respectively, and discussed in further detail below. In one aspect of the invention, a method **1000** is provided for determining the sequence of an antibody and its respective binding partner, e.g., antigen such as an autoantigen. The method entails optionally subjecting a sample to

conditions suitable for expansion of one or more ASCs or ASC precursor cells in the sample to form an expanded sample comprising antibody secreting cells (ASCs) **1001**. The sample or expanded sample is distributed into containers to provide a plurality of subsamples **1002**. Optionally, one or more subsamples, each comprising one or more ASCs or ASC precursor cells, are subjected to conditions suitable for expansion to form one or more expanded subsamples comprising ASCs **1003**. Antibodies from one or more of the subsamples (expanded or non-expanded) are captured on a solid substrate in the respective container(s) to provided captured antibodies **1004**. The captured antibodies in one or more of the containers are exposed to an antigen display library comprising a plurality of antigen display particles for a time sufficient to allow for interaction of one or more of the captured antibodies with the one of more of the display particles **1005**. Display particles are captured that interact with one or more of the captured antibodies. Unbound display particles are removed via a wash step **1006**. However, in another embodiment, a wash step **1006** is not employed. The nucleic acid sequence(s) of antibodies or antigen fragment binding portion(s) thereof, and/or antigens in containers that include captured antigen display particles is then determined **1007**. Each of the steps and components of method **1000** are discussed in further detail below.

[0026] In another aspect, a method **2000** is provided for determining the sequence of an antibody and its respective binding partner, e.g., antigen such as an autoantigen. The method entails optionally subjecting a sample to conditions suitable for expansion of one or more ASCs or ASC precursor cells in the sample to form an expanded sample comprising antibody secreting cells (ASCs) **2001**. The sample or expanded sample is distributed into containers to provide a plurality of subsamples **2002**. Optionally, one or more subsamples, each comprising one or more ASCs or ASC precursor cells, are subjected to conditions suitable for expansion to form one or more expanded subsamples comprising ASCs **2003**. A functional assay that measures a property of an antibody or ASC is performed on one or more of the subsamples **2004**. The functional assay **2004** is performed to identify a subsample(s) that exhibits the functional property of interest (i.e., to identify "functional subsamples"). Antibodies secreted by ASCs in the one or more functional subsamples are captured on a solid substrate to provide captured antibodies **2005**. Antibody capture can be accomplished in the same container as the functional assay, or a different container. For example, a functional subsample can be transferred to a different container to perform antibody capture or alternatively transferred into a plurality of different

containers to create multiple subpopulations of the transferred ASCs prior to performing the antibody capture. The captured antibodies in the containers comprising the functional subsamples are exposed to an antigen display library comprising a plurality of antigen display particles for a time sufficient to allow for interaction of one or more of the captured antibodies with the one of more of the antigen display particles **2006**. Display particles are captured that interact with one or more of the captured antibodies. Unbound display particles are removed via a wash step **2007**. The nucleic acid sequence(s) of antibodies or antigen fragment binding portion(s) thereof, and/or antigens in containers that include captured display particles is then determined **2008**. Each of the steps and components of method **2000** are discussed in further detail below.

[0027] The sample used in the methods presented herein **1000** and **2000**, contains one or more ASCs or ASC precursor cells. The sample can be subjected to conditions suitable for expansion to produce ASCs from precursor lymphocytes at various steps of the methods provided herein, as described in further detail below.

[0028] The one or more ASCs or ASC precursor cells in the sample, in one embodiment, include one or more ASCs, such as one or more plasma cells (also referred to as "plasma B-cells," "plasmocytes" and "effector B-cells"). Plasma cells are terminally differentiated, and are one type of ASC. ASCs include, for example, activated memory B-cells, plasmablasts, cells generated through the expansion of memory B-cells, cell lines that express recombinant monoclonal antibodies and hybridoma cell lines.

[0029] In one embodiment of the method **1000** or **2000**, the source of the sample is a mammal. For example, the sample can be obtained from a human, e.g., a human patient that has a disease or infection. In one embodiment, the sample source is a non-human primate. In a further embodiment, the sample is a chimpanzee, gorilla, orangutan or baboon sample. Other sources of samples include, but are not limited to a human, rat, mouse, rabbit, alpaca, camel, dog, goat, bovine, gerbil, guinea pig, hamster, llama, pig or sheep. In one embodiment, the biological source of the sample is a non-mammalian vertebrate such as an avian or reptilian species. The sample used herein can be obtained from a variety of tissues including, but not limited to, bone marrow, spleen, blood, lymph nodes. The sample may also be obtained from B-cell infiltrates or

structures isolated from tumors or tissues associated (e.g., correlated) with the particular disease state (e.g., brain and/or pancreas).

[0030] The sample used herein, once obtained from a source, can be further purified or modified prior to use in the methods provided herein. For example, ASCs or ASC precursor cells, B-cells or plasma cells may be enriched using methods that are known to those of ordinary skill in the art including bead-based affinity enrichment, flow cytometry and sorting, or density centrifugation, and further subjected to the methods described herein. For example, the one or more ASCs or ASC precursor cells from the sample in one embodiment, are purified from a sample obtained from an *in vivo* or *in vitro* source. In another embodiment, the obtained sample is expanded via monoclonal or polyclonal activation, as described below and/or by methods known to those of ordinary skill in the art.

[0031] In yet another embodiment, the sample is a recombinant cell line that includes cells engineered to express antibodies, or yeast engineered to express antibodies, or bacteria engineered to express antibodies.

[0032] In one embodiment, ASCs are produced by activating B-cells obtained from a sample or by amplifying and transferring the DNA content within B-cells to another cell line that can recombinantly express the antibodies. The produced ASCs are then further subjected to the method steps (e.g., method **1000** or method **2000**, see Figures 1 and 2) described herein.

[0033] In one embodiment, ASCs are obtained directly from a blood, primary lymphoid tissue or peripheral lymphoid tissue sample. The sample may be selected in order to enrich for antibodies associated with a particular condition that the user of the method desires to analyze, for example, an autoimmune disease or cancer. Primary lymphoid tissue samples amenable for use with the methods provided herein include bone marrow samples. Secondary lymphoid tissues amenable for use with the methods provided herein include but are not limited to, tonsils, spleen, lymph nodes, Peyer's patches, mucosa associated lymphoid tissue (MALT), gut associated lymphoid tissue (GALT), or a combination thereof. In another embodiment, a thyroid, cerebral spinal fluid, brain tissue, or tumor tissue sample is used with the methods provided herein. Appropriate tissue type can also be selected by one of ordinary skill in the art based on a patient's diagnosis and/or prognosis.

[0034] In one embodiment of the invention, the methods described herein are used to identify an autoantibody and/or autoantigen associated with an autoimmune disorder or cancer. In a further embodiment, the sample interrogated by a method provided herein is from an autoimmune patient, for example, a patient diagnosed one of the autoimmune disorders set forth in Table 1. The sample as provided above, can be obtained from blood, primary lymphoid tissue, peripheral or a secondary lymphoid tissue (e.g., lymph nodes, lymphoid follicles in tonsils, Peyer's patches, spleen, adenoids, skin) sample.

[0035] In another embodiment, the sample is obtained from a subject having one of the following autoimmune disorders: alopecia areata, autoimmune hemolytic anemia, autoimmune hepatitis, dermatomyositis, diabetes (type 1, some forms of type 2 – MODY), a form of juvenile idiopathic arthritis, glomerulonephritis, Graves' disease, Guillain-Barré syndrome, idiopathic thrombocytopenic purpura, myasthenia gravis, some forms of myocarditis, multiple sclerosis, pemphigus/pemphigoid, pernicious anemia, polyarteritis nodosa, polymyositis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma/systemic sclerosis, Sjögren's syndrome, systemic lupus erythematosus (lupus), some forms of thyroiditis, some forms of uveitis, vitiligo, or granulomatosis with polyangiitis (Wegener's). In these embodiments, the sample can be further expanded (e.g., Figure 1, **1001** or **1003**; Figure 2, **2001** or **2003**) or analyzed without an expansion step, as described below.

Table 1 – Autoimmune Disorders Amenable for Analysis by the Methods Provided Herein

Acute disseminated encephalomyelitis (ADEM)	Dermatitis herpetiformis	Linear IgA disease (LAD)	Psoriatic arthritis
Acute necrotizing hemorrhagic leukoencephalitis	Dermatomyositis	Lupus (SLE)	Idiopathic pulmonary fibrosis
Addison's disease	Devic's disease (neuromyelitis optica)	Lyme disease, chronic	Pyoderma gangrenosum
Agammaglobulinemia	Discoid lupus	Menier's disease	Pure red cell aplasia
Alopecia areata	Dressler's syndrome	Microscopic polyangiitis	Raynauds phenomenon
Amyloidosis	Endometriosis	Mixed connective tissue disease (MCTD)	Reactive arthritis

Table 1 – Autoimmune Disorders Amenable for Analysis by the Methods Provided Herein			
Ankylosing spondylitis	Eosinophilic esophagitis	Mooren's ulcer	Reflex sympathetic dystrophy
Anti-GBM/Anti-TBM nephritis	Eosinophilic fasciitis	Mucha-Habermann disease	Reiter's syndrome
Antiphospholipid syndrome (APS)	Erythema nodosum	Multiple sclerosis	Relapsing polychondritis
Autoimmune angioedema	Experimental allergic encephalomyelitis	Myasthenia gravis	Restless leg syndrom
Autoimmune aplastic anemia	Evans syndrome	Myositis	Retroperitoneal fibrosis
Autoimmune dysautonomia	Fibromyalgia	Narcolepsy	Rheumatic fever
Autoimmune hepatitis	Fibrosing alveolitis	Neuromyelitis optica (Devic's)	Rheumatoid arthritis
Autoimmune hyperlipidemia	Giant cell arteritis (temporal arteritis)	Neutropenia	Sarcoidosis
Autoimmune immunodeficiency	Giant cell myocarditis	Ocular cicatricial pemphigoid	Schmidt syndrome
Autoimmune inner ear disease (AIED)	Glomerulonephritis	Optic neuritis	Scleritis
Autoimmune myocarditis	Goodpasture's syndrome	Palindromic rheumatism	Scleroderma
Autoimmune oophoritis	Hashimoto's encephalitis	PANDAS (Pediatric autoimmune neuropsychiatric disorders associated with Streptococcus)	Sjogren's syndrome
Axonal & neuronal neuropathies	Hashimoto's thyroiditis	Paraneoplastic cerebellar degeneration	Sperm & testicular autoimmunity
Balo disease	Hemolytic anemia	Paroxysmal nocturnal hemoglobinuria (PNH)	Stiff person syndrome
Behcet's disease	Henoch-Schonlein purpura	Parry Romberg syndrome	Subacute bacterila endocarditis (SBE)
Bullous pemphigoid	Grave's disease	Parsonnage-Turner syndrome	Susac's syndrome

Table 1 – Autoimmune Disorders Amenable for Analysis by the Methods Provided Herein			
Cardiomyopathy	Guillain Barre syndrome	Pars planitis (peripheral uveitis)	Sympathetic ophthalmia
Castleman disease	Herpes gestationis	Pemphigus	Takayasu's arteritis
Celiac disease	Hypogammaglobulinemia	Peripheral neuropathy	Temporal arteritis/Giant cell arteritis
Chagas disease	Idiopathic thrombocytopenic purpura (ITP)	Perivenous encephalomyelitis	Thrombocytopenic purpura (TTP)
Chronic fatigue syndrome	IgA neuropathy	Pernicious anemia	Tolosa-Hunt syndrome
Chronic inflammatory demyelinating polyneuropathy (CIDP)	IgG4-related sclerosing disease	POEMS syndrome	Transverse myelitis
Chronic recurrent multifocal ostomyelitis (CRMO)	Immunoregulatory lipoproteins	Polyarteritis nodosa	Type 1 diabetes
Churg-Strauss syndrome	Inclusion body myositis	Type I, II, & III autoimmune polyglandular syndromes	Type 1.5 diabetes (LADA)
Cicatricial pemphigoid/benign mucosal pemphigoid	Interstitial cystitis	Polymyalgia rheumatica	MODY diabetes (types…)
Crohn's disease	Juvenile arthritis	Polymyositis	Ulcerative colitis
Cogans syndrome	Juvenile myositis	Postmycocardial infarction syndrome	Undifferentiated connective tissue disease (UCTD)
Cold agglutinin disease	Kawasaki syndrome	Postpericardiotomy syndrome	Uveitis
Congenital heart block	Lambert-Eaton syndrome	Progesterone dermatitis	Vasculitis
Coxsackie myocarditis	Leukocytoclastic vasculitis	Primary biliary cirrhosis	Vesiculobullous dermatosis
CREST disease	Lichen planus	Primary sclerosing cholangitis	Vitiligo
Essential mixed cryoglobulinemia	Lichen sclerosus	Psoriasis	Wegener's granulomatosis (aka Granulomatosis with

Table 1 – Autoimmune Disorders Amenable for Analysis by the Methods Provided Herein			
			polyangiitis (GPA))
Demyelinating neuropathies			

[0036] Once the sample is obtained, as an optional step **1001** or **2001**, the sample comprising one or more ASCs or ASC precursor cells, e.g., a sample obtained from a subject, a sample obtained from a subject that is subjected to cell sorting to contain a substantially purified lymphocyte population, or an *in vitro* sample, is subjected to conditions suitable for expansion of the ASCs or ASC precursor cells in the sample, to obtain an expanded sample comprising antibody secreting cells (ASCs) (e.g., plasmablasts or plasma cells). Determination of whether expansion is carried out can be decided by the user of the method, for example, depending on the original sample type and the types of cells in the sample.

[0037] In some embodiments, the cells from a sample may be expanded in a cell culture step in order to increase the number of ASCs expressing any given antibody. This may be done prior to (**1001** or **2001**) and/or subsequent to partitioning (**1003** or **2003**) the sample into subsamples. The expansion step may be used to increase the generated concentration of any given antibody within a sample container in order to obtain sufficient amounts of antibody to interact with antigen library particles or to implement a functional assay. Conditions suitable for expansion include both conditions for polyclonal expansion and conditions for antigen-specific expansion, as described in further detail below. In some embodiments, the ASCs may be fused with an appropriate cell line to create hybridoma cells that can be expanded to a greater extent. Methods for the generation of hybridoma cells from a variety of species, including mice, rats, gerbils, humans, and rabbits, are known to those of ordinary skill in the art. In some embodiments an alternative immortalization method, such as Epstein-Barr virus (EBV) immortalization, may be used instead of hybridoma methods, as is known to those of ordinary skill in the art.

[0038] ASCs in one embodiment are produced by activating B-cells obtained from a tissue sample or by amplifying and transferring the DNA content within an ASC to another cell line that can recombinantly express the antibodies secreted by the ASC. In some embodiments, the cells from the tissue are expanded in order to increase the number of ASCs in a sample. An

expansion step may be done prior to partitioning a sample into subsamples (where a subsample can comprise 0 or a plurality of ASCs) in discrete chambers (**1001** or **2001**), or after partitioning (**1003** or **2003**).

[0039] Activation of the sample leads to the formation of an expanded sample. In one embodiment, activation is carried out in cell culture of the one or more ASCs or ASC precursor cells in the sample (which can be subjected to one or more purification steps such as FACS). In one embodiment, in the case of a heterogeneous immune cell population, activation is employed for the entire population of lymphocytes or progeny thereof, *e.g.*, through polyclonal activation. Alternatively or additionally, activation is carried out on a subpopulation of cells, *e.g.*, with antigen specific activation and subsequent expansion. In one embodiment, where a sample includes a combination of B-cells and T-cells, only the B-cells (or a subpopulation thereof) are activated, or both the B-cells (or a subpopulation thereof) and the T-cells (or a subpopulation thereof) are activated and expanded, to form an expanded sample **1001** or **2001**, or an expanded subsample **1003** or **2003**.

[0040] Methods for performing antigen-specific expansion of B-cells and T-cells are known to those of ordinary skill in the art and the present invention is not limited by a particular expansion method. Rather, an expansion step can be carried out according to a protocol determined by the user of the method. Activation of a lymphocyte or progeny thereof causes the activated cell to divide and expand to form an expanded sample comprising an ASC (or an expanded subsample). Both polyclonal activation and antigen-specific activation are amenable for use with the present methods. The activation/expansion step can be carried out according to a protocol determined by the user of the method.

[0041] In one embodiment, a CD40L expressing feeder cell line (e.g. EL-4-B5) is used to activate and expand B-cells, and to produce ASCs. The EL-4-B5 cells express CD40-ligand and are grown with B-cells in the presence of T cell supernatants to activate the B-cells via direct cell contact to induce proliferation, differentiation, and secretion of antibody. The EL-4-B5 cell line is available commercially, for example, from Kerafast (catalog no. EVU301). In another embodiment an antibody that engages and activates CD40 may be used in the activation step, as is known to those of ordinary skill in the art.

[0042] B-cells residing primarily in peripheral lymphoid tissues in one embodiment, are activated and expanded into antibody-secreting cells (ASCs) upon antigen stimulation. In one embodiment, B-cells are activated under defined *in vitro* culture conditions resulting in polyclonal expansion and differentiation into ASCs. In the case of memory B-cells, activation and expansion in one embodiment is accomplished by treating the cells with, for example, Epstein Barr virus, CD40L, or one or more toll like receptor agonists, using protocols that are known to those of ordinary skill in the art. Protocols described in the literature that may be used to induce B-cell activation by adding supplements in the cell culture media are amenable for use with the present invention. These include different combinations of factors such as cytokines (*e.g.*, IL-21, IL-6, IL-4, IL-2, IL-10, IL-15) (Ettinger *et al.* (2005). The Journal of Immunology 175, pp. 7867-7879; Pinna *et al.* (2009). Eur. J. Immunol. 39, pp. 1260-1270 and Bernasconi *et al.* (2002). Science 298, pp. 2199-2202, each of which is incorporated by reference herein in its entirety for all purposes); cell surface ligands (*e.g.*, CD40L, BAFF, APRIL), toll-like receptor agonists (e.g. LPS, CpG, R848, PWM) (see, e.g., Pinna *et al.* (2009). Eur. J. Immunol. 39, pp. 1260-1270; Boeglin *et al.* (2011). PLOS One 6, p. e25542. doi:10.1371/journal.pone.0025542; Hartmann and Krieg (2000). J. Immunol. 164, pp. 944-953; Krieg et al. (1995). Nature 374, pp. 546-549; Crotty *et al.* (2004). J. Immunol. Methods 286, pp. 111-122; Endoh *et al.* (1987). Cell Immunol. 107, pp. 455-464, each of which is incorporated by reference herein in its entirety for all purposes), monoclonal antibodies against cell surface receptors (e.g. anti-CD40, anti-IgG) (Zhu *et al.* (2002). J. Immunol. 168, pp. 744-754; Endoh *et al.* (1987). Cell Immunol. 107, pp. 455-464, each of which is incorporated by reference herein in its entirety for all purposes), and feeder cell lines providing co-stimulation signals (e.g., cell lines expressing CD40L) (Seeber *et al.* 2014) PLOS One 9, e86184. doi:10.1371/journal.pone.0086184; Wen *et al.* (1987). Eur. J. Immunol. 17, pp. 887-892; Liebig *et al.* (2009). J. Vis. Exp. 16, pii: 1373. Doi: 10.3791/1373, each of which is incorporated by reference herein for all purposes).

[0043] In one embodiment, one or more activated cells in the expanded sample **1001** or **2001** are purified and/or enriched for. Purification/enrichment can be carried out according to methods known to those of ordinary skill in the art. In one embodiment, purification of activated cell(s) is carried out based on identification of cellular morphology or expansion marker(s), a FACS secretion assay, purified or enriched, a Milteny kit (e.g., IFN-y kit or custom), cytokine secretion

assays, cell marker assay wherein the cell marker is turned on upon activation, peptide-based purification by FACS, or a combination thereof.

[0044] The sample (e.g., purified, expanded, enriched) is distributed into a plurality of discrete containers (e.g., microwell plate well, microfluidic chambers, open wells) (Figure 1, **1002**, Figure 2, **2002**).

[0045] In the methods provided herein, the sample (e.g., cultured sample, expanded, purified, or directly from a source) is distributed into a plurality of discrete containers **1002** or **2002**. The containers can be in some embodiments, microfluidic chambers, open microwells, microfuge tubes, microdroplets, or a semi solid medium such as a matrigel. The discrete containers (also referred to as vessels) in one embodiment are open wells in a microplate, microcentrifuge tubes, or microfluidic chambers, for example, the microfluidic chambers described in PCT Publication No. WO 2014/153651, incorporated by reference herein in its entirety. Combinations of the foregoing can also be employed. In one embodiment, the discrete containers comprise a plurality of open microwells. In a further embodiment, the containers are open microwells, present in an array. In even a further embodiment, the average aspect ratio (height : width) for each open microwell is about 1:1 or greater than 1:1. The aspect ratio for each of the microwells is chosen to allow for cell retention in the respective microwell during exchange of reagents in the microwell.

[0046] In one embodiment, distributing the sample into discrete containers **1001** or **2002** results in a large number of ASCs that are maintained in spatial proximity to a substrate functionalized to capture the secreted antibodies from the ASCs, e.g., functionalized microbeads. In one embodiment, ASCs are obtained directly from a tissue sample which may be first selected in order to enrich for antibodies associated with a particular autoimmune condition. Such tissues may include, but are not limited to, tonsils, blood, bone marrow, spleen, lymph nodes, and cerebral spinal fluid. In another embodiment, a sample containing ASCs or ASC precursor cells is activated and expanded prior to distribution into containers. In some embodiments, the cells are grown with a thymoma cell line such as the murine EL4 thymoma cell line, which optionally, may have been mutagenized. For example, the thymoma cell line may have been mutagenized to obtain bromo-deoxyuiidine-resistani mutants (e.g., EL4-B5 cells, e.g., the cell line available from

Kerafast, Inc., Boston, MA, catalog no. EVU301). In some embodiments, the cells are grown with macrophages (e.g., PD3188 cells or P388D1 cells) and/or T-cells. In a further embodiment, expansion occurs via the use of an EL-4-B5 feeder cell line.

[0047] In the methods described herein, a sample comprising ASCs or ASC precursors is distributed into individual containers (*e.g.*, individual wells of a microwell plate, microfluidic chambers, individual microfuge tubes) (**1002** or **2002**). As used herein, a "container" is used interchangeably with a "vessel." The methods provided herein can be carried out in a variety of different container types. For example, in one embodiment, a container is an individual well of a multiwell plate, or an individual chamber in a microfluidic device. In one embodiment, the discrete containers are wells in a 96, 384 or 1536 microwell plate. In one embodiment, the sample (e.g., an expanded sample) is split into at least about 50, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1000, at least about 2000, at least about 5000 discrete containers, at least 20,000 discrete containers, at least 100,000 discrete containers, at least 200,000 discrete containers, at least 500,000 discrete containers or at least 1,000,000 discrete containers (e.g., open microwells or microfluidic chambers) for further processing. The number of ASCs and/or ASC precursors in each container can be the same or different. Moreover, tissues from multiple patients can be analyzed on an individual plate or device comprising multiple containers.

[0048] In one embodiment, each subsample can contain from on average, about a single cell to about 10,000 cells (ASCs or other lymphocyte cell(s)), depending on the capacity of the chamber used. It will be appreciated that microscale chambers will have less capacity than macroscopic chambers and that appropriate cell densities will be different. It should be noted that each container need not contain an ASC or ASC precursor cell. Accordingly, in some embodiments, and depending on the number of cells in the original sample and/or whether an activation/expansion step **1001** or **2001** is carried out, and how sample partitioning is carried out, one or some individual containers will have zero ASCs or zero ASCs or ASC precursor cells present, or an individual ASC or an individual ASC precursor cell present.

[0049] In one embodiment, individual single cells (e.g., individual ASCs or ASC precursors) from an expanded cell population are divided into discrete containers. In another embodiment, an average of a single ASC or ASC precursor is present in each of the containers.

[0050] Optionally, once the obtained sample is distributed into discrete containers to provide isolated subsamples, one or more of the isolated subsamples is subjected to conditions suitable for expansion of one or more ASCs or ASC precursor cells in the sample (Figure 1, **1003**; Figure 2, **2003**). For example, the EL4.B5 culture system for activation and expansion of B-cells can be employed, as known to those of ordinary skill in the art. Expansion **1003** or **2003** can be carried out with or without a feeder cell population. Examples of expansion protocols are described above for step **1001** and **2001**, and are also amenable for use at step **1003** and/or **2003**.

[0051] In embodiments where containers are microfluidic chambers, the cell culture systems of U.S. Patent Application Publication No. 2012/0009671 and/or similar cell culture systems may be employed. U.S. Patent Application Publication No. 2012/0009671 is incorporated by reference herein in its entireties for all purposes.

[0052] In some embodiments, at step **1003** and/or **2003**, the exposure of cells in subsamples to cell culture conditions results in an increase in the expression levels of mRNA, and the activation of memory B-cells to differentiate them into ASCs. A benefit of an expansion step **1003** or **2003** following sample partitioning is that the cells of the subsamples are caused to divide and make multiple copies within the well or container. This results in a greater number of total antibodies secreted by the expanded populations of ASC, and maximizes the identification of unique epitopes within a screen cell population. A cell expansion step subsequent to sample partitioning may be used to generate variability in the number of copies of antibodies derived from any clone in the starting sample. This may be done deliberately by selecting expansion conditions that favor some clones or some containers. Nevertheless, even a polyclonal activation will result in some variability of expansion of each of the clones within the sample.

[0053] A functionalized substrate is used to capture secreted antibodies in one or more of the containers (Figure 1, **1004**; Figure 2, **2005**). In one embodiment, the functionalized substrate is a functionalized container surface that has been conjugated with antibody-capture proteins such as

protein A, protein G, or polyclonal antibodies against immunoglobulins of one or more isotype from the species being studied.

[0054] In another embodiment, antibodies secreted by ASCs in a subsample (expanded or non-expanded subsample) are captured on a functionalized microscale bead substrate, present in the containers (Figure 1, **1004**; Figure 2, **2005**). Specifically, a functionalized substrate such as microscale beads that have been conjugated with antibody-capture proteins such as protein A, protein G, or polyclonal antibodies against human immunoglobulins, in one embodiment, are present in the containers or a subset thereof. ASCs are maintained in spatial proximity to the functionalized beads, and the beads capture the antibodies secreted by the ASCs. The microscale beads can be introduced into the discrete containers prior to the sample introduction, subsequent to the sample introduction, or simultaneously with the sample introduction (sample partitioning into subsamples). The cells in the subsample can be expanded at step **1003** or **2003** and when this step is carried out, in one embodiment, the microscale beads are introduced into the discrete containers after the subsamples are expanded. Following the capture of secreted antibodies in the chambers the antibodies may be interacted with a display library to allow for binding of the antibodies with recognized antigens in the display library. It will be known to those with ordinary skill in the art that efficient interaction of the display library with the antibodies requires that each antibody have the opportunity to interact with one or more members of the display library, and that this is determined by the volume and diversity of the display library, as well as the duration and mode of interaction. For instance, if the display library is comprised of a phage display library, the volume of the library that interacts with the captured antibody should be sufficient such that all members of the library are represented at least once. A phage display library may have a concentration of up to 10^{14} particles per mL (Nemudraya et al. (2016). Acta Naturae 8(1), (28), incorporated by reference herein in its entirety for all purposes), and may easily have concentration of 10^{12} particles per mL. If an interrogated ASC is analyzed in a container having a volume of 1 nL, this volume may include between approximately 10^{6} to 10^{8} phage particles. Flowing additional volume of display library through the container (e.g., microfluidic chamber) to interact with bound antibody may be performed to increase this diversity by 100 fold or more, thereby allowing for interrogation of libraries between 10^{8} and 10^{10}. It will be appreciated by those with ordinary skill in the art that larger libraries may be interrogated through the use of larger chamber volumes or by repeated steps of incubation and

flowing of additional library volume. Alternative library formats such as ribosome display libraries may be produced at higher concentrations so that library diversity is increased. Alternatively, libraries of lower total diversity but with greater concentration of each antigen may be preferred in cases where detection of binding is desired, e.g. using a fluorescently labeled reagent.

[0055] The functionalized substrate such as functionalized beads can be introduced into the containers prior to sample introduction, subsequent to sample introduction, or simultaneously with the sample. Functionalized antibody capture beads are available commercially. For example, protein A and protein G beads are available from Promega, and are amenable for use with the methods provided herein (Promega product nos. G7471, G7472, G7473, G8781, G8782 and G8783). Alternatively, container surfaces can be functionalized with an antibody capture protein prior to introducing the sample into the containers. Notably, the invention is not limited by a particular type of antibody capture bead or functionalized surface.

[0056] Secreted antibodies are captured on the functionalized surface, e.g., on the surface of a bead(s) or container surface. Moreover, as a result of distributing the sample into microscale containers, a large number of ASCs are maintained in spatial proximity to the functionalized substrate(s) that has captured the secreted antibodies. This is the case whether ASCs are present in the sample at step **1002** or **2002** or the sample is expanded at **1003** or **2003**.

[0057] Captured antibodies in one or more of the containers are exposed to antigen display library particles for a time sufficient to allow for interaction of the captured antibodies with particles of the display library (Figure 1, **1005**; Figure 2, **2006**).

[0058] The captured ASCs are exposed to a display library (Figure 1, **1005**; Figure 2, **2006**) that presents a collection of antigens to the antibodies that are captured on the solid surfaces. As discussed in further detail below, in one aspect of the invention, prior to library interrogation, captured ASCs are subjected to a functional assay to determine the presence of functional antibodies in one or more containers (e.g., Figure 2, **2005**). The results of the functional assay(s) determine which ASCs (and which containers) are interrogated with the antigen display library.

[0059] The displayed antigens in a particular library is not limited by size. For example, the displayed peptide in one embodiment is from about 10 amino acids to about 50 amino acids in length, from about 15 amino acids to about 50 amino acids in length, at least about 10 amino acids in length, etc. Size of antigens can be adjusted depending on the application studied and the method of display. Moreover, the size of a library can be increased by scrambling libraries and performing alanine scanning mutagenesis on members of the library. Displayed peptides can also be designed to overlap in sequence with other antigens.

[0060] The methods provided herein are not limited to a particular type of display library, or the number of antigens in a display library. For example, the display system can be a cell-free system, which includes libraries of (poly)peptide-nucleic acid complexes, in which each polypeptide is physically bound to its nucleic acid coding sequence, or a DNA or RNA sequence that can be used to determine its identity (sometimes referred to as a "barcode" sequence).

[0061] A barcode sequence in one embodiment, is used in the display library employed herein. In a further embodiment, the barcode sequence acts as the library source from which the displayed peptide is translated. Alternatively or additionally, the barcode sequence is used as an identifier, as described above. In another embodiment the barcode is a DNA sequence that encodes a peptide or polypeptide sequence, that may or may not include silent or non-silent mutations. Alternatively, the barcode is composed of a series of nucleotides linked to the 3' or 5' end of the nucleic acid molecules, optionally connected by a linker sequence. One of ordinary skill in the art will appreciate that when a barcode sequence is employed, it does not interfere with the expression of displayed peptides. In another embodiment, the barcode comprises a chemical or enzymatic modification, for example, a barcode comprised of a DNA sequence can be chemically linked or conjugated directly to a target protein or peptide. One method of synthesizing peptide libraries containing peptide-cDNA fusions by in vitro transcription/translation from pools of DNA templates generated by microarray-based synthesis is provided in Kozlov et al (2012) PLOS One. DOI: 10.1371/journal.pone.0037441, incorporated by reference herein in its entirety for all purposes.

[0062] Display libraries can be designed to allow for epitope mapping, i.e., linear peptide libraries can be employed to identify critical residues involved in biomolecular interactions.

Alternatively or additionally, different spatially located epitopes of an antigen can be detected by using different barcodes corresponding to different spatially located epitopes of an antigen.

[0063] In yet another embodiment, a barcoded library is designed with three levels of information: (1) amino acid sequence of displayed peptide; (2) nucleic acid barcode for identification using next generation sequencing of large pools and (3) a tag/residue such as biotin for example and subsequent fluorescent detection in well of all binders using fluorescently labelled streptavidin or any other tag.

[0064] Alternatively or additionally, the library can be generated from a cell-based system, which is based on natural or synthetic nucleotide sequences which are translated and assembled in host cells (e.g., bacteriophage or phage display). Phage display libraries are generated by basic molecular biology techniques and allow for libraries having repertories on the order of 10^{10}-10^{13} unique particles. Alternatively, more focused libraries of between 10^{3}-10^{6} members may also be employed, as well as libraries in an intermediate range of diversity between 10^{6}-10^{10} members.

[0065] Phage display technology is based on the ability to express foreign (poly)peptides as fusions to capsid proteins on the surface of bacteriophage. Surface display is achieved by inserting a peptide-encoding gene into the gene for a capsid structural protein. Billions of pooled peptides presented on phage particles form a phage-displayed peptide library, and in contrast to regular synthetic small molecule libraries, as many as 10^{10} different peptides can be screened simultaneously for the desired activity (Molek et al. (2011). Molecules **16**, pp. 857-887, incorporated by reference herein in its entirety). Lytic or filamentous phage or phagemid vectors are amenable for use herein. In one embodiment, the phage display system is based on filamentous phages in which peptides are fused to major (p8) or minor (p3) coat proteins.

[0066] Methods for producing phage display libraries are known to those of ordinary skill in the art. For example, the phage display technology originally pioneered by Cambridge Antibody Technology, as well as subsequent improvements and modification of this library, is amenable for use with the present invention. For example, see the methods described in U.S. Patent No. 5,959,108, which is hereby incorporated by reference in its entirety for all purposes.

[0067] Library formats for use with the methods provided herein include cell display, phage display, yeast display, ribosome display, mRNA display, cDNA display or a combination thereof. Construction of these libraries is well known to those of ordinary skill in the art, for example, see Carter (2006); Nature Reviews Immunology 6(5), pp. 343-357; Hoogenboom (2005) Nature Biotechnology 23(9), pp. 1105-1116; Kowalczykowski (1994). Microbiological Reviews 58(3), pp. 401-465; Ruoslahti (1996). Annual Review of Cell and Developmental Biology 12, pp. 697-715; Sarikaya (2003). Nature Materials 2(9), pp. 577-585; Smith (1997) Chemical Reviews 97(2), pp. 391-410; Smith (1993). Methods in Enzymology 217, pp. 228-257; Willats (2001). Plant Molecular Biology 47(1-2), pp. 9-27; Winter (1994). Annual Review of Immunology 12, pp. 433-455; each of which is incorporated by reference in its entirety for all purposes,

[0068] In one embodiment, the display library is a magnetic particle bead display library, e.g., a nanoparticle bead display library. In this embodiment, displayed peptides and their corresponding barcodes are conjugated to a solid support such as a nanoparticle (e.g. see Huang et al. 2013, ORBIT bead display, incorporated by reference in its entirety herein). The barcoded particles may be small enough that they remain in suspension in solution and can be easily washed away. The beads are magnetic, to allow for removal of nanoparticles with an external magnet. The strength of the magnet may be adjusted to select for affinity of the peptide interactions.

[0069] The antigen display library, in one embodiment, is a cell-free display library, e.g., a ribosome display library or other nucleic acid (cDNA or mRNA) display library.

[0070] The antigen display library in one embodiment is designed to have a known diversity of possible antigens which may be hundreds, thousands, millions, or billions of different library members. However, as a practical constraint, every library member must be represented at sufficient concentration to allow for efficient interaction of the library with the captured antibody species in a particular container.

[0071] In one embodiment, the library is a display library that presents antigens such as peptides, protein fragments or proteins that are present on the surface of cells, wherein the antigens are present in a tissue that is affected by a disorder. The antigen display library in one embodiment

is designed with knowledge of epitopes that have been implicated in the disease or indication that is being studied. For example, although specific autoantigen epitopes are unknown for a number of autoimmune diseases and cancer, the study of these diseases has uncovered surface proteins that are expressed on affected cells, for example, tumor cells in the case of a particular cancer or beta cells in the case of type 1 diabetes mellitus. The antigen display library can be designed based on the knowledge of these surface proteins.

[0072] However, in another embodiment, the display library is a random display library.

[0073] The display library in one embodiment can be constructed via entire cDNA libraries from a subset of cells of interest. For example, cDNA libraries can be used from a tumor sample where autoantibodies implicated in a particular cancer are studied.

[0074] In one embodiment, the display library is pre-enriched prior to introducing it into one or more containers. For example, in one embodiment, the library is exposed to polyclonal antibodies obtained from a patient's serum, and which are immobilized on one or more solid phases, to enrich for a subset of library particles that bind to antibodies in the serum. It is this subset of particles that is distributed into the containers.

[0075] In another embodiment, proteins and peptides for use in an antigen library can be identified based on selective expression data of an autoimmune disease positive sample as compared to a negative control sample. For example, in the case of type 1 diabetes mellitus, protein (e.g., via mass spectrometry analysis) or cDNA expression data can be compared from a beta cell sample to a negative control beta cell sample from a healthy individual. The selective expression data from the positive sample can be used to generate the antigen library. Similar strategies can be used for other autoimmune diseases, or any disease where autoantigens are implicated, e.g., a particular cancer. In a further embodiment, the disorder is an autoimmune disorder. In even a further embodiment, the autoimmune disorder is an autoimmune disorder provided in Table 1. For example, in the case of type 1 diabetes mellitus, in one embodiment, the library is a phage display library that encodes proteins expressed on the surface of islet cells.

[0076] The display library particles are exposed to the captured antibodies for a sufficient time for the antibodies to capture library particles **1005** or **2006**. Following sufficient incubation to

allow an interaction between the displayed antigens and the captured antibodies, and subsequent capture of the display particles, unbound library particles are optionally washed off of the beads **1006** or **2007**. If a captured antibody binds to a specific member of the antigen library, this member associates, and remains associated with the library particle on the solid substrate that the antibody was captured. As will be apparent to one of ordinary skill in the art, the assays provided herein can be implemented to analyze hundreds to thousands to millions of individual cells, and therefore, hundreds to thousands to millions of antibody-antigen interactions. For example, a microfluidic format, for example, a format described in PCT Publication No. WO 2014/153651, incorporated by reference herein in its entirety, can be used for the methods described herein, as well as a microwell plate format or an open microfabricated well format. For example, the assay formats described in U.S. Provisional Application Serial No. 62/309,663, filed March 17, 2016, incorporated by reference herein in its entirety may be employed.

[0077] The display library presents a collection of antigens to the antibodies that are captured on the functionalized substrate (e.g., beads) in the containers. If a captured antibody binds to a specific member of the library, this member becomes and remains associated with that library particle. In one embodiment, the antigen capture step is performed to simultaneously analyze thousands to millions of individual cells for antibody-antigen interaction, e.g., in a microfluidic format, for example, a format described in PCT Publication No. WO 2014/153651, incorporated by reference herein in its entirety. In another embodiment, an open microwell format is employed. In another embodiment a microwell plate format is employed. In another embodiment microfuge tubes are employed as the containers housing cell subsamples.

[0078] Following sufficient incubation to allow an interaction between the displayed antigens and the captured antibodies, non-interacting display particles from the library are optionally washed off the bound library particles (Figure 1, **1006**; Figure 2, **2007**).

[0079] The nucleic acid sequence of antibodies or antigen fragment binding portion(s) thereof, and/or antigens in containers that include captured display particles is then determined (**1007** or **2008**).

[0080] In one embodiment of the step **1007** or step **2008**, the contents of each of the containers are sequenced, followed by a determination of the containers that contain both antibody and antigen, as well as the sequences of the antibody and antigen.

[0081] In another embodiment, in order to determine which containers to sequence, containers are first visualized via a detection reagent to determine whether the respective container includes a bound display particle. The detection reagent can be an additional reagent, e.g., a fluorescently labeled secondary antibody that binds to a display particle. Alternatively, the display particle serves as the detection reagent. For example, the display particle in one embodiment is a fluorescently labeled display particle. ASCs, antibodies, display particles or a combination thereof are removed from container(s) that contain captured display particles, and subjected to sequencing reactions. The results of the sequencing reactions determine the identity of the antibody and/or its respective antigen binding partner **1007** or **2008**.

[0082] In one embodiment, following incubation of library particles in one or more containers, and optionally, one or more wash steps, a detection reagent is introduced into the one or more containers. The detection reagent allows for the identification of containers having ASCs that produce an antibody recognized a member of the antigen display library. Sequencing can then be employed to determine the antibody and/or antigen sequence(s) in the identified containers **1007** or **2008.** The detection reagent in one embodiment includes a fluorescently labeled secondary antibody that binds to a protein on the display particle. For example, in one embodiment, where the library is a phage library, the secondary antibody is a fluorescently labeled anti-phage antibody.

[0083] As provided above, however, in another embodiment, the library particles serve as the detection reagent. For example, the library particles in one embodiment are fluorescently labeled.

[0084] The containers, in one embodiment, are imaged, e.g., via fluorescence microscopy or a fluorescent plate reader, in order to detect the detection reagent and by extension, containers having an ASC that make an antibody which reacts to a member of the library.

[0085] The presence of a detection signal from the detection reagent is an indication that one or more display particles from the library includes an antigen that binds to an antibody in the particular container. As noted above, in some embodiments, more than one species of antigen display particle associates with a given antibody in a container, either because the antibody is poly-reactive to multiple species or because of non-specific interactions with the display particles and/or cells. Multiple species of antigen display particle can also be detected in one container in embodiments where different antibodies are secreted in the same container. The degree of enrichment of display particles in containers allows for identification of binding interactions that are significant. This may be facilitated by also analyzing antibody and display sequences obtained from "negative" wells in order to ascertain the background.

[0086] In another embodiment, a container is visualized via a detection reagent to determine whether it includes an antibody (or antigen fragment binding portion thereof) bound display particle(s). In a further embodiment, the detection reagent is visualized via imaging, e.g., via fluorescence microscopy or a fluorescent plate reader, to determine the identities of containers that include one or more ASCs that react with one or more members of the antigen display library. The detection reagent can be an additional reagent, e.g., a fluorescently labeled secondary antibody that binds to a display particle. Alternatively, the display particle serves as the detection reagent. For example, the display particle in one embodiment is a fluorescently labeled display particle. In a further embodiment, the displayed antigen is fluorescently labeled. Alternatively, the detection reagent comprises an enzyme chosen to act on a substrate in order to obtain an amplification of the detection signal.

[0087] In one embodiment of step **1007** or **2008**, ASCs and the antibody capture beads associated with display particles are recovered from containers and subjected to molecular biology protocol(s) to isolate and/or amplify nucleic acid for a subsequent sequencing reaction. This nucleic acid (e.g., RNA or DNA) material is sequenced using high-throughput sequencing instrumentation, to identify the sequence of the antibody(ies) and the identity of the associated antigen(s).

[0088] ASCs and/or antibodies and/or library particle(s) from containers are subjected to molecular biology protocol(s) to isolate and/or amplify nucleic acid that can be sequenced at step

1007 or **2008**. Sequencing reactions can be employed on nucleic acid in all containers, or for nucleic acid in containers that have been identified to contain antibody-display particle complexes. Sequencing of antigen and/or antibody sequences determines the identity of the relevant antigen(s) and/or antibody(s) at step **1007** or **2008**.

[0089] Types of sequencers and sequencing technologies amenable for use with the methods presented herein include, but are not limited to, the Genome Sequencer 20/FLX (commercialized by 454/Roche); MiSEQ instrument (Illumina), 'Solexa 1G' (later named 'Genome Analyzer' and commercialized by Illumina/Solexa), SOLiD™ system (commercialized by Applied Biosystems), and Polonator G.007 (commercialized by Dover Systems). Other protocols amenable for use with the methods provided herein include Polony sequencing, Helioscope™ single molecule sequencing, Lynx Therapeutics' Massively Parallel Signature Sequencing (MPSS), 454 pyrosequencing, Ion Torrent™ (Life Technologies), DNA nanoball sequencing (via rolling circle amplification), and VisiGen Biotechnologies approach.

[0090] In one embodiment, an Illumina MiSEQ instrument is used. High throughput sequencing protocols are well known to those of ordinary skill in the art. See, e.g., Gupta (2008). Trends in Biotechnology **26**, pp. 602-611; Metzker. (2010). Nature Reviews Genetics **11**, pp. 31-46; Schuster (2008). Nature Methods **5**, pp. 16-18; Shendure and Ji (2008). Nat. Biotechnol. **26**, pp. 1135-1145, each of which is incorporated by reference in its entirety for all purposes.

[0091] In order to determine container that contain captured display particles, in one embodiment, antibody-antigen complexes are recovered from the containers and subjected to sequencing reactions. In a further embodiment, ASCs from the containers having antibody-antigen complexes (or from a subset of containers) are recovered from the containers.

[0092] In microfluidic embodiments, recovery, in one embodiment, comprises piercing the respective microfluidic chamber comprising subsample comprising the one or more ASCs associated with one or more display particles, with a microcapillary and aspirating the chamber's contents or a portion thereof to obtain a recovered aspirated cell population.

[0093] Various methods for the recovery of one or more cells from specific containers are amenable for use herein. For example, a micropipette can be employed. In some embodiments,

the PDMS material of certain devices provided herein enables the selective recovery of cells from any chamber by piercing the upper membrane with a microcapillary. In one embodiment, the membrane reseals or substantially reseals after piercing is complete. In one embodiment, cell recovery from a chamber is carried out based in part on the methods set forth by Lecault et al. (2011). Nature Methods **8**, pp. 581-586, incorporated by reference herein in its entirety for all purposes. In one embodiment, the membrane above a particular chamber is pierced with the microcapillary and cells are aspirated. Recovered cells, antibodies and antigens can then be deposited in microfuge tubes for sequencing analysis, as known to those of ordinary skill in the art.

[0094] Recovery, in one embodiment is automated and entails the use of a robotic microcapillary instrument. However, recovery can also be accomplished manually with a microcapillary. The recovery methods provided herein in one embodiment allow for the recovery from 100 containers with >95% efficiency in about 45 minutes.

[0095] A microcapillary, in one embodiment, is used to recover one or more cells from a container. The cells in the container(s) are substantially recovered by aspirating the container contents into the microcapillary, to provide a recovered aspirated cell population. The microcapillary in one embodiment, has a diameter of from about 5 μm to about 200 μm. In a further embodiment, the microcapillary has a diameter of from about 5 μm to about 200 μm, or from about 5 μm to about 150 μm, or from about 5 μm to about 100 μm, or from about 5 μm to about 75 μm, or from about 5 μm to about 50 μm, or from about 50 μm to about 200 μm, or from about 100 μm to about 200 μm, or from about 150 μm to about 200 μm.

[0096] In some embodiments, the microcapillary has a beveled tip. In some embodiments, the microcapillary has an oval, square or circular cross section. The microcapillary in some embodiments is mounted on a robotic micromanipulation system on a microscope to provide an automated recovery apparatus. In one embodiment, the microcapillary provided herein has a single barrel. However, the microcapillary in other embodiments has multiple barrels, for example a double barrel, a triple barrel, or more than three barrels. In one embodiment the microcapillary may be used to selectively recovery a subset of cells and beads from a chamber. In one embodiment the chamber may be a well of a multiwall plate. In one embodiment the

chamber may be a microfluidic chamber. In one embodiment the chamber may be an open microwell chamber.

[0097] In another embodiment, recovery is from a microfluidic chamber and is performed by first cutting a wall of the microfluidic chamber to create an access point and then extracting cells by aspiration using a microcapillary. In yet another embodiment, the microfluidic device is fabricated such that the chambers are exposed by peeling away the material on one wall, thereby leaving an open micro-well array. ASCs and/or associated particles in identified chambers are then aspirated from their respective chambers. In order to facilitate the precise extraction of microfluidic well contents, aspiration tools such as microcapillary tubes, in one embodiment, are mounted on a robotic micromanipulator, or a manual micromanipulator. However, aspiration in other embodiments is performed manually.

[0098] Recovery of one or more ASCs from one or more microfluidic chambers, and/or recovery of antigen display particles, in one embodiment, comprises magnetic isolation/recovery. For example, in one embodiment, a microfluidic chamber is exposed to a magnetic particle (or plurality of magnetic particles) that adheres to the one or more cells or particles (e.g., antibody capture particles) within the chamber.

[0099] Sequencing of ASC genetic material and/or displayed antigens from containers can be employed to determine whether an antibody-antigen interaction has taken place at step **1007** or **2008**, and the respective sequences of the antibody and antigen. In this embodiment, if an antigen sequence is present, it can be inferred that it binds to an antibody inside the container, because unbound library particles are removed from containers at wash step **1006** or **2007**. In one embodiment, at step **1007** or **2008**, containers having antigens that interact with antibodies are first determined via a fluorescence assay. The contents of the containers that are identified as having the interaction are harvested for nucleic acid sequencing. Any sequencing protocol can be used to identify the sequence of the antibody and/or the identity of the antigen, e.g., high-throughput sequencing or single molecule sequencing. Accordingly, the invention is not limited to the type of sequencer or sequencing methodology employed. Types of sequencers and sequencing technologies are described above.

[00100] In one embodiment, an Illumina MiSEQ instrument is used. High throughput sequencing protocols are well known to those of ordinary skill in the art. For example, see the following references for protocols amenable for use with the present invention. Gupta (2008). Trends in Biotechnology 26, pp. 602-611; Metzker. (2010). Nature Reviews Genetics 11, pp. 31-46; Schuster (2008). Nature Methods 5, pp. 16-18; Shendure and Ji (2008). Nat. Biotechnol. **26**, pp. 1135-1145, each of which is incorporated by reference in its entirety for all purposes.

[00101] In one embodiment, cells and beads from individual containers are separated prior to a sequencing step. This separation may have the advantage that display particles associated non-specifically with the cell would not contribute to background signal in identifying the antigen.

[00102] In one embodiment, more than one species of antigen display particle may be associated with any given antibody, either because the antibody is poly-reactive to multiple antigen species or because of non-specific interactions with the display particles and/or cells, or because the bead has been exposed to more than one species of secreted antibody. However, the degree of enrichment of display particles in chambers allows for identification of binding interactions that are significant. This may be facilitated by also analyzing antibody and display sequences obtained from "negative" wells in order to ascertain the background. In one embodiment, cells and beads from individual chambers are separated prior to amplification and/or a sequencing step. This separation may have the advantage that display particles associated non-specifically with the cell would not contribute to background signal in identifying the antigen; this is a major issue in strategies, as presented below, where the antigen-antibody interaction occurs on the surface of a cell. In one embodiment this separation is achieved by removing beads from the reactors that contain the ASC. In another embodiment the separation is achieved by first capturing the antibodies on a substrate that is in contact with the supernatant containing the secreted antibodies. In another embodiment the separation is achieved by removing a volume of the supernatant from each container (e.g., microwell) prior to capturing the secreted antibodies onto a solid phase, such as beads of a solid substrate, and then interacting said beads or solid substrate with the display library.

[00103] As provided herein, in the methods **1000** and **2000** described herein, the sample is distributed into a plurality of discrete containers. The discrete containers can be microfluidic chambers. Amongst all microfluidics technologies, multilayer soft lithography (MSL) is unique in its rapid and inexpensive prototyping of devices having thousands of integrated microvalves (Thorsen et el. (2002). Science 298, pp. 58-584, incorporated by reference in its entirety). These valves can be used to build higher-level fluidic components including mixers, peristaltic pumps (Unger et al. (2000). Science 7, pp. 113-116) and fluidic multiplexing structures (Thorsen et el. (2002). Science 298, pp. 58-584; Hansen and Quake (2003). Curr. Opin. Struc. Biol. 13, pp. 538-544, incorporated by reference in their entireties herein) thus enabling high levels of integration and on-chip liquid handling (Hansen et al. (2004). Proc. Natl. Acad. Sci. U.S.A. 101, pp. 14431-1436; Maerkl and Quake (2007). Science **315**, pp. 233-237, each incorporated by reference in their entireties).

[00104] Figure 4 shows an optical micrograph of a valve made by MSL. Two crossing microfabricated channels, one "flow channel" for the active fluids (vertical) and one control channel for valve actuation (horizontal), create a valve structure. The flow channel is separated from the control channels by a thin elastomeric membrane to create a "pinch valve." Pressurization of the control channel deflects the membrane to close off the flow channel.

[00105] The chambers of a device, in one embodiment, have an average volume of from about 100 pL to about 100 nL. For example, in one embodiment, a sample is distributed into a plurality of microfluidic chambers and the average volume of the plurality of chambers is about 100 pL, about 200 pL, about 300 pL, about 400 pL, about 500 pL, about 600 pL, about 700 pL, about 800 pL, about 900 pL or about 1 nL. In another embodiment, the average volume of the microfluidic chambers is about 2 nL. In another embodiment, the average volume of the microfluidic chamber is from about 100 pL to about 100 nL, from about 100 pL to about 50 nL, from about 100 pL to about 10 nL, from about 100 pL to about 1 nL, from about 50 pL to about 100 nL, from about 50 pL to about 50 nL, from about 50 pL to about 10 nL or from about 50 pL to about 1 nL. In even another embodiment, the average volume of the microfluidic chambers is about 10 nL, about 20 nL, about 30 nL, about 40 nL, about 50 nL, about 60 nL, about 70 nL, about 80 nL, about 90 nL or about 100 nL.

[00106] The MSL fabrication process takes advantage of well-established photolithography techniques and advances in microelectronic fabrication technology. The first step in MSL is to draw a design of flow and control channels using computer drafting software, which is then printed on high-resolution masks. Silicon (Si) wafers covered in photoresist are exposed to ultraviolet light, which is filtered out in certain regions by the mask. Depending on whether the photoresist is negative or positive, either areas exposed (negative) or not (positive) crosslinks and the resist will polymerize. The unpolymerized resist is soluble in a developer solution and is subsequently washed away. By combining different photoresists and spin coating at different speeds, silicon wafers are patterned with a variety of different shapes and heights, defining various channels and chambers. The wafers are then used as molds to transfer the patterns to polydimethylsiloxane (PDMS). In one embodiment, prior to molding with PDMS and after defining photoresist layers, molds are parylene coated (chemical vapor deposited poly(p-xylylene) polymers barrier) to reduce sticking of PDMS during molding, enhance mold durability and enable replication of small features

[00107] In MSL, stacking different layers of PDMS cast from different molds on top of each other is used to create channels in overlapping "flow" and "control" layers. The two (or more) layers are bound together by mixing a potting prepolymer component and a hardener component at complementary stoichiometric ratios to achieve vulcanization. In order to create a simple microfluidic chip, a "thick" layer (*e.g.*, between from about 200-2000 μms) is cast from the mold containing the flow layer, and the "thin" layer (*e.g.*, between from about 25 to about 300 μms) is cast from the mold containing the control layer. After partial vulcanization of both layers, the flow layer is peeled off its mold, and aligned to the control layer (while still present on its mold, by visual inspection. The control and flow layers are allowed to bond, for example at 80 °C for about 15-60 minutes. The double slab is then peeled from the control mold, and inlet and outlet holes are punched and the double slab is bonded to a blank layer of PDMS (*i.e.*, a flat layer of PDMS with no structural features). After allowing more time to bond, the completed device is mounted on a glass slide. Fluid flow in the device is controlled using off-chip computer programmable solenoids which actuate the pressure applied to fluid in the channels of the control layer. When pressure is applied to these control channels, the flexible membrane between the overlapping orthogonal control and flow lines deflects into the flow channel,

effectively valving the flow. Different combinations of these valves can be used to create peristaltic pumps, multiplexer controls and isolate different regions of the chip.

[00108] With respect to the flow layer, assay chambers and channels for controlling fluidic flow to and from the assay chambers are defined by the photoresist layers. As will be appreciated by one of skill in the art, the thickness of a photoresist layer can be controlled in part by the speed of spin coating and the particular photoresist selected for use. The bulk of the assay chambers, in one embodiment, are defined by an SU-8 100 feature which sits directly on the Si wafer. As known to those of skill in the art, SU-8 is a commonly used epoxy-based negative photoresist. Alternatively, other photoresists known to those of skill in the art can be used to define assay chambers with the heights described above. In some embodiments, the assay chambers have a height and width of 50-500 μM and 50-500 μM, respectively, as defined by the SU-8 features.

[00109] MSL fabrication techniques allow for a wide range of device densities, and chamber volumes to be fabricated. For the devices provided herein, in one embodiment, from about 2000 to about 10,000 discrete chambers are provided in a single integrated device. In one embodiment the discrete chambers are connected in a serial format. Alternatively or additionally, the discrete chambers are individually addressable. For example, a device with 4032 individual analysis chambers (average volume of 2.25 nL) connected in serial format achieve a screening throughput of approximately 100,000 cells per run, as described in PCT Publication No. WO 2014/153651, which published October 2, 2014, incorporated by reference in its entirety for all purposes. The integrated microfluidic valves harnessed in the devices provided herein allow for chamber isolation, and programmable washing with reagents selected from a plurality of inlets, for example from 2 to about 32 inlets, 2 to about 20 inlets, 2 to about 15 inlets, 2 to about 10 inlets, or from 2 to about 9 inlets, or from 2 to about 8 inlets, or from 2 to about 7 inlets or from 2 to about 6 inlets. Additional inlets can be provided to control valve pressure.

[00110] In one embodiment, the containers, e.g., microfluidic chambers or open microwells provided herein allow for the culture and maintenance of cells, e.g., to form an expanded lymphocyte population comprising ASCs (e.g., Figure 1, 1003; Figure 2, 2003). In

one embodiment, microfluidic arrays of chambers are fabricated within a thick membrane (*e.g.*, from about 150 μm to about 500 μm thick, about 150 μm to about 200 μm thick, about 150 μm to about 300 μm thick, about 150 μm to about 400 μm thick or about 500 μm thick) of PDMS elastomer that is overlaid a reservoir of medium, for example 1 mL of medium as described previously (Lecault *et al.* (2011). Nature Methods 8, pp. 581-586, incorporated by reference herein in its entirety for all purposes). The proximity of the medium reservoir (osmotic bath) to the cell chambers effectively blocks evaporation (through the gas-permeable PDMS material) and ensures robust cell viability and where cells are not fully differentiated, growth over several days, and is critical for achieving long-term culture in nL volumes with growth rates and cellular responses that are identical to microliter volume formats.

[00111] In embodiments of method **2000**, ASC subsamples are pre-enriched, prior to interrogation with a display library by functional characterization of the ASC or antibody secreted by the ASC in a particular subsample. In one embodiment of method **2000**, prior to interrogation of antibody binding to a displayed antigen, one or more functional assays are carried out on the ASC or secreted antibody to identify functional antibodies (Figure 2, **2004**). In one embodiment of the invention, after distributing the sample or expanded sample into a plurality of containers **2002**, a functional analysis is carried out to identify one or more functional ASCs that exhibits a desired property (affinity for a particular antigen, specificity for a particular antigen, etc.) (Figure 2, **2004**). In one embodiment of step **2004**, a subsample is co-incubated with a target cell or a readout cell (e.g. cancer cell, bacteria cell), and detected with a secondary antibody to confirm the presence of a cell binding antibody or other functional feature. In another embodiment of step **2004**, ASCs may also be fixed and/or permeabilized to look for binding to intracellular antigens. Other functional assays include, but are not limited to a blocking assay to identify blocking peptides, protein-cell binding assays, affinity and/or specificity assays.

[00112] Additional functional assays as described herein, and also in WO 2014/153651, incorporated by reference herein in its entirety, may be implemented at step **2004**, to detect blocking, agonist, antagonist or neutralization activity of the ASC or secreted antibody. From the functional assay, functional subsamples are identified, antibodies from the subsamples captured **2005** and subjected to further interrogation with a display library **2006**.

[00113] The functional assay can be carried out in the same container(s) as the container(s) for assessing binding to a display particle. In other words, step **2004** can be carried out in the same or different container than step **2005** and/or step **2006**. (Figure 2).

[00114] The methods provided herein allow for the initial identification of a functional ASC in the presence of other types of cells, including other lymphocytes and non-functional ASCs. The functional ASC can be present in a homogenous population of cells, a heterogeneous population or as a single cell. The functional ASC's secreted antibodies are captured for interrogation with the display library (Figure 2, **2005** and **2006**). The functional ASC(s), once identified, can be transferred to a separate container for antibody capture and subsequent library interrogation, or can be interrogated in the same container as the functional assay.

[00115] The functional assay used in the methods described herein may be varied considerably, according to the desired property the user of the method wishes to identify. As provided herein, defined medium conditions may be used to affect a polyclonal expansion and/or differentiation of ASC precursors into ASCs (Figure 2, **2001** and **2003**). In such cases possible antibody and ASC assays include without limitation, ELISA, ELISPOT, fluorescent binding assays, cell binding assays, neutralization assays, surface plasmon resonance, complement fixation assays, cell-mediated cytotoxicity assays, cell binding assays, competition assays, agglutination assays, receptor agonist assays, receptor antagonist assays, antibody internalization assays, etc. In one embodiment, a functional assay is performed directly on ASCs using methods such as FACS, microscopy, colony assays, and other assays known to those of ordinary skill in the art.

[00116] In some instances, the expansion of ASCs and/or ASC precursors facilitates the analysis of functional properties within conventional cell culture formats having volumes between ~10 microliters and 10 mL. Formats may also include miniaturized cell analysis reactors including microfluidic devices, microdroplets, open microwells, plates, or semi-solid medium. Volumes of miniaturized cell analysis reactors may include approximately 100 pL, 1 nL, 10 nL, 100 nL, or 1 μL.

[00117] In one embodiment, the functional assay (including binding property screening, e.g., affinity, specificity) is performed in a microfluidic or open well format as described in PCT

Publication No. WO 2014/153651, which published October 2, 2014, the disclosure of which is incorporated by reference in its entirety for all purposes. The functional assays described in WO 2014/153651 can be carried out in any of the containers described herein.

[00118] In one embodiment, prior to analysis of binding to the display library, the subsample (expanded or non-expanded) or subportion thereof is subjected to a functional assay to assess the binding to one or more cell surface receptors of the cell population. Containers that include cells which bind the one or more cell surface receptors are identified and cells from those containers are processed further.

[00119] In one embodiment, the functional assay is one or more functional assays described in PCT Publication No. WO 2014/153651, incorporated by reference herein in its entirety. In another embodiment, the functional assay is a neutralization assay, a serum bactericidal antibody assay (SBA) or an opsonophagocytic assay (OPA). For example, one or more of the functional assays described in Feavers and Walker (2010). Methods Mol. Biol. 626, pp. 199-211, incorporated by reference in its entirety for all purposes, can be used with the methods described herein.

[00120] In one embodiment, the functional assay is an ELISA assay.

[00121] In another embodiment, the functional assay is a complement dependent cytotoxicity assay (CDC) assay. In another embodiment, the functional assay is a complement-dependent cytotoxicity (CDC) assay. In one CDC embodiment, a method is provided for identifying the presence of a lymphocyte or progenitor thereof (or secreted immune receptor) that binds to a readout cell in the presence of soluble factors necessary and/or sufficient to induce lysis of a readout cell via the classic complement pathway. The assay is to determine whether an antibody secreted by a lymphocyte progenitor stimulates lysis of one or more target cells by the classic complement pathway. Cell lysis by the complement pathway is quantified according to methods known to those of skill in the art. For example, cell lysis is quantified by a clonogenic assay, by the addition of a membrane integrity dye, by the loss of intracellular fluorescent molecules or by the release of intracellular molecules in solution. The released biomolecules are measured directly in solution or captured onto readout particles in the assay container.

[00122] In another embodiment, the functional assay (Figure 2, **2004**) is an antibody-dependent cell mediated cytotoxicity (ADCC) assay. ADCC is a mechanism of cell-mediated immune defense whereby an effector cell of the immune system lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies. Classical ADCC is mediated by natural killer (NK) cells. However, macrophages, neutrophils and eosinophils can also mediate ADCC, and can be provided herein as cells to be used in an ADCC functional assay. ADCC assays are known to those of ordinary skill in the art and components are commercially available. For example, the Guava Cell Toxicity Kit for Flow Cytometry (Millipore), the ADCC Reporter Bioassay Core Kit (Promega), the ADCC Assay (GenScript), the LIVE/DEAD Cell Mediated Cytotoxicity Kit (Life Technologies) and the DELFIA cell toxicity assays can be utilized in the devices provided herein.

[00123] A cell growth modulation assay can be performed as a functional assay. The cell growth modulation assay can also be performed with a single readout cell or readout particles or a heterogeneous readout cell population in a single chamber, *i.e.*, a readout cell to determine whether cell growth is modulated. As used herein, a "readout" cell or particle is any particle, including a bead or cell, e.g., a functionalized bead or a cell that reports a functionality or property, or is used in an assay to determine an ASC or antibody effect. A "readout particle" can be present as a single readout particle or within a homogeneous or heterogeneous population of readout particles within a single container (e.g., microfluidic chamber). In one embodiment, a readout particle is a bead functionalized to bind one or more antibodies secreted by an ASC, or released by an ASC upon lysis of the ASC. A single readout particle may be functionalized to capture one or more different types of biomolecules, for instance a protein and/or nucleic acid, or one or more different monoclonal antibodies.

[00124] The cell growth modulation assay, in one embodiment, is adapted to screen for cells producing biomolecules that inhibit cell growth. In another embodiment, the method is adapted to screen for cells producing molecules that modulate, *i.e.*, increase or decrease, proliferation rates of readout cells. Growth rate, in one embodiment, is measured by manual or automated cell count from light microscopy images, total fluorescence intensity of cell expressing fluorescence, average fluorescence intensity of cells labeled with a dilutive dye (*e.g.*, CFSE), nuclei staining or some other method known to those of skill in the art. Commercially

available assay to measure proliferation include the alamarBlue® Cell Viability Assay, the CellTrace™ CFSE Cell Proliferation Kit and the CellTrace™ Violet Cell Proliferation Kit (Life Technologies), each of which can be used with the methods described herein.

[00125] In another embodiment, an apoptosis functional assay is carried out to determine a functional subsample of ASCs. In one embodiment, the method is used to identify the presence of an antibody that induces apoptosis of a cell.

[00126] In one embodiment, an autophagy assay is carried out as the functional assay (Figure 2, **2004**). In one embodiment, microscopic imaging of the subpopulation(s) is carried out after the assay, to assess autophagy using cell lines engineered with autophagy reporters that are known to those of ordinary skill in the art (*e.g.*, FlowCellect™ GFP-LC3 Reporter Autophagy Assay Kit (U20S) (EMD Millipore), Premo™ Autophagy Tandem Sensor RFP-GFP-LC3B Kit (Life Technologies)).

[00127] In one embodiment, a cytokine assay is performed as a functional assay on one or more subpopulations (or subsets thereof). Examples of commercially available cytokine-dependent or cytokine-sensitive cell lines for such assays include, but are not limited to TF- 1 , NR6R-3T3, CTLL-2, L929 cells, A549, HUVEC (Human Umbilical Vein Endothelial Cells), BaF3, BW5147.G.1.4.0UAR.1, (all available from ATCC), PathHunter® CHO cells (DiscoveRx) and TANGO cells (Life Technologies). A person skilled in the art will understand that primary cells (e.g., lymphocytes, monocytes) may also be used as readout cells for a cytokine assay.

[00128] In one embodiment, a signaling assay is used to identify a functional cell. Activation of a signaling pathway can be visualized by expression of a fluorescent reporter, translocation of a fluorescent reporter within a cell, a change in growth rate, cell death, a change in morphology, differentiation, a change in the proteins expressed on the surface of the readout cell, etc. In one embodiment the signaling assay includes a measurement of RNA transcription from readout cells. Several engineered reporter cell lines are commercially available and can be used to implement such an assay. Examples include PathHunter cells® (DiscoverRx), TANGO™ cells (Life Technologies) and EGFP reporter cells (ThermoScientific).

[00129] In one embodiment, the functional assay measures binding of an antibody to a cell surface protein or membrane bound or integral membrane receptor, such as a G-protein coupled receptor or other membrane bound or integral membrane protein. In another embodiment, the functional assay measures the activation of a cell signaling protein or the phosphorylation of a target protein.

[00130] The functional assay can be carried out in the same container or a different container than the container used to interrogate antibody binding to a display library. In one embodiment, the functional assay is carried out in a microfluidic format and the functional ASC(s) is recovered for further analysis, for example, for exposure to an antigen library (Figure 2, 2005, 2006). Recovery, from microfluidic devices is discussed in detail above. In another embodiment, the functional assay is carried out in a non-microfluidic format and the functional ASCs are partitioned into different containers upon completion of the functional assay. Once functional ASCs are identified (Figure 2, 2005), these cells are subjected to library interrogation as described in detail herein. The containers used to identify the functional lymphocytes (e.g., ASCs) can be further employed to interrogate these functional cells with the antigen display library. Alternatively, the functional ASCs can be further partitioned into different containers.

[00131] It is further noted that it is not necessary that every instance of a member of one library interact with every member of another library because in either library, there may be multiple instances of each library member, as well as multiple useful interactions that may be recovered without the need to have explored all possible interactions. Further, it will be appreciated that the recurrent detection of a specific antigen antibody interaction in multiple containers, even in the presence of additional antibodies and antigens, may be used to identify the specific antigen and antibody that are responsible for the detected interactions.

[00132] In yet another aspect of the invention, a method **3000**, summarized at Figure 3, is provided to enable the screening of libraries of antibodies against one or more cell-surface antigens. The method **3000** is particularly useful for rapid antibody discovery against cell surface proteins, including integral membrane proteins. The method is provided, in one embodiment, to enable the screening of libraries of antibodies against a cell-surface antigen without the high background that is obtained when panning antibody display libraries against

cells. proteins, including integral membrane proteins. The method **3000** further provides a direct means to assess whether certain antibodies express well in mammalian cells, thereby solving a frequent issue with display technologies. Finally, the method is much faster than existing methods for selecting antibodies against membrane protein targets that harness display technologies such as phage display, followed by nucleic acid sequencing.

[00133] Method **3000** comprises enriching an antibody display library for binding to one or more targets (antigens) to provide an enriched antibody display library **3001**. The enriched antibody display library is transferred into a mammalian, bacterial or yeast cell line engineered to express the antibodies present in the enriched antibody display library to produce populations of cells that secrete different antibodies that are derived from the original display library (also referred to as an antibody secreting cell line) **3002**. ASCs from the ASC cell line are distributed into a plurality of containers to provide ASC subsamples **3003**. Antibodies secreted from the ASC subsamples are interacted with particles such as a cells that express the one or more targets **3004**. A determination is then made as to the antibody sequence(s) (or antigen binding fragment thereof) that interacts with the one or more targets **3005**. For example, if a secreted antibody interacts with the one or more targets, the container contents are harvested for downstream sequencing.

[00134] The one or more targets (antigens), in one embodiment, comprises one or more surface proteins or integral membrane proteins displayed on the surface of cells. Specifically, in one embodiment, the one or more targets comprise one or more integral membrane proteins that cannot be secreted as soluble proteins. The cells that express integral membrane proteins that cannot be secreted as soluble proteins can be engineered to produce and release into the assay medium (e.g., cell culture medium) a plurality of virus like particles that consist of nanometer scale vesicles of cell membranes that incorporate the integral membrane surface proteins and which may be captured by appropriately conjugated microbeads in the vicinity of the cells. Methods for the production of such virus like particles are known to those of ordinary skill in the art and kits for use in producing such particles are sold commercially (e.g., MembranePro™ Functional Protein Expression System).

[00135] In one embodiment, the antibody display library subjected to method **3000** is a display library comprising Fab fragments. In another embodiment, the antibody display library is a mammalian display library. In another embodiment, the antibody display library expresses full length antibodies. In another embodiment, the antibody display library is a yeast antibody expression library or a yeast display library that can be induced to secrete antibodies. In another embodiment, the antibody display library is a library expressing single chain antibodies. In another embodiment, the antibody display library is a library expressing VHH class antibodies derived from the immune response of a camel, llama, or alpaca.

[00136] In one embodiment, it is advantageous to have the library of antibodies (or antigen binding fragments thereof) highly enriched for binding to the antigens secreted by the cells, or displayed by the cells (e.g., as a cell receptor or membrane protein) **3001**. This is accomplished in one embodiment, by design of the library of antibodies to have specific properties thought to enrich for binding to the one or more targets. In another embodiment, enrichment is accomplished by first enriching the library by panning on cells or collections of antigens that comprise, at least in part, the target epitope.

[00137] Such library enrichment may also be applied to the one or more target antigens. This may be achieved by judicious selection of the one or more target antigens that is relevant for the biological question under consideration. For example, the one or more target antigens can be present in tissue obtained from a subject having the particular disease.

[00138] Enriching libraries of antibodies may be of particular benefit when working from large libraries of antibodies in order to reduce the complexity of the library and thus ensure efficient interaction with the one or more target antigens. An enriched library may for example be produced by panning a display library such as a phage library expressing fab fragments of antibodies against one or more populations of cells or particles or surfaces that display the one or more targets, thereby positively enriching for active library members. Alternatively, an enriched antibody library may be fabricated by panning against particles, cells, or surfaces that do not express the one or more targets and recovering the unbound library members, thereby creating a library that is depleted for non-specific library members. Alternatively, a combination of depletion and positive panning may be used to create the enriched library.

[00139] As one example of enriching prior to screening **3001**, the application of finding antibodies that bind to one or more integral membrane protein targets is instructive. A display library that expresses a large number of antibody fab fragments may be first enriched for members that bind to the one or more integral membrane targets by first panning against a cell line that does not express the one or more targets, thereby depleting the library of irrelevant library members. This library may then be panned against the same cell line that expresses the one or more integral membrane protein targets in order to effect a positive enrichment. The resulting fab fragment library may then optionally isolated and amplified and then transferred into a mammalian, yeast or bacterial cell line engineered to express and secrete the fab library **3002**. Expression in the cell line may be done in fab format or alternatively, reformatted to produce conventional antibodies of a desired isotype. This library of reduced complexity in a further embodiment is interacted with a library of cells that express the integral membrane protein target(s) on their surface, or with virus like particles (VLPs) that contain the targets of interest. Detection of the interaction of a member of the library of reduced complexity with the particles (e.g., cells or VLPs) that contain the target may then be achieved using a detection reagent. Alternatively, detection of the interaction may be detected by a functional assay which may include a signaling response or lack of response from cells that express the one or more targets of interest.

[00140] In VLP embodiments, the VLPs may be immobilized in the vicinity of the ASCs. The VLPs can be further used to query if the library of antibody producing cells that are in close proximity to the VLPs produce antibodies that bind to the targets presented in the VLPs. In embodiments where an antigen expression cell or antigen expressing cells present their antigens on the cell surface, the antigen expressing cells are used to assess if the antibodies secreted by cells in close proximity to these cells bind to one or more of the targets. Labeled detection reagents are used to identify containers that include antigen and antibody binding interactions. Once containers with interactions are identified, the cells are sequenced to identify the antigens and antibodies that participate in these interactions. In another embodiment where the antigen expressing cell or library of antigen expressing cells present their antigens on the cell surface, the assessment of whether the antibodies secreted by cells in the close proximity to these cells bind to one or more of the targets may include a functional assay.

[00141] Nucleic acid expressing antibodies from the enriched antibody display library is transferred into a cell line (e.g., mammalian, bacterial or yeast cell line) engineered to secrete the respective antibody **3002**. Mammalian cell lines for expressing antibodies are known to those of ordinary skill in the art. For example, the cell lines described by Lai et al. and Li et al. can be used in the method **3000** described herein (Lai et al. (2013). Pharmaceuticals 6, pp. 579-603; Li et al. (2010). mAbs 2:5, pp. 466-477, each of which is incorporated by reference herein in its entirety for all purposes).

[00142] In one embodiment, antibodies are produced in a mammalian host cell line selected from NS0 murine myeloma cells, PER.C6® human cells, and Chinese hamster ovary (CHO) cells. With respect to Murine NS0 cells, both cholesterol auxotrophs and cholesterol-independent cell lines can be used with method **3000** and step **3002**.

[00143] The ASCs are distributed into a plurality of discrete containers (e.g., microwell plate well, microfluidic chambers, open wells) **3003**. The containers are in some embodiments, microfluidic chambers, open microwells, microfuge tubes, microdroplets, or a semi solid medium such as a matrigel. The discrete containers, in one embodiment, are open wells in a microplate, microcentrifuge tubes, or microfluidic chambers, for example, the microfluidic chambers described in PCT Publication No. WO 2014/153651, incorporated by reference herein in its entirety. Combinations of the foregoing can also be employed. In one embodiment, the discrete chambers comprise a plurality of microfluidic chambers connected to microchannels that are controlled by integrated pneumatic microvalves. In one embodiment, the discrete containers comprise a plurality of open microwells. In even a further embodiment, the average aspect ratio (height : width) for each microfluidic chamber is about 1:1 or greater than 1:1. In even a further embodiment, the average aspect ratio (height : width) for each open microwell is about 1:1 or greater than 1:1. The aspect ratio for each of the microfluidic chamber or microwell is chosen to allow for cell retention in the respective microwell during exchange of reagents in the microwell.

[00144] Method **3000** can be carried out in a variety of different container types. For example, in one embodiment, a container is an individual well of a multiwell plate, or an individual chamber in a microfluidic device. In one embodiment, the discrete containers are wells in a 96, 384 or 1536 microwell plate. In one embodiment, the ASCs are split into at least

about 50, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1000, at least about 2000, at least about 5000 discrete containers, at least about 10,000 discrete chambers, at least about 20,000 discrete chambers, at least about 50,000 discrete chambers, at least about 100,000 discrete chambers, at least about 500,000 discrete chambers or at least about 1,000,000 discrete chambers (e.g., open microwells or microfluidic chambers) for further processing. The number of ASCs in each container can be the same or different. Moreover, tissues from multiple patients can be analyzed on an individual plate or device comprising multiple containers.

[00145] In one embodiment, each container includes from on average, about a single ASC to about 10,000 ASCs. It should be noted that each container need not contain an ASC. Accordingly, in some embodiments, one or some individual containers will have zero ASCs or zero lymphocytes present, or an individual ASC or an individual lymphocyte present.

[00146] Once the ASCs are distributed into a plurality of containers, antibodies secreted by the ASCs are interacted with a library of cells or particles that express one or more targets of interest **2004**. In one embodiment, the one or more targets of interest comprise an integral membrane protein. The target in one embodiment, is an ion-channel receptor, ABC transporter, a G-protein coupled receptor (GPCR), a receptor tyrosine kinase (RTK) or a receptor with intrinsic enzymatic activity such as intrinsic guanylate cyclase activity. In a further embodiment, the integral membrane protein is a GPCR.

[00147] The one or more targets is selected by the user of the method **3000**. In order to detect binding of an antibody to the target **3004**, such as an integral membrane protein, the target can be expressed on the surface of a particle such as a cell. For example, cell lines that express particular GPCRs, engineered to provide a readout of binding, activation or inhibition, are commercially available for example, from Life Technologies (GeneBLAzer® and Tango™ cell lines), DiscoveRx, Cisbio, Perkin Elmer, etc., and are amenable for use in method **3000**. In another embodiment, a particle population comprises a vesicle or a bead functionalized with a membrane extracts (available from Integral Molecular), or a stabilized solubilized GPCR (e.g., Heptares). Antibody binding to the particle population is then assessed at **3004**.

[00148] In one embodiment, the target is expressed or tethered to the surface of cells, or secreted by the cells, captured locally, and then interacted with antibodies. The tested antibody library may be Fab fragments that are expressed by bacteria, yeast, or mammalian cells as known to those of ordinary skill in the art. In another embodiment, of this aspect, the antibody library may be derived from an antibody display library fabricated using genetic material from a patient, for example, a patient having one of the autoimmune diseases set forth in Table 1, from an immunized animal, or from a synthetic source.

[00149] Without wishing to be bound by theory, it is thought that one advantage of this method is that the cells present the antigen in a more native format. This can be critical if recognized epitopes include glycosylation or other modifications that would be lost in phage, or if the phage cannot express some of the classes of proteins, such as integral membrane proteins such as G protein coupled receptors (GPCRs).

[00150] The analysis of the antibody library may be done to identify antibodies that have desired functional properties. In one embodiment, a β-arrestin GPCR assay that can be universally used for the detection of antagonists and agonists of GPCR signaling is used in method step **3004** (Rossi et a/. (1997). Proc. Natl. Acad. Sci. U.S.A. 94, pp. 8405-841 0, incorporated by reference in its entirety for all purposes). This assay is based on a β-galactosidase (β-Gal) enzyme-complementation technology, now commercialized by DiscoveRx. The GPCR target is fused in frame with a small N-terminal fragment of the β-Gal enzyme. Upon GPCR activation, a second fusion protein, containing β-arrestin linked to the N-terminal sequences of β-Gal, binds to the GPCR, resulting in the formation of a functional β-Gal enzyme. The β-Gal enzyme then rapidly converts non-fluorescent substrate Di-D-Galactopyranoside (FDG) to fluorescein, providing large amplification and excellent sensitivity. In this embodiment, cells (with GPCRs) are preloaded with cell-permeable pro-substrate (acetylated FDG) which is converted to cell-impermeable FDG by esterase cleavage of acetate groups.

[00151] In one embodiment, activation of a GPCR an antibody is determined by measuring the increase in cytosolic calcium in one or more readout cells **3005**. In a further embodiment, the increase in cytosolic calcium is detected with one or more calcium sensitive dyes. Calcium sensitive dyes have a low level of fluorescence in the absence of calcium and

undergo an increase in fluorescent properties once bound by calcium. The fluorescent signal peaks at about one minute and is detectable over a 5 to 10 minute window. Thus, to detect activity using fluorescent calcium the detection and addition of the agonist are closely coupled. In order to achieve this coupling, the ASCs are exposed simultaneously to the population of readout cells and the one or more calcium sensitive dyes. In one embodiment, the one or more calcium sensitive dyes are one provided in a FLIPR™ calcium assay (Molecular Devices).

[00152] The recombinant expressed jellyfish photoprotein, aequorin, in one embodiment, is used in a GPCR screen **3004** and **3005**. Aequorin is a calcium-sensitive reporter protein that generates a luminescent signal when a coelenterazine derivative is added. Engineered cell lines with GPCRs expressed with a mitochondrially targeted version of apoaequorin are available commercially (Euroscreen). In one embodiment, the one or more of the cell lines available from Euroscreen is used as a the cells expressing the target(s) of interest **3004**.

[00153] In one embodiment, antibody interaction with a GPCR is determined by using one of the ACTOne cell lines (Codex Biosolutions), expressing a GPCR and a cyclic nucleotide-gated (CNG) channel **3004** and **3005**. In this embodiment, determining the antibody interaction is accomplished with cell lines that contain an exogenous Cyclic Nucleotide-Gated (CNG) channel. The channel is activated by elevated intracellular levels of cAMP, which results in ion flux (often detectable by calcium-responsive dyes) and cell membrane depolarization which can be detected with a fluorescent membrane potential (MP) dye. The ACTOne cAMP assay allows both end-point and kinetic measurement of intracellular cAMP changes with a fluorescence microplate reader.

[00154] A reporter gene assay, in one embodiment, is used to determine whether an antibody binds to a particular GPCR **3005**. A reporter gene assay, in one embodiment, is based on a GPCR second messenger such as calcium (API or NFAT response elements) or cAMP (CRE response element) to activate or inhibit a responsive element placed upstream of a minimal promoter, which in turn regulates the expression of the reporter protein chosen by the user. Expression of the reporter, in one embodiment, is coupled to a response element of a transcription factor activated by signaling through a GPCR. For example, reporter gene expression can be coupled to a responsive element for one of the following transcription factors:

ATF2/ATF3/AFT4, CREB, EL 1/SRF, FOS/JUN, MEF2, GLI, FOXO, STAT3, NFAT, NFKB. In a further embodiment, the transcription factor is NFAT. Reporter gene assays are available commercially, for example from SA Biosciences

[00155] Reporter proteins are known in the art and include, for example, β-galactosidase, luciferase (see, e.g., Paguio et al. (2006). "Using Luciferase Reporter Assays to Screen for GPCR Modulators," Cell Notes Issue 16, pp. 22-25 ; Dual-Glo™ Luciferase Assay System Technical Manual #TM058; pGL4 Luciferase Reporter Vectors Technical Manual #TM259, each incorporated by reference in their entireties for all purposes), GFP, YFP, CFP, β-lactamase. Reporter gene assays for measuring GPCR signaling are available commercially and can be used in the methods and devices described herein. For example, the GeneBLAzer® assay from Life Technologies is amenable for use with the present invention.

[00156] In one embodiment, overexpression of a G protein in a reporter cell is carried out to force a cAMP coupled GPCR to signal through calcium. This is referred to as force coupling.

[00157] In one embodiment, a Gq coupled cell line is used as a readout cell line in the methods described herein. In one embodiment, the Gq coupled cell line reports GPCR signaling through β-lactamase, for example, one of the cell-based GPCR reporter cell lines (GeneBLAzer®, Life Technologies). The reporter cell line can be division arrested or include normal dividing cells.

[00158] Cellular events that result from GPCR receptor activation or inhibition can also be detected to determine an antibody's ability to activate or antagonize a readout cell **3005**. For example, in the case of the Gq coupled receptors, when the GPCR is activated, the Gq protein is activated, which results in the phospholipase C cleavage of membrane phospholipids. This cleavage results in the generation of inositol triphosphates 3 (IP3). Free IP3 binds to its target at the surface of the endoplasmic reticulum causing a release of calcium. The calcium activates specific calcium responsive transcription vectors such as nuclear factor of activated T-cells (NFAT). Thus, by monitoring NFAT activity or expression, an indirect readout of the GPCR in a readout cell is established. See. e.g., Crabtree and Olson (2002). Cell 109, pp. S67-S79, incorporated by reference herein in its entirety.

[00159] ASCs and/or antibodies from containers that display the interaction **3004** are subjected to molecular biology protocol(s) to isolate and/or amplify and sequence the antibody nucleic acid. Sequencing of antibody determines the identity of the relevant antibody(s) at step **3005**.

[00160] Types of sequencers and sequencing technologies amenable for use with the methods presented herein include, but are not limited to, the Genome Sequencer 20/FLX (commercialized by 454/Roche); MiSEQ instrument (Illumina), 'Solexa 1G' (later named 'Genome Analyzer' and commercialized by Illumina/Solexa), SOLiD™ system (commercialized by Applied Biosystems), and Polonator G.007 (commercialized by Dover Systems). Other protocols amenable for use with the methods provided herein include Polony sequencing, Helioscope™ single molecule sequencing, Lynx Therapeutics' Massively Parallel Signature Sequencing (MPSS), 454 pyrosequencing, Ion Torrent™ (Life Technologies), DNA nanoball sequencing (via rolling circle amplification), and VisiGen Biotechnologies approach.

[00161] In one embodiment, an Illumina MiSEQ instrument is used. High throughput sequencing protocols are well known to those of ordinary skill in the art. See, e.g., Gupta (2008). Trends in Biotechnology **26**, pp. 602-611; Metzker. (2010). Nature Reviews Genetics **11**, pp. 31-46; Schuster (2008). Nature Methods **5**, pp. 16-18; Shendure and Ji (2008). Nat. Biotechnol. **26**, pp. 1135-1145, each of which is incorporated by reference in its entirety for all purposes.

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[00162] All, documents, patents, patent applications, publications, product descriptions, and protocols which are cited throughout this application are incorporated herein by reference in their entireties for all purposes.

[00163] The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Modifications and variation of the above-described embodiments of the invention are possible without departing from the invention, as appreciated by those skilled in the art in light

of the above teachings. It is therefore understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.

CLAIMS

1. A method comprising

optionally, subjecting a sample comprising one or more antibody secreting cells (ASCs) or ASC precursor cells to conditions suitable for expansion of the one or more ASCs or ASC precursor cells, to form an expanded sample comprising ASCs;

distributing the sample into a plurality of containers to provide a plurality of subsamples;

optionally, subjecting one or more subsamples to conditions suitable for expansion of the one or more ASCs or ASC precursor cells in the one or more subsamples to form one or more expanded subsamples comprising ASCs;

capturing antibodies, present in one or more of the subsamples, on a solid substrate in one or more of the containers to provide captured antibodies;

exposing the captured antibodies in one or more of the containers to an antigen display library comprising a plurality of display particles for a time sufficient to allow for interaction of the captured antibodies with the one of more of the display particles;

capturing one or more display particles that interact with one or more of the antibodies;

optionally washing the solid substrate to remove unbound display particles; and

sequencing an antibody nucleic acid or antigen binding fragment thereof that interacts with one or more of the display particles.

2. A method, comprising

optionally, subjecting a sample comprising one or more antibody secreting cells (ASCs) or ASC precursor cells to conditions suitable for expansion of the one or more of the lymphocytes to form an expanded sample comprising ASCs;

distributing the sample into a plurality of containers to provide a plurality of subsamples;

optionally, subjecting one or more subsamples to conditions suitable for expansion of one or more ASCs or ASC precursor cells in the one or more subsamples to form one or more expanded subsamples comprising ASCs;

performing a functional assay on one or more of the subsamples to identify one or more functional subsamples, wherein the functional assay measures a property of an antibody secreted by an ASC in the one or more subsamples;

identifying one or more functional subsamples based on the results of the functional assay;

capturing antibodies from one or more functional subsamples on a solid substrate in one or more containers to provide captured antibodies;

exposing the captured antibodies in one or more of the functional subsamples to an antigen display library comprising a plurality of display particles for a time sufficient to allow for interaction of the captured lymphocyte receptors with the one of more of the display particles;

capturing one or more display particles that interact with one or more of the captured antibodies;

optionally washing the solid substrate to remove unbound display particles; and

sequencing an antibody nucleic acid or antigen binding fragment thereof that interacts with one or more of the display particles.

3. The method of claim 1 or 2, further comprising determining the containers that contain the captured display particles prior to the sequencing step.

4. The method of claim 3, wherein determining the containers that contain the captured display particles comprises

imaging containers to detect a signal from a detection reagent to identify positive containers that include captured display particles.

5. The method of claim 4, wherein the detection reagent is a labeled display particle.

6. The method of claim 5, wherein the labeled display particle comprises a labeled secondary antibody that binds to the display particle.

7. The method of any one of claims 2-6, wherein the functional assay is an ELISA, ELISPOT, fluorescent binding, cell binding assays, neutralization assay, surface plasmon resonance, complement fixation, cell-mediated cytotoxicity, cell binding assay, competition assay, agglutination assay, or a combination thereof.

8. The method of any one of claims 2-6, wherein the functional assay is an ELISA assay.

9. The method of any one of claims 2-6, wherein the functional assay is an antibody affinity or specificity assay.

10. The method of any one of claims 2-6, wherein the functional assay is an antibody ELISPOT assay.

11. The method of any one of claims 2-6, wherein the functional assay is a cytokine neutralization assay, a virus neutralization assay or an enzyme neutralization assay.

12. The method of any one of claims 2-11, wherein the antibodies from the one or more functional subsamples are captured in the same container as the container used for the functional assay.

13. The method of any one of claims 2-11, wherein the antibodies from the one or more functional subsamples are captured in a different container from the container used for the functional assay.

14. The method of any one of claims 1-13, comprising subjecting the sample comprising one or more antibody secreting cells (ASCs) or ASC precursor cells to conditions suitable for expansion of the one or more of the ASCs or ASC precursor cells, to form an expanded sample comprising ASCs.

15. The method of any one of claims 1-14, wherein one or more subsamples are subjected to conditions suitable for expansion of one or more antibody secreting cells (ASCs) or ASC precursor cells in the one or more subsamples to form one or more expanded subsamples comprising ASCs.

16. The method of any one of claim 1-15, wherein the conditions suitable for expansion comprise conditions suitable for polyclonal activation.

17. The method of any one of claim 1-15, wherein the conditions suitable for expansion comprise conditions suitable for antigen-specific activation.

18. The method of any one of claims 1-15, wherein the conditions suitable for expansion comprise the use of an EL-4-B5 cell line as a feeder layer.

19. The method of any one of claims 1-15, wherein the conditions suitable for expansion comprise the use of CD40 ligand.

20. The method of any one of claims 1-15, wherein the conditions suitable for expansion comprise treatment with Epstein Barr virus, CD40L, one or more toll like receptor agonists, phorbol 12-myristate 13-acetate (PMA) in combination with ionomycin or phytohaemagglutinin (PHA) activation, irradiated allogeneic peripheral blood mononuclear cells (PBMC) in combination with soluble anti-CD3 mAB, or a combination thereof.

21. The method of any one of claims 1-15, wherein the conditions suitable for expansion comprise treatment with one or more cytokines, a cell surface ligand selected from CD40L, BAFF and APRIL, a Toll-like receptor agonists selected from LPS, CpG, R848, PWM, a monoclonal antibody against a cell surface receptor selected from anti-CD40 and anti-IgG, or a feeder cell line providing co-stimulation signals.

22. The method of any one of claims 1-21, wherein the ASCs comprise plasma cells, plasmablasts, memory B-cells, cells that recombinantly express monoclonal antibodies, hybridoma cells, or a combination thereof.

23. The method of any one of claims 1-22, wherein the ASCs comprise plasma cells.

24. The method of any one of claims 1-23, wherein the ASCs comprise plasmablasts.

25. The method of any one of claims 1-24, wherein the ASCs comprise memory B-cells.

26. The method of any one of claims 1-25, wherein the ASCs comprise plasma cells.

27. The method of any one of claims 1-26, wherein the ASCs comprise cells that recombinantly express monoclonal antibodies

28. The method of any one of claims 1-27, wherein the ASCs comprise hybridoma cells.

29. The method of any one of claims 1-28, wherein the solid substrate comprises a functionalized bead.

30. The method of claim 29, wherein the functionalized bead comprises a protein A functionalized bead.

31. The method of claim 29 or 30, wherein the functionalized bead comprises a protein G functionalized bead.

32. The method of any one of claims 29-31, wherein the functionalized bead comprises polyclonal antibody against human immunoglobulin.

33. The method of any one of claims 29-32, wherein the functionalized bead is magnetic.

34. The method of any one of claims 1-33, wherein the solid substrate comprises a functionalized container surface.

35. The method of claim 34, wherein the functionalized container surface comprises a protein A functionalized container surface.

36. The method of claim 34 or 35, wherein the functionalized container surface comprises a protein G functionalized container surface.

37. The method of any one of claims 34-36, wherein the functionalized container surface comprises a polyclonal antibody against human immunoglobulin functionalized container surface.

38. The method of any one of claims 1-37, wherein the antigen display library is a phage display library.

39. The method of any one of claims 1-37, wherein the antigen display library is a yeast display library.

40. The method of any one of claims 1-39, wherein the antigen display library is a phage display library, yeast display library, ribosome display library, mRNA display library, cDNA display library, or a combination thereof.

41. The method of any one of claims 1-40, wherein sequencing comprises a high throughput sequencing reaction.

42. The method of any one of claims 1-41, wherein the sample is obtained from a human subject.

43. The method of claim 42, wherein the human subject has one of the autoimmune diseases set forth in Table 1.

44. The method of claim 42, wherein the human subject has cancer.

45. A method, comprising,

enriching an antibody display library for binding to one or more targets to provide an enriched antibody display library;

transferring the enriched antibody display library into a mammalian, yeast or bacterial cell line engineered to express the antibodies present in the enriched antibody display library to produce a cell line comprising antibody secreting cells (ASCs);

distributing the ASCs into a plurality of containers to provide ASC subsamples;

interacting one or more antibodies in one or more ASC subsamples with particles that express the one or more targets; and

determining the nucleic acid sequence(s) of an antibody that interacts with the one or more targets of interest.

46. The method of claim 45, wherein the antibody display library is a display library comprising Fab fragments.

47. The method of claim 45 or 46, wherein the one or more targets comprises an integral membrane protein.

48. The method of claim 47, wherein the integral membrane protein is a G-protein coupled receptor (GPCR).

49. The method of claim 47, wherein the one or more targets is an ion channel.

50. The method of any one of claims 45-49, wherein enriching comprises panning the antibody display library against a cell line that does not express the one or more targets of interest to deplete the library of irrelevant library members.

51. The method of any one of claims 45-50, wherein the enriched antibody display library is transferred to a mammalian cell line, and the mammalian cell line expresses the antibodies as Fab fragments.

52. The method of any one of claims 45-50, wherein the enriched antibody display library is transferred to a yeast cell line, and the yeast cell line expresses the antibodies as Fab fragments.

53. The method of any one of claims 45-51, wherein the particles that express the one or more targets of interest are virus like particles (VLPS).

54. The method of any one of claims 45-52, further comprising,

imaging containers to detect a signal from a detection reagent to identify positive containers that include antibody bound to the one or more of the targets.

55. The method of any one of claims 45-53, wherein the containers comprise open microwells.

56. The method of any one of claims 45-53, wherein the containers comprise microfluidic chambers.

57. The method of any one of claims 45-56, wherein the detection reagent is a labeled antibody.

58. The method of claim 57, wherein the labeled antibody comprises a labeled secondary antibody.

FIGURE 1

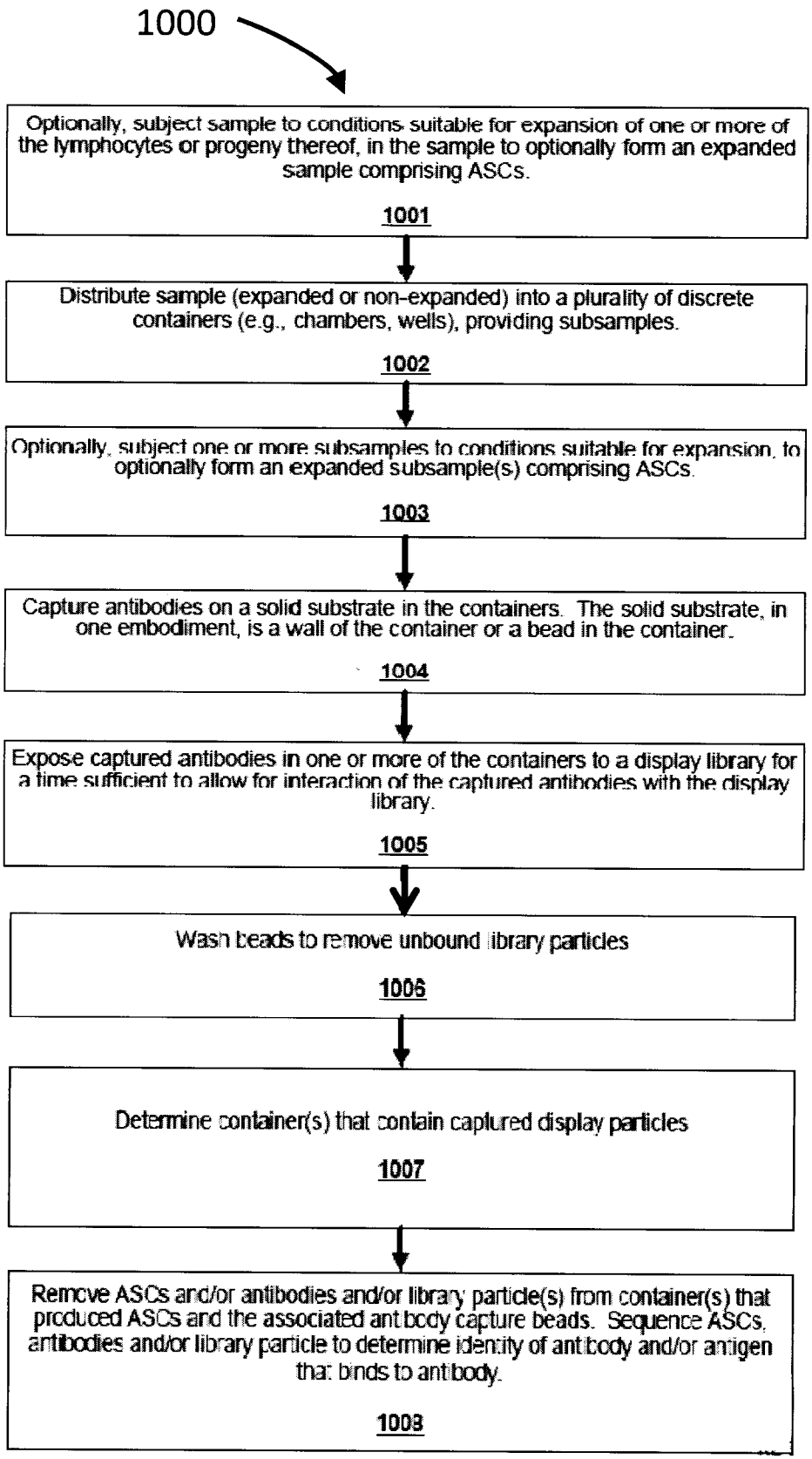

1000
Optionally, subject sample to conditions suitable for expansion of one or more of the lymphocytes or progeny thereof, in the sample to optionally form an expanded sample comprising ASCs.
1001
Distribute sample (expanded or non-expanded) into a plurality of discrete containers (e.g., chambers, wells), providing subsamples.
1002
Optionally, subject one or more subsamples to conditions suitable for expansion, to optionally form an expanded subsample(s) comprising ASCs.
1003
Capture antibodies on a solid substrate in the containers. The solid substrate, in one embodiment, is a wall of the container or a bead in the container.
1004
Expose captured antibodies in one or more of the containers to a display library for a time sufficient to allow for interaction of the captured antibodies with the display library.
1005
Wash beads to remove unbound library particles
1006
Determine container(s) that contain captured display particles
1007
Remove ASCs and/or antibodies and/or library particle(s) from container(s) that produced ASCs and the associated antibody capture beads. Sequence ASCs, antibodies and/or library particle to determine identity of antibody and/or antigen that binds to antibody.
1008

FIGURE 2

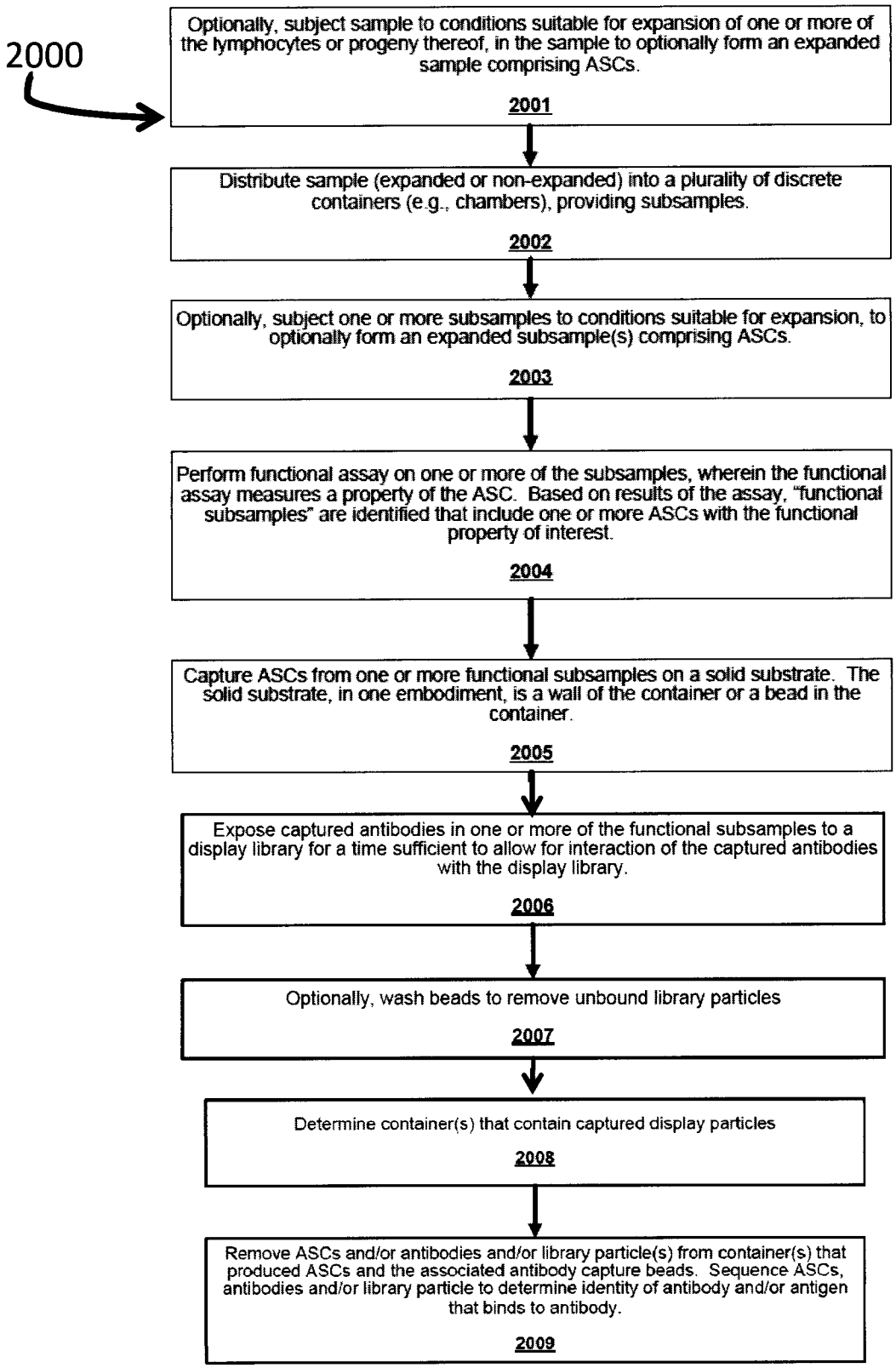

2000
Optionally, subject sample to conditions suitable for expansion of one or more of the lymphocytes or progeny thereof, in the sample to optionally form an expanded sample comprising ASCs.
2001
Distribute sample (expanded or non-expanded) into a plurality of discrete containers (e.g., chambers), providing subsamples.
2002
Optionally, subject one or more subsamples to conditions suitable for expansion, to optionally form an expanded subsample(s) comprising ASCs.
2003
Perform functional assay on one or more of the subsamples, wherein the functional assay measures a property of the ASC. Based on results of the assay, "functional subsamples" are identified that include one or more ASCs with the functional property of interest.
2004
Capture ASCs from one or more functional subsamples on a solid substrate. The solid substrate, in one embodiment, is a wall of the container or a bead in the container.
2005
Expose captured antibodies in one or more of the functional subsamples to a display library for a time sufficient to allow for interaction of the captured antibodies with the display library.
2006
Optionally, wash beads to remove unbound library particles
2007
Determine container(s) that contain captured display particles
2008
Remove ASCs and/or antibodies and/or library particle(s) from container(s) that produced ASCs and the associated antibody capture beads. Sequence ASCs, antibodies and/or library particle to determine identity of antibody and/or antigen that binds to antibody.
2009

FIGURE 3

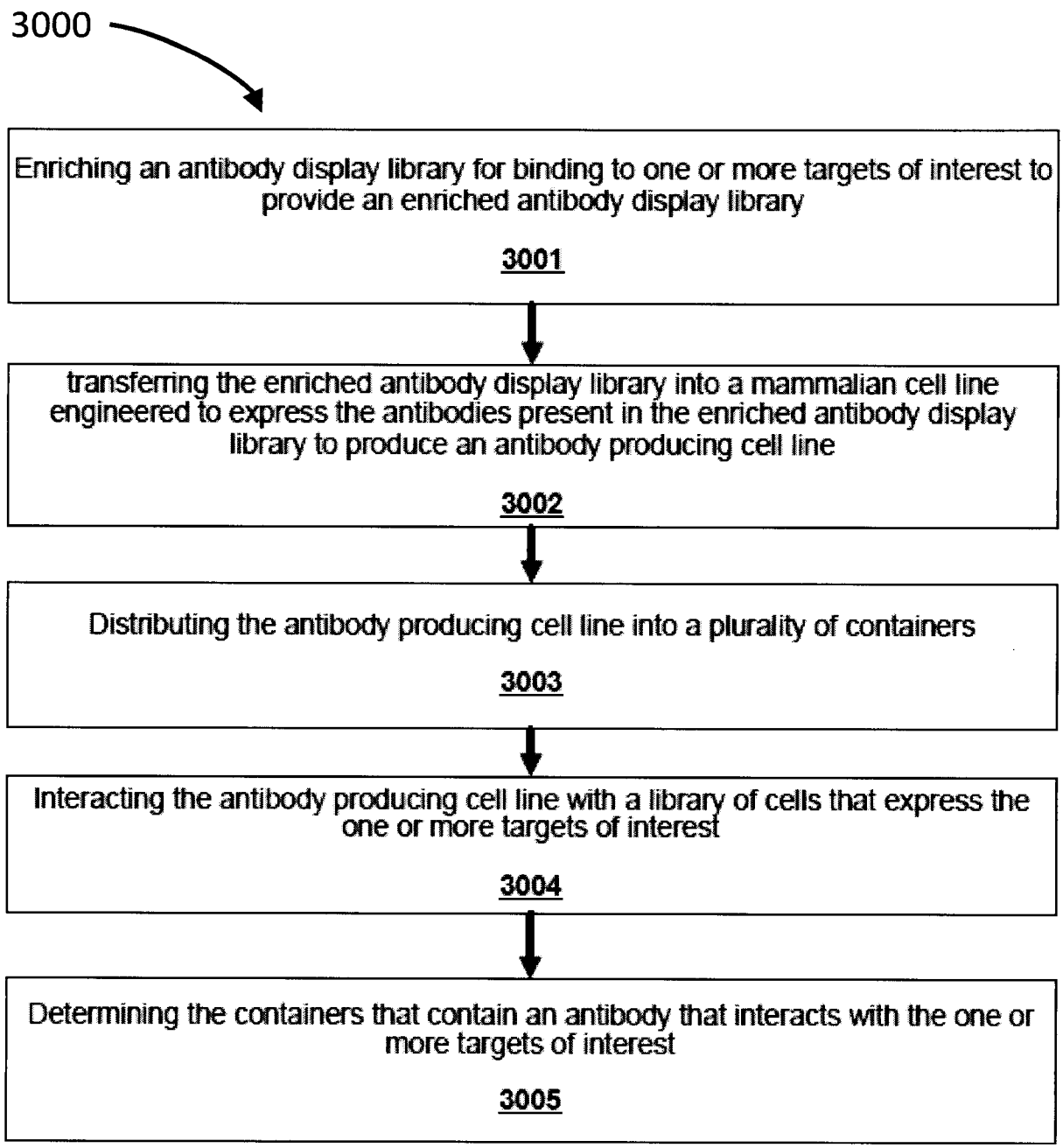
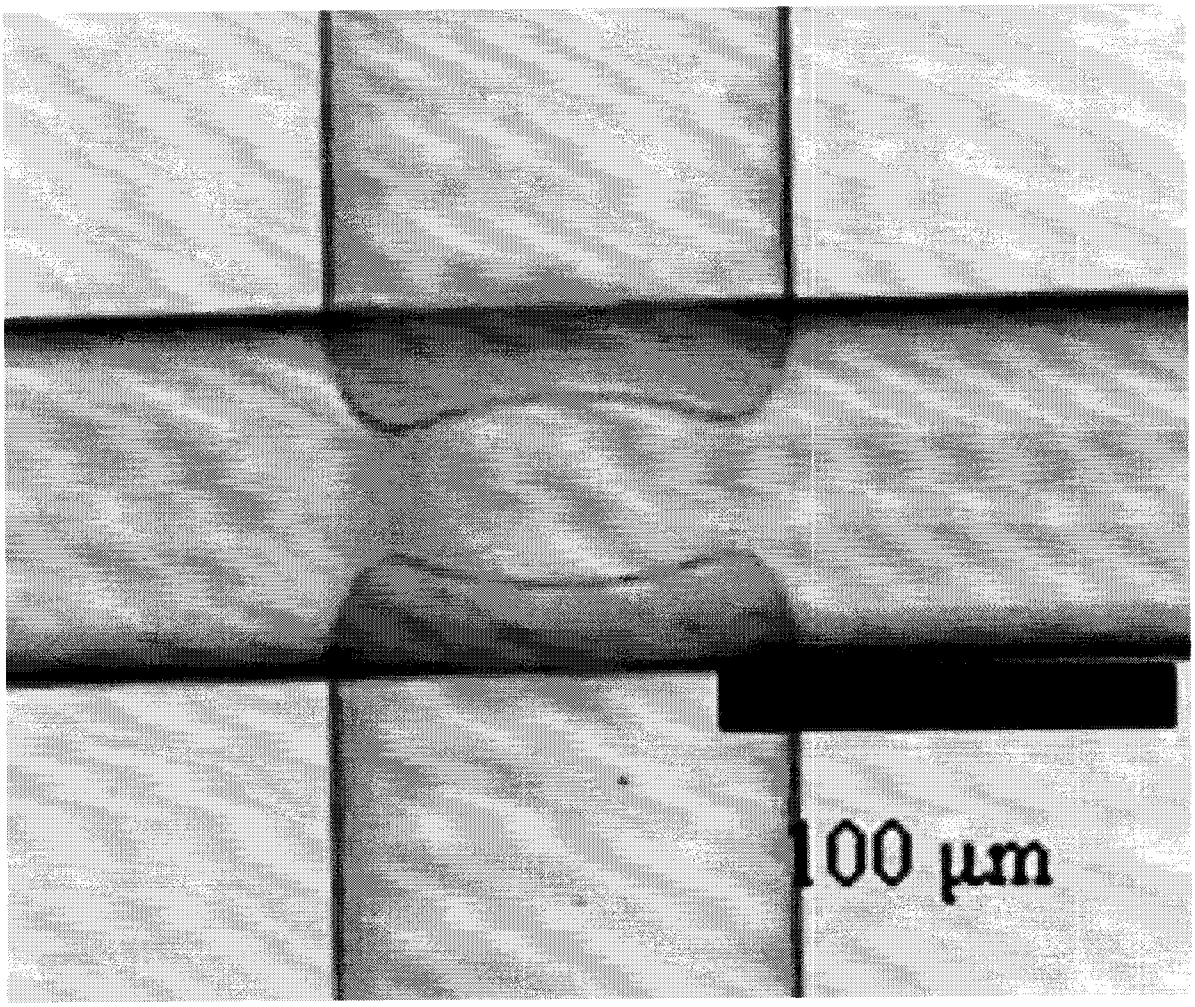

3000
Enriching an antibody display library for binding to one or more targets of interest to provide an enriched antibody display library
3001
transferring the enriched antibody display library into a mammalian cell line engineered to express the antibodies present in the enriched antibody display library to produce an antibody producing cell line
3002
Distributing the antibody producing cell line into a plurality of containers
3003
Interacting the antibody producing cell line with a library of cells that express the one or more targets of interest
3004
Determining the containers that contain an antibody that interacts with the one or more targets of interest
3005

FIGURE 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/000323

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C40B 30/04*** (2006.01) , ***C12N 5/078*** (2010.01) , ***C12Q 1/02*** (2006.01) , ***C12Q 1/68*** (2006.01) , ***C40B 20/00*** (2006.01) , ***C40B 40/02*** (2006.01) (more IPCs on the last page)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C40B 30/04, and keywords used across the whole IPC

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian patent database, Questel Orbit, Scopus and STN: Biosis, CaPlus, Medline, Inpadoc and DWPI
keywords: antibody secreting cells (ASC), display library, sample, lymphocyte, solid substrate, high throughput
Carl Hansen, Véronique Lecault and Kevin Heyries with University of British Columbia and AbCellera

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/153651 (The University of British Columbia) 2 October 2014 (02-10-2014) Whole document	1-17, 21-37, 40-45, 47-49 and 54-58
Y		18-20, 38, 39, 46 and 50-53
Y	CROWE, J.E., Technical sheet for the EL4-B5 cell line sold by Kerafast (catalog number: EVU301), 24 December 2009 (24-12-2009), [retrieved on 23 February 2017]. Retrieved from https://www.kerafast.com/product/2291/cd40l-expressing-feeder-cell-line-el-4-b5 Whole document	18-20
Y	HOOGENBOOM, H.R., "*Selecting and screening recombinant antibody libraries*". Nature Biotechnology, September 2005 (09-2005), Vol. 23(9), pp. 1105-1116. Whole document	38, 39, 46 and 50-53

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 22 February 2017 (20-02-2017)	Date of mailing of the international search report 08 March 2017 (08-03-2017)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Nathalie Chartrand (819) 639-7781

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2016/000323

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

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

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☑ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Box III

Group A - Claims 1 (completely) and 3-6, 14-44 (partially) are directed to a method wherein optionally a sample comprising antibody secreting cells (ASCs) is subjected to conditions suitable for expansion of the ASCs, the sample is then distributed into a plurality of containers to provide a plurality of subsamples; optionally, subjecting the subsamples to conditions suitable for expansion of the ASCs; capturing antibodies present in the subsamples on a solid substrate to provide captured antibodies; exposing the captured antibodies to an antigen display library comprising a plurality of display particles; capturing one or more display particles that interact with one or more of the antibodies and sequencing an antibody nucleic acid or antigen binding fragment thereof that interacts with one or more of the display particles;
Group B - Claims 2, 7-13 (completely) and 3-6, 14-44 (partially) are directed to a method wherein optionally a sample comprising antibody secreting cells (ASCs) is subjected to conditions suitable for expansion of the ASCs, the sample is then distributed into a plurality of containers to provide a plurality of subsamples; optionally, subjecting the subsamples to conditions suitable for expansion of the ASCs; performing a functional assay on one or more subsamples to identify one or more functional subsamples, wherein the functional assay measures a property of an antibody secreted by an ASC in one or more subsamples; identifying one or more functional subsamples based on the results of the functional assay; capturing antibodies from one or more functional subsamples on a solid substrate to provide captured antibodies; exposing the captured antibodies to an antigen display library comprising a plurality of display particles; capturing one or more display particles that interact with one or more of the antibodies and sequencing an antibody nucleic acid or antigen binding fragment thereof that interacts with one or more of the display particles; and
Group C - Claims 45-58 are directed to a method comprising enriching an antibody display library for binding to one or more targets to provide an enriched antibody display library; transferring the enriched antibody display library into a mammalian, yeast or bacterial cell line engineered to express the antibodies to produce a cell line comprising ASCs; distributing the ASCs into a plurality of containers to provide a plurality of subsamples; interacting one or more antibodies in one or more ASC subsamples with particles that express the one or more targets; and determining the nucleic acid sequence(s) of an antibody that interacts with the one or more targets of interest.

The claims must be limited to one inventive concept as set out in PCT Rule 13.
An a posteriori analysis has confirmed that the steps of the methods as defined in Groups A-C were known in the prior art (see D1). Therefore, the claimed methods of Groups A-C do not form a single general inventive concept.

C40B 50/06 (2006.01) , ***G01N 33/543*** (2006.01) , ***G01N 33/566*** (2006.01) , ***G01N 33/574*** (2006.01)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2016/000323

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2014153651A1	02 October 2014 (02-10-2014)	WO2014153651A1	02 October 2014 (02-10-2014)
		WO2014153651A8	06 November 2014 (06-11-2014)
		WO2014153651A9	10 March 2016 (10-03-2016)
		AU2014245806A1	24 September 2015 (24-09-2015)
		AU2014245806A9	16 June 2016 (16-06-2016)
		CA2906231A1	02 October 2014 (02-10-2014)
		CN105492621A	13 April 2016 (13-04-2016)
		EP2978855A1	03 February 2016 (03-02-2016)
		EP2978855A4	26 October 2016 (26-10-2016)
		JP2016515823A	02 June 2016 (02-06-2016)
		KR20150134429A	01 December 2015 (01-12-2015)
		US2016252495A1	01 September 2016 (01-09-2016)