

Claims:

1. A method of detecting a B cell lymphocyte expressing an antibody that specifically binds to an antigen of interest for preparation of a cDNA library, the method comprising:
introducing a sample comprising B cell lymphocytes into a microfluidic device, the microfluidic device comprising:
an enclosure having a flow region and a sequestration pen, wherein said sequestration pen comprises an isolation region having a single opening and a connection region, said connection region providing a fluidic connection between said isolation region and said flow region, and wherein said isolation region of said sequestration pen is an unswept region of said micro-fluidic device; wherein said isolation region of said sequestration pen comprises at least one conditioned surface;
~~loading-disposing~~ a B cell lymphocyte from said sample into said isolation region of said sequestration pen;
introducing said antigen of interest into said flow region of said enclosure such that said antigen of interest is proximal to said disposed B cell lymphocyte;
monitoring binding of said antigen of interest to said antibody expressed by said disposed B cell lymphocyte, ~~wherein said isolation region of said sequestration pen comprises at least one conditioned surface~~ thereby detecting an antibody-expressing B cell lymphocyte of interest;
and lysing the antibody-expressing B cell lymphocyte of interest thereby releasing RNA of sufficient quality for preparation of a cDNA library ~~to prepare a cDNA library~~.
2. The method of claim 1, wherein said at least one conditioned surface comprises a layer of covalently linked hydrophilic molecules.
3. The method of claim 2, wherein said hydrophilic molecules comprise polyethylene glycol (PEG)-containing polymers.
4. The method of any one of claims 1 to 3, wherein said enclosure of said microfluidic device further comprises a dielectrophoresis (DEP) configuration.
5. The method of claim 1, wherein said sample comprising B cell lymphocytes is a sample of peripheral blood, a spleen biopsy, a bone marrow biopsy, a lymph node biopsy, or a tumor biopsy.
6. The method of claim 5, wherein said B cell lymphocytes of said sample is-are a-plasma B cells.
7. The method of claim 1, wherein said sample comprising B cell lymphocytes is obtained from a human, mouse, rat, guinea pig, gerbil, hamster, rabbit, ferret, goat, sheep, pig, horse, cow, llama, camel, monkey, or chicken, or turkey.

~~chicken.~~

8. The method of claim 1, wherein said sample comprises B cell lymphocytes is from a mammal, and said mammal has been immunized against said antigen of interest, wherein said mammal has been exposed to or immunized against a pathogen associated with said antigen of interest, wherein said mammal has cancer and said cancer is associated with said antigen of interest, or wherein said mammal has an auto-immune disease and said auto-immune disease is associated with said antigen of interest.
9. The method of claim 1, wherein said sample comprising B cell lymphocytes has been contacted with DNase prior to being introduced into said microfluidic device and is depleted of cell types other than B cell lymphocytes.
10. The method of claim 1 or 9, wherein said sample comprising B cell lymphocytes has been enriched for B cell lymphocytes expressing CD138.
11. The method of claim 1, further comprising: ~~providing culture medium to contacting~~ said ~~disposed~~ B cell lymphocyte, ~~wherein the culture medium comprises with~~ a growth-inducing agent.
12. The method of claim 11, wherein said ~~growth-inducing agent~~~~culture medium~~ comprises IL-6 and/or April.
13. The method of claim 12, wherein said ~~disposed~~ B cell lymphocyte is provided ~~the~~ culture medium for a period of one to 3 to 5 days.
14. The method of claim 4, wherein ~~loading-disposing~~ said B cell lymphocyte into said isolation region of said sequestration pen comprises moving said B cell lymphocyte from said flow region to said isolation region using DEP force.
15. The method of claim 1, wherein ~~providing-introducing~~ said antigen of interest comprises flowing a solution comprising soluble antigen of interest into or through said flow region, wherein said antigen of interest is covalently bound to a first detectable label.
16. The method of claim 15 further comprising providing a micro-object comprising a first antibody-binding agent, wherein said first antibody-binding agent binds to said antibody expressed by said B cell lymphocyte without inhibiting the binding of antigen of interest to said antibody expressed by said B cell lymphocyte, and wherein monitoring of binding of said antigen of interest to said antibody expressed by said B cell lymphocyte comprises detecting indirect binding of said antigen of interest to said micro-object.
17. The method of claim 16, wherein said first antibody-binding agent binds to an ~~Fe-Fc~~ domain of said antibody expressed by said B cell lymphocyte.

18. The method of claim 16, wherein providing said micro-object comprises flowing a solution comprising said micro-object into said flow region and stopping said flow when said micro-object is located proximal to said sequestration pen.
19. The method of claim 16, wherein said solution comprising said micro-object and said solution comprising said soluble antigen of interest are the same solution.
20. The method of claim 16, further comprising: providing a second antibody-binding agent, wherein said second antibody-binding agent comprises a second detectable label; and monitoring indirect binding of said second antibody—binding agent to said micro-object, wherein said first detectable label is different from said second detectable label.
21. The method of claim 20, wherein said second antibody-binding agent binds to IgG antibodies.
22. The method of claim 1, wherein providing said antigen of interest comprises providing a micro-object that comprises said antigen of interest, wherein said micro-object is a cell, a liposome, a lipid nanoraft, or a bead; and providing a labeled antibody-binding agent prior to or concurrently with said antigen of interest, wherein said monitoring of binding of said antigen of interest to said antibody expressed by said B cell lymphocyte comprises detecting indirect binding of said labeled antibody—binding agent to said antigen of interest.
23. The method of claim 22, wherein said labeled antibody-binding agent binds to anti-IgG antibodies.
24. The method of claim 1, wherein monitoring binding of said antigen of interest to said antibody expressed by said disposed B cell lymphocyte comprises imaging all or part of said sequestration pen of said microfluidic device.
25. The method of claim 24, wherein said imaging comprises fluorescence imaging.
26. The method of claim 24, wherein said imaging comprises taking a plurality of images.
27. The method of claim 1, wherein said microfluidic device comprises a plurality of said sequestration pens, each having an isolation region and a connection region, each said connection region providing a fluidic connection between said isolation region and said flow region, said method further comprising: ~~loading~~ disposing one or more of said plurality of B cell lymphocytes into said isolation region of each of two or more sequestration pens of said plurality; introducing said antigen of interest into said microfluidic device such that

said antigen of interest is proximal to each of said two or more sequestration pens ~~loaded~~ disposed with one or more B cell lymphocytes; monitoring of binding of said antigen of interest to said antibody expressed by each of said ~~loaded-disposed~~ B cell lymphocytes; detecting binding of said antigen of interest to said antibody expressed by said ~~loaded-disposed~~ B cell lymphocyte, or ones of said ~~loaded-disposed~~ B cell lymphocytes; identifying said ~~loaded-disposed~~ B cell lymphocyte, or said ones of said ~~loaded-disposed~~ B cell lymphocytes, as expressing an antibody that specifically binds to said antigen of interest; and lysing the one or more B cell lymphocytes identified as binding to said antigen of interest.

28. A method of characterizing an antibody that specifically binds to an antigen of interest, the method comprising:

identifying a B cell lymphocyte that expresses an antibody that specifically binds to said antigen of interest, wherein said identifying is performed according to the method of claim 27;

isolating from said B cell lymphocyte, a nucleic acid encoding an immunoglobulin heavy chain variable region (V_H) and/or an immunoglobulin light chain variable region (V_L); and

sequencing at least a portion of said nucleic acid encoding said immunoglobulin heavy chain variable region (V_H) and/or at least a portion of said nucleic acid encoding said immunoglobulin light chain variable region (V_L).

29. The method of claim 28, wherein sequencing said immunoglobulin heavy chain variable region (V_H) comprises: lysing said identified B cell lymphocyte; reverse transcribing mRNA isolated from said B cell lymphocyte, wherein said mRNA encodes said immunoglobulin heavy chain variable region (V_H), thereby forming heavy chain variable region V_H cDNA; and sequencing at least a portion of said heavy chain variable region V_H cDNA.

30. The method of claim 28, wherein sequencing said immunoglobulin light chain variable region (V_L) comprises: lysing said identified B cell lymphocyte; reverse transcribing mRNA isolated from said B cell lymphocyte, wherein said mRNA encodes said immunoglobulin light chain variable region (V_L), thereby forming light chain variable region V_L cDNA; and sequencing at least a portion of said light chain variable region V_L cDNA.

31. The method of claim 29 or 30, wherein reverse transcribing said mRNA comprises contacting said mRNA with a capture/priming oligonucleotide.

32. The method of claim 31, wherein said reverse transcribing is performed in the presence of a transcript switching oligonucleotide.
33. The method of claim 29 or 30, wherein said identified B cell lymphocyte, is(are) exported from said microfluidic device prior to being lysed, wherein exporting said identified B cell lymphocyte comprises: moving said identified B cell lymphocyte from said isolation region of said sequestration pen into said flow region of said microfluidic device; and flowing said identified B cell lymphocyte through said flow region and out of said microfluidic device.
34. The method of claim 33, wherein moving said identified B cell lymphocyte from said isolation region of said sequestration pen comprises capturing and moving said identified B cell lymphocyte using DEP force.
35. The method of claim 29 or 30, further comprising: providing one or more capture beads in close proximity to said identified B cell lymphocyte, where said one or more capture beads each comprises oligonucleotides capable of binding said V_H -heavy chain variable region mRNA and/or said V_L -light chain variable region mRNA; lysing said identified B cell lymphocyte; and allowing said V_H -heavy chain variable region mRNA and/or said V_L -light chain variable region mRNA from said lysed B cell lymphocyte to be bound by said one or more capture beads, and wherein said identified B cell lymphocyte is lysed within said microfluidic device.
36. The method of claim 35, wherein said bound- V_H -heavy chain variable region mRNA and/or said bound- V_L -light chain variable region mRNA is reverse transcribed into V_H -heavy chain variable region cDNA and/or V_L -light chain variable region cDNA while bound to said one or more capture beads.
37. The method of claim 36, wherein said V_H -heavy chain variable region cDNA and/or V_L -light chain variable region cDNA is exported from said microfluidic device while bound to said one or more capture beads.
38. The method of claim 29 or 30, further comprising amplifying said V_H -heavy chain variable region cDNA and/or said V_L -light chain variable region cDNA prior to said sequencing, wherein said amplifying comprises increasing the representation of V_H -heavy chain variable region cDNA and/or V_L -light chain variable region cDNA, or fragments thereof, in the reverse transcribed mRNA isolated from said B cell lymphocyte.
39. The method of claim 38, wherein said amplifying comprises:
a first round of amplification which increases the representation of V_H -heavy chain variable region cDNA and/or V_L -light chain variable region cDNA, or fragments thereof, in the reverse transcribed mRNA isolated from said B cell lymphocyte; and

a second round of amplification which introduces barcode sequences into the heavy chain variable region V_H cDNA and/or light chain variable region V_L cDNA, or fragments thereof, amplified in the first round.