In WO 2007/047578 the cell display of antibody libraries is reported.

Summary ~~of the Invention~~

It has been found that full length antibodies can be expressed and displayed on eukaryotic cells by using a lentiviral virus particle comprising a bicistronic expression cassette. The expression of full length antibodies on eukaryotic cells using a lentiviral virus particle as reported herein is possible by using the IRES (internal ribosomal entry site) element of the EV71, which is linking either the expression cassettes of an antibody light chain and an antibody heavy chain or the expression cassettes of two antibody heavy chains.

In case of the presence of an antibody light chain expression cassette and an antibody heavy chain expression cassette in the lentiviral vector the antibody heavy chain expression cassette may comprises after the exon encoding the C-terminal antibody domain a non-constitutive splice site providing a means for expressing beside the soluble antibody heavy chain also a membrane-bound antibody heavy chain resulting in the presentation of membrane-bound full length antibody.

In case of the presence of two antibody heavy chain expression cassettes in the lentiviral vector either both or only the second antibody heavy chain expression cassette may comprises after the exon encoding the C-terminal antibody domain an exon encoding a transmembrane domain resulting in the presentation of membrane-bound full length antibody.

Further are provided herein methods for generating and selecting a eukaryotic cell expressing and displaying on its surface an antibody, especially a full length monoclonal antibody.

The disclosure relates to ~~One aspect as reported herein is~~ a lentiviral vector comprising a bicistronic expression cassette, which comprises in 5'- to 3'-direction

- a promoter,
- a first nucleic acid encoding a full length antibody light chain,
- the EV71-IRES,
- a second nucleic acid encoding a full length antibody heavy chain,
- a spliceable intron, and
- a transmembrane domain or a GPI-anchor.

This lentiviral vector as reported herein is an expression vector in that it comprises a bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain, wherein the IRES separating the two expression cassettes is the EV71-IRES.

It has been found that only the EV71-IRES can be used for the expression of full length antibody in a bicistronic expression cassette in a lentiviral expression system.

Due to the provision of the spliceable intron a soluble form of the antibody heavy chain as well as a membrane-bound form of the antibody heavy chain can be expressed from the expression vector as reported herein.

By expression of a soluble form and a membrane-bound form of the antibody heavy chain the cell on the one hand secretes a full length antibody, which can be tested e.g. in an ELISA, and at the same time presents a membrane-bound form of the full length antibody on its surface, which can be used for the selection of the cell e.g. by FACS enabling the isolation of a single cell clone.

The disclosure relates to ~~One aspect as reported herein is~~ a lentiviral vector comprising a bicistronic expression cassette, which comprises in 5'- to 3'-direction

- a promoter,
- a first nucleic acid encoding a first full length antibody heavy chain,
- the EV71-IRES,
- a second nucleic acid encoding a second full length antibody heavy chain, and
- a transmembrane domain or a GPI-anchor.

This lentiviral vector as reported herein is an expression vector in that it comprises a bicistronic expression cassette for the expression of two different full length antibody heavy chains, wherein the IRES separating the two expression cassettes is the EV71-IRES.

By expression of a membrane-bound form of the antibody heavy chain the cell presents a membrane-bound form of the full length antibody on its surface, which can be used for the selection of the cell e.g. by FACS enabling the isolation of a single cell clone.

In one embodiment the antibody is an antibody that specifically binds to an antigen. Thus, in one embodiment the antibody or the antibody encoding nucleic acid, respectively, is obtained from a B-cell that has been selected for that specifically binds to an antigen.

In one embodiment the antibody is a bivalent monospecific antibody. In one embodiment the antibody specifically binds to an antigen.

In one embodiment the antibody is a bivalent bispecific antibody. In one embodiment the antibody specifically binds to two different antigens or to two epitopes on the same antigen.

In one embodiment the antibody is a tetravalent bispecific antibody. In one embodiment the antibody specifically binds to two different antigens or to two epitopes on the same antigen.

In one embodiment the expression vector is a lentiviral (expression) vector.

The disclosure relates to ~~One aspect as reported herein is~~ a lentiviral vector library comprising two or more lentiviral particles each comprising an expression vector as reported herein, wherein the antibodies encoded by each vector differ in at least one amino acid from each other.

In one embodiment the vector library comprises of from 1,000 to 1,000,000 different expression vectors.

In one embodiment the antibodies encoded by the vectors of the vector library differ in at least one amino acid residue in the variable domains of the antibody.

In one embodiment the antibodies encoded by the vectors of the vector library differ in at least one amino acid residue in one of the CDRs of the antibody. In one embodiment the CDR is the heavy chain CDR3.

The disclosure relates to ~~One aspect as reported herein is~~ a eukaryotic cell comprising a bicistronic expression cassette as reported herein. In one embodiment the bicistronic expression cassette has been transduced into the cell.

The disclosure relates to ~~One aspect as reported herein is~~ a eukaryotic cell library comprising two or more eukaryotic cells each comprising a bicistronic expression cassette or a vector as

reported herein, wherein the antibodies expressed by each cell differ in at least one amino acid from each other.

In one embodiment the eukaryotic cell library comprises of from 1,000 to 1,000,000 different mammalian cells.

In one embodiment the antibodies expressed by the cells of the eukaryotic cell library differ in at least one amino acid residue in the variable domains of the antibody.

In one embodiment the antibodies encoded by the eukaryotic cells of the eukaryotic cell library differ in at least one amino acid residue in one of the CDRs of the antibody. In one embodiment the CDR is the heavy chain CDR3.

The disclosure relates to ~~One aspect as reported herein is~~ a eukaryotic cell library comprising the vector library as reported herein.

In one embodiment the eukaryotic cells of the eukaryotic cell library express and display a single antibody.

In one embodiment the eukaryotic cells of the eukaryotic cell library display a single antibody.

In one embodiment the eukaryotic cell library is a population of eukaryotic cells expressing a library of antibodies wherein the encoding nucleic acid is derived from B-cells of an immunized animal. In one embodiment the B-cells are pre-selected for their specificity towards the antigen of interest.

In one embodiment the eukaryotic cell library is a population of eukaryotic cells wherein each cell comprises a first expression cassette encoding a full length antibody that specifically binds to a first antigen and a second expression cassette encoding a full length antibody that specifically binds to a second antigen.

In one embodiment the eukaryotic cell library is a population of eukaryotic cells wherein each cell comprises a first expression cassette encoding a first full length antibody light chain and a first full length antibody heavy chain binding to a first antigen and a second expression cassette encoding a second full length antibody light chain and a second full length antibody heavy chain that specifically binds to a second antigen.

In one embodiment the eukaryotic cell library is a population of eukaryotic cells wherein each cell comprises an expression cassette encoding a first full length antibody heavy chain that specifically binds to a first antigen and a second full length antibody heavy chain that specifically binds to a second antigen, wherein the eukaryotic cell expresses a common light chain.

In one embodiment the first full length antibody heavy chain comprises a hole mutation and the second antibody heavy chain comprises a knob mutation.

In one embodiment the first full length antibody light chain comprises as constant domain a CH1 domain and the first full length antibody heavy chain comprises as first constant domain a CL domain, or the second full length antibody light chain comprises as constant domain a CH1 domain and the second full length antibody heavy chain comprises as first constant domain a CL domain.

In one embodiment the expression vector library is obtained by randomization of one or more amino acids residues in one or more CDRs of a parent expression vector.

In one embodiment the expression vector library is obtained by combination of two different half antibodies.

The disclosure relates to ~~One aspect as reported herein is~~ a method for the isolation or selection of an antibody which specifically binds to one or more, especially two, antigen(s) of interest.

It has been found that the screening method reported herein can be performed in a "one antibody per cell" format, which is advantageous because it allows the screen to be completed in one single round of selection.

Herein is reported a method of generating, selecting, and/or isolating a cell expressing an antibody, which specifically binds to an antigen.

In one embodiment the antibody is a monoclonal full length antibody. In one embodiment the antibody is a bispecific monoclonal full length antibody.

The methods as reported herein allow cloning the variable regions of the antibody or the entire antibody from the selected cell.

The disclosure relates to ~~One aspect as reported herein is~~ a method to recombinantly produce antibodies selected with the method as reported herein.

In one embodiment the full length antibody comprises a constant region of human origin, especially of human IgG1, IgG2, or IgG4 class.

The methods as reported herein allow to recombinantly produce antibodies with desired specificity in a fully species specific form, especially as fully human antibodies.

The disclosure relates to ~~One aspect as reported herein is~~ a method of selecting a cell expressing an antibody which specifically binds to an antigen of interest comprising the steps of

(a) optionally selecting from a population of B-cells a sub-population of B-cells or a single B-cell or a clonal population of B-cells secreting an antibody that specifically binds one or more antigens,

(b) generating a lentiviral expression library, wherein each member of the lentiviral expression library encodes a variant of the antibody or the antibodies that specifically binds one or more antigens, by

(i) generating a multitude of DNA molecules, wherein the generating comprises the step of amplifying a pool of DNA molecules from the sub-population of B-cells or the step of generating a library of DNA molecules from the DNA encoding a single antibody that specifically binds to one or two antigens of interest by randomization of the encoding nucleic acid sequence, and

(ii) cloning the multitude of DNA molecules into a lentiviral expression vector comprising an EV71-IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain in a soluble as well as membrane bound form;

(c) transducing a population of eukaryotic cells with a population of lentiviral virus particles each comprising a member of the lentiviral expression library;

(d) displaying antibodies encoded by the lentiviral expression library on the surface of the eukaryotic mammalian cells; and

(e) isolating from the population of eukaryotic cells a cell, wherein the cell is selected for the capability of the antibody displayed on its surface to specifically bind the antigen or antigens of interest or a fragment or antigenic determinant thereof.

The disclosure relates to ~~One aspect as reported herein is~~ a method of selecting a cell expressing a bispecific antibody (which specifically binds to two antigens of interest) comprising the steps of

(a) generating a lentiviral expression library, wherein each member of the lentiviral expression library encodes a variant of the bispecific antibody, by
    (i) generating a multitude of DNA molecules from the DNA encoding a single bispecific antibody by randomization of the encoding nucleic acid sequence, and
    (ii) cloning the multitude of DNA molecules into a lentiviral expression vector comprising an EV71-IRES linked bicistronic expression cassette for the expression of a full length bispecific antibody as membrane bound form;
(b) transducing a population of eukaryotic cells with a population of lentiviral virus particles each comprising a member of the lentiviral expression library;
(c) displaying antibodies encoded by the lentiviral expression library on the surface of the eukaryotic mammalian cells; and
(d) isolating from the population of eukaryotic cells a cell, wherein the cell is selected for the capability of the antibody displayed on its surface to specifically bind the antigens of interest or a fragment or antigenic determinant thereof.

The use of a lentiviral expression library in combination with a lentiviral expression vector comprising an EV71-IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain in a soluble as well as membrane bound form allows for a high screening efficiency.

In one embodiment the method comprises generating a multitude of DNA molecules encoding antibodies, the generating a multitude of DNA molecules comprising the steps of:

(1) amplifying from a sub-population of B-cells a first pool of DNA molecules encoding heavy chain variable regions (HCVRs); and
(2) amplifying from the sub-population of B-cells a second pool of DNA molecules encoding light chain variable regions (LCVRs);
(3) cloning a combination of the multitude of DNA molecule encoding LCVRs and of the multitude of DNA molecules encoding HCVRs into a

antibody light chain and a full length antibody heavy chain simultaneously in a soluble as well as membrane bound form.

In one embodiment the method comprises the generating of a lentiviral expression library, the generating comprising the steps of:

(i) generating a multitude of DNA molecules encoding antibodies that specifically binds to one or two antigens, the generating comprising the steps of:
  (1) isolating mRNA from a single B-cell or a clonal population of B-cells;
  (2) transcribing the mRNA to cDNA;
  (3) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding region; and
  (4) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding region;
  (5) randomizing the first and/or the second DNA molecule and thereby generating a first pool of DNA molecules and a second pool of DNA molecules,

(ii) cloning a pair of DNA molecules of the first and the second pool of DNA molecules into a lentiviral expression vector comprising an EV71-IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain simultaneously in a soluble as well as membrane bound form.

The disclosure relates to~~One aspect as reported herein is~~ a method of selecting a cell expressing a bispecific antibody (which specifically binds to two antigens of interest) comprising the steps of

(a) generating a lentiviral expression library, wherein each member of the lentiviral expression library encodes variants of the heavy chains of a bispecific antibody, by
  (i) generating a multitude of DNA molecules from the DNA encoding the heavy chains of a single bispecific antibody by randomization of the encoding nucleic acid sequence, and
  (ii) cloning the multitude of DNA molecules into a lentiviral expression vector comprising an EV71-IRES linked bicistronic expression cassette for the expression of the two heavy chains of the bispecific

antibody, wherein the nucleic acid downstream of the EV71-IRES encodes an antibody heavy chain with a C-terminal transmembrane domain;

(b) transducing a population of eukaryotic cells expressing an antibody light chain that can form an antigen binding site with any of the antibody heavy chains with a population of lentiviral virus particles each comprising a member of the lentiviral expression library;

(c) displaying antibodies encoded by the lentiviral expression library on the surface of the eukaryotic mammalian cells; and

(d) isolating from the population of eukaryotic cells a cell, wherein the cell is selected for the capability of the antibody displayed on its surface to specifically bind the antigens of interest or a fragment or antigenic determinant thereof.

The disclosure relates to ~~One aspect as reported herein is~~ a lentiviral expression vector for displaying a full length antibody on the surface of a eukaryotic cell.

In one embodiment the expression vector comprises DNA elements encoding a signal peptide, an EV71-IRES, a transmembrane region and, optionally, a detection tag.

In one embodiment the expression vector comprises a restriction site allowing the cloning, especially the orientation specific cloning, of DNA molecules encoding a full length antibody heavy chain and a full length antibody light chain into the expression vector.

The disclosure relates to ~~One aspect as reported herein is~~ an expression library comprising the expression vector as reported herein.

The disclosure relates to ~~One aspect as reported herein is~~ a eukaryotic cell comprising the expression vector as reported herein or comprising at least one member of the expression library as reported herein.

Monoclonal antibodies produced by the method as reported herein can be used for research purposes, diagnostic purposes or the treatment of diseases.

In one embodiment the eukaryotic cell is a mammalian cell or a yeast cell. In one embodiment the mammalian cell is a CHO cell or a HEK cell.

The disclosure relates to ~~One aspect as reported herein is~~ a method of selecting a cell expressing an antibody comprising the steps of

(a) generating a population of eukaryotic cells by transduction with a population of lentiviral virus particles, whereby each cell of the population of cells displays a membrane-bound full length antibody which is encoded by the lentiviral nucleic acid, and which specifically binds to one or more antigens or one or more epitopes on the same antigen, and

(b) selecting from the population of eukaryotic cells a cell depending on the properties of the displayed membrane-bound full length antibody,

whereby each lentiviral virus particle of the population of lentiviral virus particles comprises a bicistronic expression cassette comprising the EV71-IRES for the expression of the membrane-bound antibody.

In one embodiment each bicistronic expression cassette of the lentiviral virus particle of the population of lentiviral virus particles encodes a different variant of a parent antibody, which specifically binds to one or more antigens or one or more epitopes on the same antigen.

In one embodiment the bicistronic expression cassettes comprises in 5'- to 3'-direction

- a promoter,
- a first nucleic acid encoding a full length antibody light chain,
- the EV71-IRES,
- a second nucleic acid encoding a full length antibody heavy chain,
- a spliceable intron, and
- a transmembrane domain or a GPI-anchor.

In one embodiment each cell of the population of eukaryotic cells displays a membrane-bound full length antibody and secretes a full length antibody.

In one embodiment each cell of the population of eukaryotic cells displays and secretes a single full length antibody.

In one embodiment the antibody is a bispecific antibody.

The disclosure relates to ~~One aspect as reported herein is~~ a lentiviral vector comprising a bicistronic expression cassette, which comprises in 5'- to 3'-direction

- a promoter,
- a first nucleic acid encoding a full length antibody light chain,

- the EV71-IRES,
- a second nucleic acid encoding a full length antibody heavy chain,
- an alternatively spliceable intron for the simultaneous production of membrane-bound antibody and a secreted antibody, and
- a transmembrane domain or a GPI-anchor.

The disclosure relates to ~~One aspect as reported herein is~~ a lentiviral vector comprising a bicistronic expression cassette, which comprises in 5'- to 3'-direction

- a promoter,
- a first nucleic acid encoding a first full length antibody heavy chain,
- the EV71-IRES,
- a second nucleic acid encoding in 5'- to 3'-direction a second full length antibody heavy chain and a transmembrane domain or a GPI-anchor.

The disclosure relates to~~One aspect as reported herein is~~ the use of a lentiviral vector according to previous aspects for the generation of a population of eukaryotic cells displaying and secreting or displaying a full length antibody.

One aspect as reported herein is a method of selecting a cell expressing a bispecific antibody comprising the steps:

(a) generating a population of eukaryotic cells by transduction with a population of lentiviral virus particles, whereby each of the lentiviral virus particles comprises a bicistronic expression cassette, which comprises a first heavy chain variable domain encoding nucleic acid in a hole- or knob-locus, which is upstream of the EV71-IRES, and a second heavy chain variable domain encoding nucleic acid in the respective other locus, which is downstream of the EV71-IRES, whereby the first heavy chain variable domain binds to a first antigen and the second variable domain binds to a second antigen, whereby the first antigen and the second antigen can be the same or different, whereby the eukaryotic cell expresses a common light chain, whereby one or both of the heavy chains further comprise a transmembrane domain at their C-terminus, and

(b) selecting from the population of eukaryotic cells a cell depending on the properties of the displayed membrane-bound full length bispecific antibody.

In one embodiment the method comprises the step:

- performing a PCR of the complete first heavy chain encoding nucleic acid and the variable domain of the second heavy chain encoding nucleic acid (2.2 kbp) including the EV71-IRES and cloning into second shuttle vector without transmembrane-domain with the removal of the transmembrane-domain of the first heavy chain by restriction cutting and religation of the vector.

One aspect as reported herein is use of a cell selected with a method as defined herein for the production of an antibody.

All embodiments as reported herein shall refer to all aspects of the invention and may be combined in any possible combination.

Detailed Description of the Invention

Herein is reported a selection method using the expression of full length antibodies in their natural environment, i.e. the secretory pathway of mammalian cells, that ensures that all the cellular components normally involved in antibody synthesis and processing (folding, disulfide bond formation, glycosylation etc.) are available in a physiological form and concentration.

GENERAL ASPECTS

As known to a person skilled in the art enables the use of recombinant DNA technology the production of numerous derivatives of a nucleic acid and/or polypeptide. Such derivatives can, for example, be modified in one individual or several positions by substitution, alteration, exchange, deletion, or insertion. The modification or derivatization can, for example, be carried out by means of site directed mutagenesis. Such modifications can easily be carried out by a person skilled in the art (see e.g. Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, USA (1999)). The use of recombinant technology enables a person skilled in the art to transform various host cells with heterologous nucleic acid(s). Although the transcription and translation, i.e. expression, machinery of different cells use the same elements, cells belonging to different species may have among other things a different so-called codon usage. Thereby identical polypeptides (with respect to amino acid sequence) may be encoded by different nucleic acid(s). Also, due to the degeneracy of the genetic code, different nucleic acids may encode the same polypeptide.

20. The method according to any one of claims 1 to 19, characterized in that the antibody is a humanized or human antibody, especially a human antibody.

21. A method of selecting a cell expressing an antibody comprising the steps of

(a) generating a population of eukaryotic cells by transduction with a population of lentiviral virus particles, whereby each cell of the population of cells displays a membrane-bound full length antibody, whereof at least two chains are encoded by a bicistronic expression cassette, and which specifically binds to one or more antigens or one or more epitopes on the same antigen, and

(b) selecting from the population of eukaryotic cells a cell depending on the properties of the displayed membrane-bound full length antibody,

whereby each lentiviral virus particle of the population of lentiviral virus particles comprises a bicistronic expression cassette comprising the EV71-IRES for the expression of the membrane-bound antibody.

22. The method according to claim 21, characterized in that each bicistronic expression cassette of the lentiviral virus particle of the population of lentiviral virus particles encodes a different variant of a parent antibody, which specifically binds to one or more antigens or one or more epitopes on the same antigen.

~~23. The method according to any one of claims 21 to 22, characterized in that the bicistronic expression cassettes comprises in 5'- to 3'-direction~~

~~— a promoter,~~

~~— a first nucleic acid encoding a full-length antibody light chain,~~

~~— the EV71-IRES,~~

~~— a second nucleic acid encoding a full-length antibody heavy chain,~~

~~— a spliceable intron, and~~

~~— a nucleic acid encoding a transmembrane domain or a GPI-anchor.~~

~~24. The method according to any one of claims 21 to 22, characterized in that the bicistronic expression cassettes comprises in 5'- to 3'-direction~~

~~— a promoter,~~

~~— a first nucleic acid encoding a first full-length antibody heavy chain,~~

~~— optionally a nucleic acid encoding a transmembrane domain or a GPI-anchor,~~

~~— the EV71-IRES,~~

~~— a second nucleic acid encoding a second full-length antibody heavy chain, and~~

~~— a nucleic acid encoding a transmembrane domain or a GPI-anchor.~~

~~25. The method according to any one of claims 21 to 22, characterized in that the bicistronic expression cassettes comprises in 5'- to 3'-direction~~

~~- a promoter,~~

~~- a first nucleic acid encoding a full length antibody light chain,~~

~~- the EV71-IRES,~~

~~- a second nucleic acid encoding a full length antibody heavy chain linked at its C-terminus to a scFv,~~

~~- a spliceable intron, and~~

~~- a nucleic acid encoding a transmembrane domain or a GPI-anchor.~~

~~26. The method according to any one of claims 21 to 22, characterized in that the bicistronic expression cassettes comprises in 5'- to 3'-direction~~

~~- a promoter,~~

~~- a first nucleic acid encoding a full length antibody light chain,~~

~~- the EV71-IRES,~~

~~- a second nucleic acid encoding a full length antibody heavy chain linked at its C-terminus to a scFab,~~

~~- a spliceable intron, and~~

~~- a nucleic acid encoding a transmembrane domain or a GPI-anchor.~~

~~27~~23. The method according to ~~any one~~ claims 21 ~~to 26~~or 22, characterized in that each cell of the population of eukaryotic cells displays a membrane-bound full length antibody and secretes a full length antibody.

~~28~~24. The method according to ~~any one of~~ claims 21 ~~to 26~~or 22, characterized in that each cell of the population of eukaryotic cells displays and secretes a single full length antibody.

~~29~~25. The method according to any one claims 21 to ~~28~~24, characterized in that the antibody specifically binds to an antigen.

~~30~~26. The method according to ~~any one~~ claims 21 or 22~~to 23~~, characterized in that the antibody is a bivalent monospecific antibody.

~~31~~27. The method according to ~~any one~~ claims 21 ~~to~~ or 22 ~~and 24~~, characterized in that the antibody is a bivalent, bispecific antibody.

~~32~~28. The method according to ~~any one~~ claims 21 ~~to~~ or 22 ~~and 25 to 26~~, characterized in that the antibody is a tetravalent, bispecific antibody.

~~33~~29. The method according to any one of claims 21 to ~~28~~ 24 and ~~31~~ 27 to ~~32~~28, characterized in that the antibody specifically binds to two different antigens or to two epitopes on the same antigen.

~~34~~30. The method according to any one claims ~~31-~~27 to ~~33~~29, characterized in that the first full length antibody heavy chain comprises a hole mutation and the second antibody heavy chain comprises a knob mutation.

~~35~~31. The method according to any one claims ~~31-~~27 to ~~34~~30, characterized in that the first full length antibody light chain comprises as constant domain a CH1 domain and the first full length antibody heavy chain comprises as first constant domain a CL domain, or the second full length antibody light chain comprises as constant domain a CH1 domain and the second full length antibody heavy chain comprises as first constant domain a CL domain.

~~36~~32. The method according to any one of claims 21 to ~~35~~31, characterized in that the full length antibody comprises a constant region of human origin, especially of human IgG1, IgG2, or IgG4 class.

~~37~~33. The method according to any one of claims 21 to ~~36~~32, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

~~38~~34. The method according to any one of claims 21 to ~~36~~32, characterized in that the mammalian cell is a CHO cell or a HEK cell.

~~39~~35. The method according to any one of claims 21 to ~~38~~34, characterized in that the nucleic acid encoding an immunoglobulin heavy chain comprises all exons and all but one intron of the genomically organized immunoglobulin heavy chain gene.

~~40~~36. The method according to any one of claims 21 to ~~39~~35, characterized in that the transmembrane domain is a fragment of a transmembrane domain or a signal peptide for a GPI-anchor, whereby the fragment of the transmembrane domain is encoded by a single exon.

~~41~~37. The method according to any one of claims 21 to ~~40~~36, characterized in that the transmembrane domain an immunoglobulin transmembrane domain encoded by an M1-M2-exon-fusion of a single exon without the genomically intervening intron.

~~42~~38. The method according to any one of claims 21 to ~~41~~37, characterized in that the transmembrane domain is encoded by a cDNA.

~~43~~39. The method according to any one of claims 21 to ~~42~~38, characterized in that the antibody is a humanized or human antibody, especially a human antibody.

40. Use of a cell selected with a method according to any one of claims 1 to 39 for the production of an antibody.

~~44. A bicistronic expression cassette, which comprises in 5'- to 3'-direction~~

~~a promoter,~~

~~a first nucleic acid encoding a full length antibody light chain,~~

~~the EV71 IRES,~~

~~a second nucleic acid encoding a full length antibody heavy chain,~~

- a spliceable intron, and
- a nucleic acid encoding a transmembrane domain or a GPI-anchor.

45. A bicistronic expression cassette, which comprises in 5'- to 3'-direction
- a promoter,
- a first nucleic acid encoding a first full-length antibody heavy chain,
- the EV71-IRES,
- a second nucleic acid encoding a second full-length antibody heavy chain, and
- a nucleic acid encoding a transmembrane domain or a GPI-anchor.

46. The bicistronic expression cassette according to any one of claims 44 to 45, characterized in that the first full-length antibody heavy chain comprises a hole mutation and the second antibody heavy chain comprises a knob mutation.

47. The bicistronic expression cassette according to any one of claims 44 to 46, characterized in that the first full-length antibody light chain comprises as constant domain a CH1 domain and the first full-length antibody heavy chain comprises as first constant domain a CL domain, or the second full-length antibody light chain comprises as constant domain a CH1 domain and the second full-length antibody heavy chain comprises as first constant domain a CL domain.

48. The bicistronic expression cassette according to any one of claims 44 to 47, characterized in that the full-length antibody comprises a constant region of human origin, especially of human IgG1, IgG2, or IgG4 class.

49. The bicistronic expression cassette according to any one of claims 44 to 48, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

50. The bicistronic expression cassette according to any one of claims 44 to 49, characterized in that the mammalian cell is a CHO cell or a HEK cell.

51. The bicistronic expression cassette according to any one of claims 44 to 50, characterized in that the nucleic acid encoding an immunoglobulin heavy chain comprises all exons and all but one intron of the genomically organized immunoglobulin heavy chain gene.

52. The bicistronic expression cassette according to any one of claims 44 to 51, characterized in that the transmembrane domain is a fragment of a transmembrane domain or a signal peptide for a GPI-anchor, whereby the fragment of the transmembrane domain is encoded by a single exon.

53. The bicistronic expression cassette according to any one of claims 44 to 52, characterized in that the transmembrane domain an immunoglobulin

transmembrane domain encoded by an M1-M2-exon-fusion of a single exon without the genomically intervening intron.

54. The bicistronic expression cassette according to any one of claims 44 to 53, characterized in that the transmembrane domain is encoded by a cDNA.

55. The bicistronic expression cassette according to any one of claims 44 to 54, characterized in that the antibody is a humanized or human antibody, especially a human antibody.

56. A eukaryotic cell comprising a bicistronic expression cassette according to any one of claims 44 to 55.

57. The eukaryotic cell according to claim 56, characterized in that the bicistronic expression cassette has been transduced into the cell.

58. The eukaryotic cell according to any one of claims 56 to 57, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

59. The eukaryotic cell according to claim 58, characterized in that the mammalian cell is a CHO cell or a HEK cell.

60. A lentiviral vector comprising a bicistronic expression cassette, which comprises in 5'- to 3'-direction

- a promoter,
- a first nucleic acid encoding a full length antibody light chain,
- the EV71-IRES,
- a second nucleic acid encoding a full length antibody heavy chain,
- a spliceable intron, and
- a nucleic acid encoding a transmembrane domain or a GPI-anchor.

61. A lentiviral vector comprising a bicistronic expression cassette, which comprises in 5'- to 3'-direction

- a promoter,
- a first nucleic acid encoding a first full length antibody heavy chain,
- the EV71-IRES,
- a second nucleic acid encoding a second full length antibody heavy chain, and
- a nucleic acid encoding a transmembrane domain or a GPI-anchor.

62. The lentiviral vector according to any one of claims 60 to 61, characterized in that the full length antibody comprises a constant region of human origin, especially of human IgG1, IgG2, or IgG4 class.

63. The lentiviral vector according to any one of claims 60 to 62, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

64. The lentiviral vector according to any one of claims 60 to 63, characterized in that the mammalian cell is a CHO cell or a HEK cell.

65. The lentiviral vector according to any one of claims 60 to 64, characterized in that the nucleic acid encoding an immunoglobulin heavy chain comprises all exons and all but one intron of the genomically organized immunoglobulin heavy chain gene.

66. The lentiviral vector according to any one of claims 60 to 65, characterized in that the transmembrane domain is a fragment of a transmembrane domain or a signal peptide for a GPI anchor, whereby the fragment of the transmembrane domain is encoded by a single exon.

67. The lentiviral vector according to any one of claims 60 to 66, characterized in that the transmembrane domain an immunoglobulin transmembrane domain encoded by an M1-M2-exon fusion of a single exon without the genomically intervening intron.

68. The lentiviral vector according to any one of claims 60 to 67, characterized in that the transmembrane domain is encoded by a cDNA.

69. The lentiviral vector according to any one of claims 60 to 68, characterized in that the antibody is a humanized or human antibody, especially a human antibody.

70. A eukaryotic cell comprising a lentiviral vector according to any one of claims 60 to 69.

71. The eukaryotic cell according to claim 70, characterized in that the lentiviral vector has been transduced into the cell.

72. The eukaryotic cell according to any one of claims 70 to 71, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

73. The eukaryotic cell according to claim 72, characterized in that the mammalian cell is a CHO cell or a HEK cell.

74. Use of a lentiviral vector according to any one of claims 60 to 69 for the generation of a population of eukaryotic cells for displaying or secreting or both of a full-length antibody.

75. The use according to claim 74, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

76. The use according to claim 75, characterized in that the mammalian cell is a CHO cell or a HEK cell.

77. A lentiviral vector library comprising two or more lentiviral particles each comprising an expression vector according to any one of claims 60 to 69,

wherein the antibodies encoded by each vector differ in at least one amino acid from each other.

78. The lentiviral vector library according to claim 77, characterized in that the vector library comprises of from 1,000 to 1,000,000 different expression vectors.

79. The lentiviral vector library according to any one of claims 77 to 78, characterized in that the antibodies encoded by the vectors of the vector library differ in at least one amino acid residue in one of the CDRs of the antibody.

80. The lentiviral vector library according to claim 79, characterized in that the CDR is the heavy chain CDR3.

81. The lentiviral vector library according to any one of claims 77 to 80, characterized in that the expression vector library is obtained by randomization of one or more amino acids residues in one or more CDRs of a parent expression vector.

82. The lentiviral vector library according to any one of claims 77 to 81, characterized in that the lentiviral expression vector library is obtained by combination of nucleic acids encoding two different half antibodies.

83. The lentiviral vector library according to any one of claims 77 to 82, characterized in that the diversity of the lentiviral vector library is generated by using the HCVR and LCVR encoding nucleic acids obtained from a pool of B-cells producing an antibody that specifically binds to one antigen, or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

84. The lentiviral vector library according to any one of claims 77 to 82, characterized in that the diversity of the lentiviral vector library is generated by using pairs of HCVR and LCVR encoding nucleic acids selected from pools of HCVR and LCVR encoding nucleic acids, which are obtained by randomizing at least one codon of the HCVR and the LCVR encoding nucleic acid obtained from a single B-cell producing an antibody that specifically binds to one antigen, or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

85. The lentiviral vector library according to any one of claims 77 to 82, characterized in that the diversity of the lentiviral vector library is generated by using pairs of different HCVR encoding nucleic acids and a single LCVR encoding nucleic acid, whereby the different HCVR encoding nucleic acids

are obtained by randomizing at least one codon of the HCVR encoding nucleic acid obtained from a single B-cell producing an antibody that specifically binds to one antigen, or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

86. The lentiviral vector library according to any one of claims 77 to 82, characterized in that the diversity of the lentiviral vector library is generated by using pairs of different LCVR encoding nucleic acids and a single HCVR encoding nucleic acid, whereby the different LCVR encoding nucleic acids are obtained by randomizing at least one codon of the LCVR encoding nucleic acid obtained from a single B-cell producing an antibody that specifically binds to one antigen, or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

87. The lentiviral vector library according to any one of claims 77 to 86, characterized in that the single B-cell is a clonal population of B-cells.

88. The lentiviral vector library according to any one of claims 77 to 87, characterized in that the generating of the diversity of the lentiviral expression library comprises the steps of

(a):

(i) isolating RNA from the sub-population of B-cells,

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first pool of DNA molecules using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second pool of DNA molecules using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions; and

(v) providing pairs of one member of the first pool of DNA molecules and one member of the second pool of DNA molecules;

or (b):

(i) isolating RNA from a single B-cell, or from a clonal population of B-cells,

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions;

(v) generating a first pool of DNA molecules by randomizing at least one codon of the first DNA molecule;

(vi) generating a second pool of DNA molecules by randomizing at least one codon of the second DNA molecule, and

(vii) providing pairs of one member of the first pool of DNA molecules and one member of the second pool of DNA molecules;

or (e):

(i) isolating RNA from a single B-cell, or from a clonal population of B-cells;

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions;

(v) generating a pool of DNA molecules by randomizing at least one codon of the first DNA molecule, and

(vi) providing pairs of one member of the pool of DNA molecules and the second DNA molecule;

or (d):

(i) isolating RNA from a single B-cell, or from a clonal population of B-cells;

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions;

(v) generating a pool of DNA molecules by randomizing at least one codon of the second DNA molecule, and

(vi) providing pairs of one member of the pool of DNA molecules and the first DNA molecule.

89. The lentiviral vector library according to any one of claims 77 to 88, characterized in that the nucleic acid encoding an immunoglobulin heavy chain comprises all exons and all but one intron of the genomically organized immunoglobulin heavy chain gene.

90. The lentiviral vector library according to any one of claims 77 to 89, characterized in that the transmembrane domain is a fragment of a transmembrane domain or a signal peptide for a GPI-anchor, whereby the fragment of the transmembrane domain is encoded by a single exon.

91. The lentiviral vector library according to any one of claims 77 to 90, characterized in that the transmembrane domain an immunoglobulin transmembrane domain encoded by an M1-M2-exon-fusion of a single exon without the genomically intervening intron.

92. The lentiviral vector library according to any one of claims 77 to 91, characterized in that the transmembrane domain is encoded by a cDNA.

93. A eukaryotic cell library comprising two or more eukaryotic cells each comprising a bicistronic expression cassette according to any one of claims 44 to 55 or a lentiviral vector according to any one of claims 60 to 69, wherein the antibodies expressed by each cell differ in at least one amino acid from each other.

94. A eukaryotic cell library comprising the lentiviral vector library according to claims 77 to 92.

95. The eukaryotic cell library according to claim 94, characterized in that each eukaryotic cell of the eukaryotic cell library expresses a single antibody.

96. The eukaryotic cell library according to any one of claims 94 to 95, characterized in that each eukaryotic cell of the eukaryotic cell library displays a single antibody.

97. The eukaryotic cell library according to any one of claims 94 to 96, characterized in that the eukaryotic cell library is a population of eukaryotic cells expressing a library of antibodies wherein the encoding nucleic acids are derived from a population of B-cells of an immunized animal.

98. The eukaryotic cell library according to claim 97, characterized in that the B-cells are pre-selected for their specificity towards the one or more antigens of interest.

99. The eukaryotic cell library according to any one of claims 94 to 98, characterized in that the eukaryotic cell library is a population of eukaryotic cells wherein each cell comprises a first expression cassette encoding a full length antibody that specifically binds to a first antigen and a second expression cassette encoding a full length antibody that specifically binds to a second antigen.

100. The eukaryotic cell library according to any one of claims 94 to 99, characterized in that the eukaryotic cell library is a population of eukaryotic cells wherein each cell comprises a first expression cassette encoding a first full length antibody light chain and a first full length antibody heavy chain binding to a first antigen and a second expression cassette encoding a second full length antibody light chain and a second full length antibody heavy chain that specifically binds to a second antigen.

101. The eukaryotic cell library according to any one of claims 94 to 100, characterized in that the eukaryotic cell library is a population of eukaryotic cells wherein each cell comprises a expression cassette encoding a first full length antibody heavy chain that specifically binds to a first antigen and a second full length antibody heavy chain that specifically binds to a second antigen, wherein the eukaryotic cell expresses a common light chain.

102. The eukaryotic cell library according to any one of claims 94 to 102, characterized in that the first full length antibody heavy chain comprises a hole mutation and the second antibody heavy chain comprises a knob mutation.

103. The eukaryotic cell library according to any one of claims 94 to 101, characterized in that the first full length antibody light chain comprises as constant domain a CH1 domain and the first full length antibody heavy chain comprises as first constant domain a CL domain, or the second full length antibody light chain comprises as constant domain a CH1 domain and the second full length antibody heavy chain comprises as first constant domain a CL domain.

104. The eukaryotic cell library according to any one of claims 94 to 103, characterized in that the full length antibody comprises a constant region of human origin, especially of human IgG1, IgG2, or IgG4 class.

105. The eukaryotic cell library according to any one of claims 94 to 104, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

106. The eukaryotic cell library according to any one of claims 94 to 105, characterized in that the mammalian cell is a CHO cell or a HEK cell.

107. The eukaryotic cell library according to any one of claims 94 to 106, characterized in that the population of isolated B-cells or the single B-cells or the clonal population of B-cells is derived from a source selected from: (a) blood; (b) secondary lymphoid organs, especially spleen or lymph node; (c) bone marrow; and (d) tissue comprising memory B-cells.

108. The eukaryotic cell library according to claim 107, characterized in that the population of isolated B-cells comprises or especially consists of peripheral blood mononuclear cells (PBMCs).

109. The eukaryotic cell library according to any one of claims 94 to 108, characterized in that the animal is a mammal, especially a rat, a mouse, a rabbit, or a human.

110. The eukaryotic cell library according to claim 109, characterized in that the animal is a transgenic mouse or a transgenic rabbit or a human.

111. The eukaryotic cell library according to any one of claims 94 to 110, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or a fragment or antigenic determinant thereof; and

(b) selecting B-cells or a single B-cell that specifically binds the antigen of interest or fragment or antigenic determinant thereof.

112. The eukaryotic cell library according to any one of claims 94 to 111, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) coating a carrier with the antigen of interest or fragment or antigenic determinant thereof;

(b) contacting the population of isolated B-cells with the carrier and allowing the B-cells to bind to the carrier via the antigen of interest or fragment or antigenic determinant thereof;

(c) removing unbound B-cells, wherein especially the carrier comprises or further especially consists of beads, wherein still further especially the beads are paramagnetic beads; and

(d) recovering the sub-populations of B-cells or the single B-cell from the paramagnetic beads.

113. The eukaryotic cell library according to any one of claims 94 to 112, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell is performed by FACS-sorting.

114. The eukaryotic cell library according to any one of claims 94 to 113, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or fragment or antigenic determinant thereof, wherein the antigen of interest or fragment or antigenic determinant thereof is labeled with a fluorescence dye; and

(b) separating B-cells bound to the antigen of interest or fragment or antigenic determinant thereof by FACS sorting.

115. The eukaryotic cell library according to any one of claims 94 to 114, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or a fragment or antigenic determinant thereof;

(b) selecting a population of B-cells or a single B-cell that specifically binds the antigen of interest or fragment or antigenic determinant thereof; and

(c) selecting the B-cells for at least one additional parameter, wherein especially the selection for the at least one additional parameter is

(i) a positive selection for a parameter selected from presence of a B cell specific marker, especially CD19 or B220, and vitality of the B-cells; and/or

(ii) a negative selection for a parameter selected from: presence of IgM antibodies; presence of IgD antibodies, presence of cell death markers, and presence of apoptosis markers.

116. The eukaryotic cell library according to any one of claims 94 to 115, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells further comprises the step of selecting for class switched B-cells, especially for IgM and/or IgD-negative B-cells, most especially for IgM- and IgD-negative B-cells.

117. The eukaryotic cell library according to any one of claims 94 to 116, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or fragment or antigenic determinant thereof, wherein the antigen of interest or fragment or antigenic determinant thereof is labeled with a

first fluorescence dye, wherein especially the fluorescence dye is Alexa 647 nm, Alexa 488 or Alexa 546 nm;

(b) contacting the cells of the population of isolated B-cells with anti-IgM and/or anti-IgD antibodies, wherein the anti-IgM and/or anti-IgD antibodies are labeled with a second and/or a third fluorescence dye, wherein the second and/or the third fluorescence dye emits fluorescence at a wavelength which is different from the wavelength of the fluorescence emitted by the first fluorescence dye; and

(c) separating a population of B-cells or a single B-cell bound to the antigen of interest or fragment or antigenic determinant thereof but not bound to the anti-IgM and/or not bound to the anti-IgD antibodies by FACS sorting.

118. The eukaryotic cell library according to any one of claims 94 to 117, characterized in that the library is a viral library, especially an lentiviral library, and the introducing the library into a first population of eukaryotic, especially mammalian cells is performed by infecting the eukaryotic, especially mammalian cell with the viral library, especially with the lentiviral library, wherein further especially the infecting is performed at a multiplicity of infection of at most 10, especially at most 1, more especially at most 0.2, and most especially at most 0.1.

119. The eukaryotic cell library according to claim 118, characterized in that the multiplicity of infection is about 0.1.

120. The eukaryotic cell library according to any one of claims 94 to 119, characterized in that the isolating of the cell is performed by FACS sorting. In one embodiment the isolating of the cell comprises the steps of:

(a) staining the first population of eukaryotic, especially mammalian cells with the antigen of interest or fragment or antigenic determinant thereof, wherein the antigen of interest or fragment or antigenic determinant thereof is labeled with a fluorescence dye; and

(b) separating an individual cell that specifically binds the antigen of interest, or fragment or antigenic determinant thereof, by means of FACS sorting.

121. The eukaryotic cell library according to any one of claims 94 to 120, characterized in that the separating an individual cell that specifically binds the antigen of interest, or fragment or antigenic determinant thereof, by means of FACS sorting comprises the step of further selecting the cell at least one additional parameter.

122. The eukaryotic cell library according to any one of claims 94 to 122, characterized in that the method further comprises the steps of:

(a) cultivating at least one, especially exactly one, of the individual cells in the presence of a second population of eukaryotic cells, especially mammalian cells;

(b) verifying the capability of the second population of eukaryotic, especially mammalian cells of that specifically binds the antigen of interest, or fragment or antigenic determinant thereof.

123. The eukaryotic cell library according to any one of claims 94 to 122, characterized in that the first population of eukaryotic, especially mammalian cells and/or, especially and, the second population of eukaryotic, especially mammalian cells comprises or especially consists of cells selected from: (a) BHK-21 cells, especially ATCC CCL-10; (b) Neuro-2a cells; (c) HEK-293T cells, especially ATCC CRL-11268; (d) CHO-K1 cells, especially ATCC CRL-62; and (e) HEK293 cells.

124. The eukaryotic cell library according to any one of claims 94 to 123, characterized in that the first population of eukaryotic, especially mammalian cells and/or the second population of eukaryotic, especially mammalian cells comprises or especially consists of CHO-K1 cells, wherein further especially the expression library is an lentiviral expression library.

125. A method of selecting a cell expressing an antibody which specifically binds to an antigen of interest comprising the steps of

(a) optionally selecting from a population of B-cells a sub-population of B-cells or a single B-cell or a clonal population of B-cells secreting an antibody that specifically binds one or more antigens,

(b) generating a lentiviral expression library, wherein each member of the lentiviral expression library encodes a variant of the antibody or the antibodies that specifically binds one or more antigens, by

(i) generating a multitude of DNA molecules, wherein the generating comprises the step of amplifying a pool of DNA molecules from the sub-population of B-cells or the step of generating a library of DNA molecules from the DNA encoding a single antibody that specifically binds to one or two antigens of interest by randomization of the encoding nucleic acid sequence, and

(ii) cloning the multitude of DNA molecules into a lentiviral expression vector comprising an EV71 IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a

full-length antibody heavy chain in a soluble as well as membrane bound form;

(c) transducing a population of eukaryotic cells with a population of lentiviral virus particles each comprising a member of the lentiviral expression library;

(d) displaying antibodies encoded by the lentiviral expression library on the surface of the eukaryotic mammalian cells; and

(e) isolating from the population of eukaryotic cells a cell, wherein the cell is selected for the capability of the antibody displayed on its surface to specifically bind the antigen or antigens of interest or a fragment or antigenic determinant thereof.

126. A method of selecting a cell expressing an bispecific antibody (which specifically binds to two antigens of interest) comprising the steps of

(a) generating a lentiviral expression library, wherein each member of the lentiviral expression library encodes a variant of the bispecific antibody, by

(i) generating a multitude of DNA molecules from the DNA encoding a single bispecific antibody by randomization of the encoding nucleic acid sequence, and

(ii) cloning the multitude of DNA molecules into a lentiviral expression vector comprising an EV71 IRES linked bicistronic expression cassette for the expression of a full length bispecific antibody as membrane bound form;

(b) transducing a population of eukaryotic cells with a population of lentiviral virus particles each comprising a member of the lentiviral expression library;

(c) displaying antibodies encoded by the lentiviral expression library on the surface of the eukaryotic mammalian cells; and

(d) isolating from the population of eukaryotic cells a cell, wherein the cell is selected for the capability of the antibody displayed on its surface to specifically bind the antigens of interest or a fragment or antigenic determinant thereof.

127. The method according to any one of claims 125 to 126, characterized in that the method comprises generating a multitude of DNA molecules encoding antibodies, the generating a multitude of DNA molecules comprising the steps of:

(1) amplifying from a sub-population of B-cells a first pool of DNA molecules encoding heavy chain variable regions (HCVRs); and

(2) amplifying from the sub-population of B-cells a second pool of DNA molecules encoding light chain variable regions (LCVRs);

(3) cloning a combination of the multitude of DNA molecule encoding LCVRs and of the multitude of DNA molecules encoding HCVRs into a lentiviral expression vector comprising an EV71 IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain in a soluble as well as membrane bound form.

128. The method according to any one of claims 125 to 127, characterized in that the method comprises generating a multitude of DNA molecules encoding antibodies that specifically binds to one or two antigens of interest, the generating a multitude of DNA molecules comprising the steps of:

(1) amplifying from a single B-cell or a clonal population of B-cells a DNA molecule encoding the HCVR and a DNA molecule encoding the LCVR, and

(2) randomization the DNA molecule encoding the HCVR and/or the DNA molecule encoding the LCVR by randomizing at least one codon and thereby generating a multitude of DNA molecules encoding the HCVR and a multitude of DNA molecules encoding the LCVR;

(3) cloning a combination of the randomized multitude of DNA molecule encoding LCVRs and of the multitude of DNA molecules encoding HCVRs into a lentiviral expression vector comprising an EV71 IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain in a soluble as well as membrane bound form.

129. The method according to any one of claims 125 to 128, characterized in that the method comprises the generating of a lentiviral expression library, the generating comprising the steps of:

(i) generating a multitude of DNA molecules encoding antibodies, the generating comprising the steps of:

(1) isolating mRNA from the sub-population of B-cells;

(2) transcribing the mRNA to cDNA;

(3) amplifying from the cDNA a first pool of DNA molecules using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions; and

(4) amplifying from the cDNA a second pool of DNA molecules using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions;

(ii) cloning a pair of DNA molecules of the first and the second pool of DNA molecules into a lentiviral expression vector comprising an EV71-IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain in a soluble as well as membrane bound form.

130. The method according to any one of claims 135 to 129, characterized in that the method comprises the generating of a lentiviral expression library, the generating comprising the steps of:

(i) generating a multitude of DNA molecules encoding antibodies that specifically binds to one or two antigens, the generating comprising the steps of:

(1) isolating mRNA from a single B-cell or a clonal population of B-cells;

(2) transcribing the mRNA to cDNA;

(3) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding region; and

(4) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding region;

(5) randomizing the first and/or the second DNA molecule and thereby generating a first pool of DNA molecules and a second pool of DNA molecules,

(ii) cloning a pair of DNA molecules of the first and the second pool of DNA molecules into a lentiviral expression vector comprising an EV71-IRES linked bicistronic expression cassette for the expression of a full length antibody light chain and a full length antibody heavy chain in a soluble as well as membrane bound form.

131. The method according to any one of claims 125 to 130, characterized in that the eukaryotic cell is a mammalian cell or a yeast cell.

132. The method according to claim 131, characterized in that the mammalian cell is a CHO cell or a HEK cell.

133. The method according to any one of claims 125 to 132, characterized in that the animal is selected from the group consisting of sheep, elk, deer, donkey, mule deer, mink, horse, cattle, pig, goat, dog, cat, rat, hamster, guinea pig, and mouse. In one embodiment the animal is a mouse, a rat or, a primate.

134. The method according to any one of claims 125 to 133, characterized in that the animal is a non-human primate or a human.

135. The method according to any one of claims 125 to 134, characterized in that the animal is a transgenic animal with a human immunoglobulin locus.

136. The method according to any one of claims 125 to 135, characterized in that the nucleic acid is obtained by selecting from a population of isolated B-cells a sub-population of B-cells by selecting B-cells for their capability of that specifically binds the antigen of interest.

137. The method according to any one of claims 125 to 136, characterized in that the nucleic acid is obtained by selecting from a population of isolated B-cells a single B-cell by selecting a B-cell for its capability of that specifically binds the one or two antigens of interest.

138. The method according to any one of claims 125 to 137, characterized in that the single B-cell is a clonal B-cell population.

139. The method according to any one of claims 125 to 138, characterized in that the nucleic acid is obtained by amplifying the variable domain encoding nucleic acid from the isolated mRNA of a single B-cell or a clonal B-cell population and transcribing the amplified mRNA into cDNA.

140. The method according to any one of claims 125 to 139, characterized in that the diversity of the lentiviral vector library is generated by using the HCVR and LCVR encoding nucleic acids obtained from a pool of B-cells producing an antibody that specifically binds to one antigen, or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

141. The method according to any one of claims 125 to 140, characterized in that the diversity of the lentiviral vector library is generated by using pairs of HCVR and LCVR encoding nucleic acids selected from pools of HCVR and LCVR encoding nucleic acids, which are obtained by randomizing at least one codon of the HCVR and the LCVR encoding nucleic acid obtained from a single B-cell producing an antibody that specifically binds to one antigen,

or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

142. The method according to any one of claims 125 to 140, characterized in that the diversity of the lentiviral vector library is generated by using pairs of different HCVR encoding nucleic acids and a single LCVR encoding nucleic acid, whereby the different HCVR encoding nucleic acids are obtained by randomizing at least one codon of the HCVR encoding nucleic acid obtained from a single B-cell producing an antibody that specifically binds to one antigen, or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

143. The method according to any one of claims 125 to 142, characterized in that the diversity of the lentiviral vector library is generated by using pairs of different LCVR encoding nucleic acids and a single HCVR encoding nucleic acid, whereby the different LCVR encoding nucleic acids are obtained by randomizing at least one codon of the LCVR encoding nucleic acid obtained from a single B-cell producing an antibody that specifically binds to one antigen, or two different antigens, or that specifically binds to two different, non-overlapping epitopes of the same antigen.

144. The method according to any one of claims 125 to 143, characterized in that the single B-cell is a clonal population of B-cells.

145. The method according to any one of claims 125 to 144, characterized in that the generating of the diversity of the lentiviral expression library comprises the steps of

(a):

(i) isolating RNA from the sub-population of B-cells,

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first pool of DNA molecules using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second pool of DNA molecules using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions; and

(v) providing pairs of one member of the first pool of DNA molecules and one member of the second pool of DNA molecules;

or (b):

(i) isolating RNA from a single B-cell, or from a clonal population of B-cells;

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions;

(v) generating a first pool of DNA molecules by randomizing at least one codon of the first DNA molecule,

(vi) generating a second pool of DNA molecules by randomizing at least one codon of the second DNA molecule, and

(vii) providing pairs of one member of the first pool of DNA molecules and one member of the second pool of DNA molecules;

or (c):

(i) isolating RNA from a single B-cell, or from a clonal population of B-cells,

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions;

(v) generating a pool of DNA molecules by randomizing at least one codon of the first DNA molecule, and

(vi) providing pairs of one member of the pool of DNA molecules and the second DNA molecule;

or (d):

(i) isolating RNA from a single B-cell, or from a clonal population of B-cells,

(ii) transcribing the RNA to cDNA;

(iii) amplifying from the cDNA a first DNA molecule using a first mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying HCVR coding regions;

(iv) amplifying from the cDNA a second DNA molecule using a second mixture of oligonucleotides comprising at least two oligonucleotides capable of amplifying LCVR coding regions;

(v) generating a pool of DNA molecules by randomizing at least one codon of the second DNA molecule, and

(vi) providing pairs of one member of the pool of DNA molecules and the first DNA molecule.

146. The method according to any one of claims 125 to 145, characterized in that the variability of antigen-specific antibodies is increased by randomly combining different light and heavy chain variable regions.

147. The eukaryotic cell library according to any one of claims 125 to 146, characterized in that the population of isolated B-cells or the single B-cells or the clonal population of B-cells is derived from a source selected from: (a) blood; (b) secondary lymphoid organs, especially spleen or lymph node; (c) bone marrow; and (d) tissue comprising memory B-cells.

148. The eukaryotic cell library according to claim 147, characterized in that the population of isolated B-cells comprises or especially consists of peripheral blood mononuclear cells (PBMCs).

149. The eukaryotic cell library according to any one of claims 125 to 148, characterized in that the animal is a mammal, especially a rat, a mouse, a rabbit, or a human.

150. The eukaryotic cell library according to claim 149, characterized in that the animal is a transgenic mouse or a transgenic rabbit or a human.

151. The eukaryotic cell library according to any one of claims 125 to 150, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or a fragment or antigenic determinant thereof; and

(b) selecting B-cells or a single B-cell that specifically binds the antigen of interest or fragment or antigenic determinant thereof.

152. The eukaryotic cell library according to any one of claims 125 to 151, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) coating a carrier with the antigen of interest or fragment or antigenic determinant thereof;

(b) contacting the population of isolated B-cells with the carrier and allowing the B-cells to bind to the carrier via the antigen of interest or fragment or antigenic determinant thereof;

(c) removing unbound B-cells, wherein especially the carrier comprises or further especially consists of beads, wherein still further especially the beads are paramagnetic beads; and

(d) recovering the sub-populations of B-cells or the single B-cell from the paramagnetic beads.

153. The eukaryotic cell library according to any one of claims 125 to 152, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell is performed by FACS-sorting.

154. The eukaryotic cell library according to any one of claims 125 to 153, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or fragment or antigenic determinant thereof, wherein the antigen of interest or fragment or antigenic determinant thereof is labeled with a fluorescence dye; and

(b) separating B-cells bound to the antigen of interest or fragment or antigenic determinant thereof by FACS-sorting.

155. The eukaryotic cell library according to any one of claims 125 to 154, characterized in that the selecting from the population of isolated B-cells a sub-population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or a fragment or antigenic determinant thereof;

(b) selecting a population of B-cells or a single B-cell that specifically binds the antigen of interest or fragment or antigenic determinant thereof; and

(c) selecting the B-cells for at least one additional parameter, wherein especially the selection for the at least one additional parameter is

(i) a positive selection for a parameter selected from presence of a B cell specific marker, especially CD19 or B220, and vitality of the B-cells; and/or

(ii) a negative selection for a parameter selected from: presence of IgM antibodies; presence of IgD antibodies, presence of cell death markers, and presence of apoptosis markers.

156. The eukaryotic cell library according to any one of claims 125 to 155, characterized in that the selecting from the population of isolated B-cells a sub population of B-cells further comprises the step of selecting for class switched B-cells, especially for IgM-and/or IgD-negative B-cells, most especially for IgM- and IgD-negative B-cells.

157. The eukaryotic cell library according to any one of claims 125 to 156, characterized in that the selecting from the population of isolated B-cells a sub population of B-cells or a single B-cell comprises the steps of:

(a) contacting the population of isolated B-cells with the antigen of interest or fragment or antigenic determinant thereof, wherein the antigen of interest or fragment or antigenic determinant thereof is labeled with a first fluorescence dye, wherein especially the fluorescence dye is Alexa 647 nm, Alexa 488 or Alexa 546 nm;

(b) contacting the cells of the population of isolated B-cells with anti-IgM and/or anti-IgD antibodies, wherein the anti-IgM and/or anti-IgD antibodies are labeled with a second and/or a third fluorescence dye, wherein the second and/or the third fluorescence dye emits fluorescence at a wavelength which is different from the wavelength of the fluorescence emitted by the first fluorescence dye; and

(c) separating a population of B-cells or a single B-cell bound to the antigen of interest or fragment or antigenic determinant thereof but not bound to the anti-IgM and/or not bound to the anti-IgD antibodies by FACS sorting.

158. The eukaryotic cell library according to any one of claims 125 to 157, characterized in that the library is a viral library, especially an lentiviral library, and the introducing the library into a first population of eukaryotic, especially mammalian cells is performed by infecting the eukaryotic, especially mammalian cell with the viral library, especially with the lentiviral library, wherein further especially the infecting is performed at a multiplicity of infection of at most 10, especially at most 1, more especially at most 0.2, and most especially at most 0.1.

159. The eukaryotic cell library according to claim 158, characterized in that the multiplicity of infection is about 0.1.

160. The eukaryotic cell library according to any one of claims 125 to 159, characterized in that the isolating of the cell is performed by FACS sorting. In one embodiment the isolating of the cell comprises the steps of:

(a) staining the first population of eukaryotic, especially mammalian cells with the antigen of interest or fragment or antigenic determinant thereof, wherein the antigen of interest or fragment or antigenic determinant thereof is labeled with a fluorescence dye; and

(b) separating an individual cell that specifically binds the antigen of interest, or fragment or antigenic determinant thereof, by means of FACS sorting.

161. The eukaryotic cell library according to any one of claims 125 to 160, characterized in that the separating an individual cell that specifically binds the antigen of interest, or fragment or antigenic determinant thereof, by means of FACS sorting comprises the step of further selecting the cell at least one additional parameter.

162. The eukaryotic cell library according to claim 161, characterized in that the at least one additional parameter is selected from

(i) a positive selection for vitality of the cell; and/or

(ii) a negative selection for a parameter selected from: presence of IgM antibodies; presence of IgD antibodies, presence of cell death markers, and presence of apoptosis markers.

163. The eukaryotic cell library according to any one of claims 125 to 162, characterized in that the method further comprises the steps of:

(a) cultivating at least one, especially exactly one, of the individual cells in the presence of a second population of eukaryotic cells, especially mammalian cells;

(b) verifying the capability of the second population of eukaryotic, especially mammalian cells of that specifically binds the antigen of interest, or fragment or antigenic determinant thereof.

164. The eukaryotic cell library according to any one of claims 125 to 163, characterized in that the first population of eukaryotic, especially mammalian cells and/or, especially and, the second population of eukaryotic, especially mammalian cells comprises or especially consists of cells selected from: (a) BHK-21 cells, especially ATCC CCL-10; (b) Neuro-2a cells; (c) HEK-293T

cells, especially ATCC CRL-11268; (d) CHO-K1 cells, especially ATCC CRL-62; and (e) HEK293 cells.

165. The eukaryotic cell library according to any one of claims 125 to 164, characterized in that the first population of eukaryotic, especially mammalian cells and/or the second population of eukaryotic, especially mammalian cells comprises or especially consists of CHO-K1 cells, wherein further especially the expression library is an lentiviral expression library.

166. A workflow/method for the display of full length antibodies comprising a common light chain on the surface of eukaryotic cells and the selection of cells and thereby the selection of an antibody comprising the following steps:

- immunization of an experimental animal, such as a transgenic rabbit,
- selection of antigen-specific B-cells (by FACS, bulk sort),
- PCR amplification of heavy chain encoding nucleic acid: two separate polymerase chain reactions introducing unique restriction sites to enable directed cloning into the shuttle vector, one for the knob chain using one or more or all of the primer of SEQ ID NO: 6 to SEQ ID NO: 10 and the primer of SEQ ID NO: 11 and one for the hole chain using one or more or all of the primer of SEQ ID NO: 1 to SEQ ID NO: 4 and the primer of SEQ ID NO: 5; ligation: first heavy chain variable domain encoding nucleic acid into hole locus without transmembrane domain, i.e. of the EV71-IRES and second heavy chain variable domain encoding nucleic acid into knob locus with a transmembrane domain, i.e. downstream of the EV71-IRES,
- virus generation, infection of a mammalian cell stably expressing a common light chain, selection of bispecific antibodies displayed on the surface of the mammalian cell (by off-rate screening), bulk sort of hits (mammalian cell clones) using FACS,
- PCR of the complete first heavy chain encoding nucleic acid and the variable domain encoding nucleic acid (which has no TM domain) of the second heavy chain including the EV71-IRES and cloning into second shuttle vector without transmembrane domain, using e.g. primers of SEQ ID NO: 29 and SEQ ID NO: 30,
- virus generation, infection of a mammalian cell expressing a common light chain, single cell sort of cells, screening of the bispecific antibody in the supernatant, and selection of bispecific antibody.

167. A workflow/method for the display of full length antibodies comprising a common light chain on the surface of eukaryotic cells and the selection of cells and thereby the selection of an antibody comprising the following steps:

- immunization of an experimental animal, such as a transgenic rabbit,
- selection of antigen-specific B-cells (by FACS, bulk sort),
- PCR amplification of heavy chain encoding nucleic acid (two separate polymerase chain reactions introducing unique restriction sites to enable directed cloning into the shuttle vector: ligation: first heavy chain variable domain encoding nucleic acid into hole-locus with transmembrane-domain, i.e. upstream of the EV71-IRES, and second heavy chain variable domain encoding nucleic acid into knob-locus with a transmembrane domain, i.e. downstream of the EV71-IRES,
- virus generation, infection of a mammalian cell expressing a common light chain, selection of mammalian cell membrane-displayed bispecific antibodies (by off-rate screening), bulk sort of hits (mammalian cell clones) using FACS,
- PCR of the complete first heavy chain encoding nucleic acid and the variable domain of the second heavy chain encoding nucleic acid (2.2 kbp) including the EV71-IRES and cloning into second shuttle vector without transmembrane-domain; removal of the transmembrane-domain of the first heavy chain by restriction cutting and religation of the vector,
- virus generation, infection of a mammalian cell expressing a common light chain, single cell sort of cells, screening of the bispecific antibody in the supernatant, selection of bispecific antibody.

168. A workflow/method for the display of full length antibodies comprising a common light chain on the surface of eukaryotic cells and the selection of cells and thereby the selection of an antibody comprising the following steps:

- immunization of an experimental animal, such as a transgenic rabbit,
- selection of antigen-specific B-cells (by FACS, bulk sort),
- PCR amplification of heavy chain encoding nucleic acid: two separate polymerase chain reactions introducing unique restriction sites to enable directed cloning into the shuttle vectors; ligation: first heavy chain variable domain encoding nucleic acid into a first shuttle vector into a hole-locus with or without transmembrane domain, and second heavy chain variable domain encoding nucleic acid into a second

shuttle vector into a knob locus with or without a transmembrane domain, but at least one has a transmembrane domain,

virus generation (one for the first shuttle vector and one for the second shuttle vector), sequential infection of a mammalian cell expressing a common light chain with the first virus and the second virus, selection of bispecific antibodies displayed on the surface of the mammalian cell (by off-rate screening), bulk sort of hits (mammalian cell clones) using FACS,

PCR of the heavy chain variable domains encoding nucleic acid and cloning into a third shuttle vector in a bicistronic expression unit without transmembrane domain and EV71-IRES,

virus generation, infection of a mammalian cell expressing a common light chain, single cell sort of cells, screening of the bispecific antibody in the supernatant, and selection of bispecific antibody.

169. A workflow/method for the display of full length bispecific antibodies comprising a common light chain on the surface of eukaryotic cells and the selection of such eukaryotic cells and thereby also the selection of a bispecific antibody comprising the following steps:

a first experimental animal, in one embodiment a transgenic mouse or a transgenic rabbit, is immunized with a first antigen of interest, in one embodiment an extracellular receptor domain, wherein the B-cells of the experimental animal express the same light chain,

a second experimental animal, in one embodiment a transgenic mouse or a transgenic rabbit, is immunized with a second antigen of interest, in one embodiment an extracellular receptor domain, wherein the B-cells of the experimental animal express the same light chain,

whereby the first antigen and the second antigen are different,

selecting the B-cells of the first and second immunized experimental animal, in one embodiment by bulk sorting by FACS,

obtaining the heavy chain encoding nucleic acid of each B-cell by individual PCR amplification by two separate/sequential polymerase chain reactions introducing unique restriction sites to enable directed cloning into a shuttle vector/lentiviral expression vector,

ligation of a first heavy chain variable domain encoding nucleic acid into a shuttle vector/lentiviral expression vector upstream of an IRES, in one embodiment the IRES is the EV71-IRES, into a hole- or knob-locus with transmembrane domain, and ligation of a second heavy

chain variable domain encoding nucleic acid into a the same shuttle vector/lentiviral expression vector downstream of the IRES into the respective other locus with a transmembrane domain, i.e. if the heavy chain upstream of the IRES has a hole locus the heavy chain downstream of the IRES has a knob locus and vice versa, whereby the first heavy chain variable domain binds to a first antigen and the second variable domain binds to a second antigen, whereby the first antigen and the second antigen are different,

- virus generation,
- infection of a mammalian cell expressing a common light chain with the virus,
- selection of cells displaying bispecific antibodies on their surface by FACS of doubly labeled transduced cells,
- PCR of the complete first heavy chain encoding nucleic acid and the variable domain of the second heavy chain encoding nucleic acid (2.2 kbp) including the EV71 IRES and cloning into second shuttle vector without transmembrane domain; removal of the transmembrane domain of the first heavy chain by restriction cutting and religation of the vector,
- virus generation, infection of a mammalian cell expressing a common light chain, single cell sort of cells, screening of the bispecific antibody in the supernatant, and selection of a bispecific antibody.

170. A workflow/method for the display of full length bispecific antibodies comprising a common light chain on the surface of eukaryotic cells and the selection of such eukaryotic cells and thereby also the selection of a bispecific antibody comprising the following steps:

- an experimental animal, in one embodiment a transgenic mouse or a transgenic rabbit, is immunized with an antigen of interest, in one embodiment an extracellular receptor domain, wherein the B-cells of the experimental animal express the same light chain,
- selecting the B-cells of the immunized experimental animal, in one embodiment by bulk sorting by FACS,
- obtaining the heavy chain encoding nucleic acid of each B-cell by individual PCR amplification by two separate/sequential polymerase chain reactions introducing unique restriction sites to enable directed cloning into a shuttle vector/lentiviral expression vector,

- ligation of the heavy chain variable domain encoding nucleic acid into a shuttle vector/lentiviral expression vector downstream of an IRES, in one embodiment the IRES is the EV71 IRES, into a heavy chain locus with transmembrane domain, wherein the shuttle vector/lentiviral expression vector comprises upstream of the IRES the common light chain encoding nucleic acid,
- virus generation,
- infection of a mammalian cell expressing a common light chain with the virus,
- selection of cells displaying antibodies on their surface by FACS of antigen specific labeled transduced cells, in one embodiment by bulk sorting by FACS,
- obtaining the heavy chain encoding nucleic acid of each selected cell by individual PCR amplification by two separate/sequential polymerase chain reactions introducing unique restriction sites to enable directed cloning into a shuttle vector/lentiviral expression vector,
- ligation of a first heavy chain variable domain encoding nucleic acid into a shuttle vector/lentiviral expression vector upstream of an IRES, in one embodiment the IRES is the EV71 IRES, into a hole- or knob-locus without transmembrane domain, and ligation of a second heavy chain variable domain encoding nucleic acid into a the same shuttle vector/lentiviral expression vector downstream of the IRES into the respective other locus without a transmembrane domain, i.e. if the heavy chain upstream of the IRES has a hole locus the heavy chain downstream of the IRES has a knob locus and vice versa, whereby the first heavy chain variable domain binds to a first antigen and the second variable domain binds to a second antigen, whereby the first antigen and the second antigen can be the same or different,
- virus generation,
- infection of a mammalian cell expressing a common light chain with the virus,
- selection of a cell secreting a bispecific antibody.

171. Use of a cell selected with a method according to any one of claims 1 to 170 for the production of an antibody.