

CLAIMS

1. A method for the isolation and identification of at least one nucleotide sequence encoding an antibody or fragment thereof specific for a desired antigen or ligand, comprising the steps of:

(a) transducing at least one retroviral expression construct encoding an antibody or fragment thereof into vertebrate host cells by using replication incompetent retroviral particles, wherein the at least one construct stably integrates into the host cell genome such that the transduced host cells are capable of expressing and displaying said antibody or fragment thereof on their cell surface;

(b) stably expressing said antibody or fragment thereof in said vertebrate host cells and displaying the same on their cell surface;

(c) enriching vertebrate host cells expressing said antibody or fragment thereof on the basis of their ability to bind to said desired antigen or ligand by separating cells that exhibit specific antigen binding from a non-binding cell population thus selectively isolating strong antibody binders having high affinity to said desired antigen or ligand; and

(d) isolating and identifying said at least one nucleotide sequence encoding said antibody or fragment thereof from the retrovirally transduced and enriched vertebrate host cells.

2. The method according to claim 1, wherein step (d) is preceded by expanding said enriched vertebrate host cells in tissue culture.

3. The method according to claim 1, wherein step (c) is followed by expanding said enriched vertebrate host cells in

tissue culture, after which step (c) as defined in claim 1 is repeated at least once.

4. The method according to any of claims 1 to 3, in which the
5 retroviral transduction is performed at a multiplicity of infection of equal to or less than 0.1.

5. The method according to any one of claims 1 to 4, in which the antibody is a full-length antibody.

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6. The method according to any one of claims 1 to 4, in which the fragment of an antibody is selected from the group consisting of a heavy chain, a light chain, a single V_H domain, a single V_L domain, a scFv fragment, a Fab fragment, and a
15 $F(ab')_2$ fragment.

7. The method according to any one of claims 1 to 6, wherein the vertebrate host cells are derived from a group of species consisting of cartilaginous fish, bony fish, amphibians, reptilia, birds, mammals including pigs, sheep, cattle, horses
20 and rodents including mice, rats, rabbits and guinea pigs.

8. The method according to claim 7, wherein the preferred vertebrate host cell species is mice.

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9. The method according to any one of claims 1 to 8, wherein the vertebrate host cells are lymphocytes.

10. The method according to claim 9, wherein the lymphocytes
30 are precursor B lymphocytes unable to express endogenous antibody polypeptides and/or at least one surrogate light chain component.

11. The method according to any one of claims 1 to 9, wherein
35 the vertebrate host cells express either endogenous $Ig\alpha$ and $Ig\beta$ molecules or ectopically expressed $Ig\alpha$ and $Ig\beta$ molecules.

12. The method according to claim 5, wherein the full-length antibody is selected from the group consisting of a fully human antibody, a humanized antibody, and a chimeric antibody.

5 13. The method according to any one of claims 1 to 12, wherein the at least one nucleotide sequence is a plurality of nucleotide sequences that encode (i) an antibody heavy chain sequence and multiple antibody light chain sequences, or (ii) an antibody light chain sequence and multiple antibody heavy
10 chain sequences.

14. The method according to any one of claims 1 to 13, wherein the antibody or fragment thereof comprises a variable binding domain encoded by said at least one retroviral expression
15 construct enabling V(D)J recombination in order to generate a coding sequence for a variable binding domain upon retroviral transduction.

15. The method according to any one of claims 1 to 14, wherein
20 step (b) is performed under mutagenizing conditions.

16. The method according to any one of claims 1 to 15, wherein the expression of said antibody or fragment thereof in the retrovirally transduced vertebrate host cells is operably
25 linked to

(a) at least one antibiotic selection marker,

(b) at least one screening marker, and/or to

(c) a combination thereof,

and wherein the expression of said antibody or fragment
30 thereof is coupled using at least one internal ribosomal entry sequence (IRES).

17. The method according to claim 16, wherein the at least one screening marker is

35 (i) a fluorescent protein selected from the group consisting of green fluorescent protein (GFP), yellow fluorescent

protein (YFP), red fluorescent protein (RFP), and blue fluorescent protein (BFP); or

- (ii) a cell surface marker selected from the group consisting of CD7, CD34, and the low affinity nerve growth factor receptor.

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18. The method according to any one of claims 1 to 17 wherein the enrichment step (c) is performed by physical separation of cells from a non-binding cell population using:

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- (i) fluorescent activated cell sorting (FACS);
(ii) micromanipulation; or
(iii) panning methods to immobilized desired antigen or ligand.

Claims

1. A method for the isolation and identification of at least one nucleotide sequence encoding an antibody or fragment thereof specific for a desired antigen or ligand, comprising the steps of:
 - (a) transducing at least one retroviral expression construct encoding an antibody or fragment thereof into vertebrate host cells by using replication incompetent retroviral particles, wherein the at least one construct stably integrates into the host cell genome such that the transduced host cells are capable of expressing and displaying said antibody or fragment thereof on their cell surface, and wherein the vertebrate host cells are precursor B lymphocytes that endogenously express Ig α and Ig β molecules facilitating membrane deposition of said antibody or fragment thereof, and are unable to express endogenous antibody polypeptides and at least one surrogate light chain component;
 - (b) stably expressing said antibody or fragment thereof in said vertebrate host cells and displaying the same on their cell surface;
 - (c) enriching vertebrate host cells expressing said antibody or fragment thereof on the basis of their ability to bind to said desired antigen or ligand by separating cells that exhibit specific antigen binding from a non-binding cell population thus selectively isolating strong antibody binders having high affinity to said desired antigen or ligand; and
 - (d) isolating and identifying said at least one nucleotide sequence encoding said antibody or fragment thereof from the retrovirally transduced and enriched vertebrate host cells.
2. The method according to claim 1, wherein step (d) is preceded by expanding said enriched vertebrate host cells in tissue culture.
3. The method according to claim 1, wherein step (c) is followed by expanding said enriched vertebrate host cells in tissue culture, after which step (c) as defined in claim 1 is repeated at least once.
4. The method according to any of claims 1 to 3, in which the retroviral transduction is performed at a multiplicity of infection of equal to or less than 0.1.
5. The method according to any one of claims 1 to 4, in which the antibody is a full-length antibody.
6. The method according to any one of claims 1 to 4, in which the fragment of an antibody is selected from the group consisting of a heavy chain, a light chain, a single V_H domain, a single V_L domain, a scFv fragment, a Fab fragment, and a F(ab')₂ fragment.
7. The method according to any one of claims 1 to 6, wherein the vertebrate host cells are derived from a group of species consisting of cartilaginous fish, bony fish, amphibians, reptilia, birds, mammals including pigs, sheep, cattle, horses and rodents including mice, rats, rabbits and guinea pigs.
8. The method according to claim 7, wherein the preferred vertebrate host cell species is mice.
9. The method according to claim 5, wherein the full-length antibody is selected from the group consisting of a fully human antibody, a humanized antibody, and a chimeric antibody.
10. The method according to any one of claims 1 to 9, wherein the at least one nucleotide sequence is a plurality of nucleotide sequences that encode (i) an antibody heavy chain sequence and multiple antibody light chain sequences, or (ii) an antibody light chain sequence and multiple antibody heavy chain sequences.
11. The method according to any one of claims 1 to 10, wherein the antibody or fragment thereof comprises a variable binding domain encoded by said at least one retroviral expression construct enabling V(D)J recombination in order to generate a coding sequence for a variable binding domain upon retroviral transduction.
12. The method according to any one of claims 1 to 11, wherein step (b) is performed under mutagenizing conditions.

13. The method according to any one of claims 1 to 12, wherein the expression of said antibody or fragment thereof in the retrovirally transduced vertebrate host cells is operably linked to

- (a) at least one antibiotic selection marker,
- (b) at least one screening marker, and/or to
- (c) a combination thereof,

and wherein the expression of said antibody or fragment thereof is coupled using at least one internal ribosomal entry sequence (IRES).

14. The method according to claim 13, wherein the at least one screening marker is

- (i) a fluorescent protein selected from the group consisting of green fluorescent protein (GFP), yellow fluorescent protein (YFP), red fluorescent protein (RFP), and blue fluorescent protein (BFP); or
- (ii) a cell surface marker selected from the group consisting of CD7, CD34, and the low affinity nerve growth factor receptor.

15. The method according to any one of claims 1 to 14, wherein the enrichment step (c) is performed by physical separation of cells from a non-binding cell population using:

- (i) fluorescent activated cell sorting (FACS);
- (ii) micromanipulation; or
- (iii) panning methods to immobilized desired antigen or ligand.