expression of recombinant IgG antibodies is reported by Li et al. (J. Immunol. Meth. 318 (2007) 113-124). Ho, S.C.L., et al. report "IRES-mediated tricistronic vectors for enhancing generation of high monoclonal antibody expressing CHO cell lines (J. Biotechnol. 157 (2011) 130-139). Production of anti-CD2 chimeric antibody by recombinant animal cells is reported by Hotta, A., et al. (J. Biosci. Bioeng. 98 (2004) 298-303). Lee, J-C., et al. report "High-efficiency protein expression mediated by enterovirus 71 internal ribosome entry (Biotechnol. Bioeng. 90 (2005) 656-662). In WO 2008/142124 recombinant protein production in Avian EBX® cells is reported.

Summary ~~of the Invention~~

It has been found that for the recombinant production of antibodies the position of the heavy chain expression cassette in front of the light chain expression cassette (HC-LC (5'-3')) provides for better expression results compared to the inverse order (LC-HC (5'-3')). Additionally it has been found that the position of the selection marker after both antibody chain expression cassettes provides for better expression results (HC-LC-SM (5'-3')) compared to the bidirectional position in front of the first antibody chain (SM (3'-5')-HC-LC (5'-3')).

It has been found that for stable transfections the in row arrangement of 1) antibody heavy chain, 2) antibody light chain and 3) selection marker has shown to be optimal. But whereas the hEF1α promoter is clearly superior to the hCMV promoter in stable pools, we have seen the clear opposite effect on single clone level. Here, the human cytomegalovirus immediate early promoter/enhancer (hCMV) generated clones with highest productivity. Moreover, its performance can further be improved by combining it with the bGH polyA signal and the terminator sequence of the human gastrin gene (hGT), which increases both productivity and stability of expression.

It has been found that the use of an expression vector comprising an expression cassettes for an antibody heavy chain and an expression cassette for an antibody light chain each comprising a promoter, a structural gene and a polyA signal sequence and optionally a terminator sequence, results in a higher number of antibody producing/secreting cell clones after transfection if 1) the promoter is the human cytomegalovirus promoter (hCMV), the polyA signal sequence is the bovine growth hormone polyA signal sequence (bGH polyA) and the terminator sequence is the human gastrin gene transcription terminator sequence (hGT), or 2)

~~One aspect a~~As reported herein is the use of an expression vector as reported herein for the recombinant production of an antibody.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein for the generation of a stable cell line.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein for the generation of a production cell line.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the generation of a stable cell line.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the generation of a production cell line.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the recombinant production of an antibody.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human CMV promoter for the generation of a production cell line.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human CMV promoter for the generation of a stable cell line.

~~One aspect a~~As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human CMV promoter for the production of an antibody.

~~One aspect a~~As reported herein is a method for the transfection of a eukaryotic cell with an expression vector, characterized in that the expression vector is linearized prior to the transfection by cleavage in the prokaryotic origin of replication.

In one embodiment the prokaryotic origin of replication is between one antibody light chain expression cassette and one antibody heavy chain expression cassette.

~~One aspect a~~As reported herein is the use of a method as reported herein for the generation of a eukaryotic cell for the recombinant production of an antibody.

~~One aspect a~~As reported herein is a method for the selection of a eukaryotic cell comprising a nucleic acid encoding an antibody, characterized in that the selection agent is added to the cultivation medium for the first time about 24 hours after transfection.

~~One aspect a~~As reported herein is the use of a method as reported herein for the generation of a eukaryotic cell for the recombinant production of an antibody.

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

~~One aspect a~~As reported herein is a method for the production of an antibody comprising the following steps:

- cultivating a eukaryotic cell comprising an expression vector as reported herein, and
- recovering the antibody from the cell or the cultivation medium.

~~One aspect a~~As reported herein is a method for the production of an antibody comprising the following step:

- cultivating a eukaryotic cell, which has been obtained by the transfection with an expression vector that has been linearized prior to the transfection by cleavage in the prokaryotic origin of replication, and
- recovering the antibody from the cell or the cultivation medium.

~~One aspect a~~As reported herein is a method for the production of an antibody comprising the following step:

- cultivating a eukaryotic cell, which has been selected by the addition of a selection agent about 24 hours after transfection to the cultivation, and
- recovering the antibody from the cell or the cultivation medium.

~~One aspect a~~As reported herein is a method for the transfection of a eukaryotic cell with an expression vector comprising prokaryotic and eukaryotic nucleic acid sequences, characterized in that the prokaryotic nucleic acid sequences are removed from the expression vector prior to the transfection of the eukaryotic cell with the expression vector.

One aspect as reported herein is a method for the transfection of a eukaryotic cell with an expression vector encoding an antibody whereby the expression vector comprises prokaryotic and eukaryotic nucleic acid sequences, characterized in that the prokaryotic nucleic acid sequences are removed from the expression vector prior to the transfection of the eukaryotic cell with the expression vector.

~~One aspect a~~As reported herein is the use of a linearized expression vector comprising no prokaryotic nucleic acid sequences for the transfection of a eukaryotic cell.

One aspect as reported herein is the use of a linearized expression vector encoding an antibody comprising no prokaryotic nucleic acid sequences for the transfection of a eukaryotic cell.

~~One aspect a~~As reported herein is the use of an expression vector comprising only eukaryotic nucleic acid sequences for the generation of a eukaryotic cell for the recombinant production of an antibody.

One aspect as reported herein is the use of an expression vector encoding an antibody comprising only eukaryotic nucleic acid sequences for the generation of a eukaryotic cell for the recombinant production of an antibody.

In one embodiment ~~of all aspects~~ as reported herein the antibody is a bispecific antibody.

In one embodiment the bispecific antibody has a first binding specificity or binding site that specifically binds to a first antigen or a first epitope on an antigen and the bispecific antibody has a second binding specificity or binding site that specifically binds to a second antigen or second epitope on the antigen.

If one embodiment the expression vector comprises either

- a first expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a first antibody light chain, a polyA signal sequence, and optionally a terminator sequence,
- a second expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a second antibody light chain, a polyA signal sequence, and optionally a terminator sequence,

- a third expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a first antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence,

- a fourth expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a second antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence,

or

- a first expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding an antibody light chain, a polyA signal sequence, and optionally a terminator sequence,
- a second expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a first antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence, and

- a third expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a second antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence,

whereby the antibody light chain is a common light chain for both antibody heavy chains.

In one embodiment ~~of all aspects~~ as reported herein the expression vector comprises

- an antibody light chain expression cassette,
- a first antibody heavy chain expression cassette,
- a second antibody heavy chain expression cassette, and
- a selection marker expression cassette,

wherein at least one of the antibody heavy chain expression cassettes, the antibody light chain expression cassette, and the selection marker expression cassette are arranged unidirectional, and

wherein the unidirectional expression cassettes are arranged in the 5' to 3' sequence of antibody heavy chain expression cassette, antibody light chain expression cassette and selection marker expression cassette.

In one embodiment ~~of all aspects~~ as reported herein the expression vector comprises

- a first antibody light chain expression cassette,
- a second antibody light chain expression cassette,
- a first antibody heavy chain expression cassette,
- a second antibody heavy chain expression cassette, and
- a selection marker expression cassette,

wherein one of the antibody heavy chain expression cassettes, one of the antibody light chain expression cassette, and the selection marker expression cassette are arranged unidirectional, and

wherein the unidirectional expression cassettes are arranged in the 5' to 3' sequence of antibody heavy chain expression cassette, antibody light chain expression cassette and selection marker expression cassette.

In one embodiment encodes the one of the antibody heavy chain expression cassettes an antibody heavy chain comprising a hole mutation.

Patent Claims

1. An expression vector comprising

an antibody light chain expression cassette,

an antibody heavy chain expression cassette, and

a selection marker expression cassette,

wherein the expression cassettes are arranged unidirectional, and

wherein the expression cassettes are arranged in the 5' to 3' sequence of antibody heavy chain expression cassette, antibody light chain expression cassette and selection marker expression cassette.

2. The expression vector according to claim 1, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other comprise a promoter selected from the human elongation factor 1 alpha promoter, the human CMV promoter, and the SV40 promoter.

3. The expression vector according to any one of claims 1 to 2, characterized in that one, two, or all three expression cassettes comprise the human elongation factor 1 alpha promoter.

4. The expression vector according to any one of claims 1 to 3, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human elongation factor 1 alpha promoter.

5. The expression vector according to any one of claims 1 to 4, characterized in that the expression cassettes do not comprise a terminator sequence.

6. The expression vector according to claim 5, characterized in that the terminator sequence is the human gastrin gene transcription terminator sequence (hGT).

7. The expression vector according to any one of claims 1 to 2, characterized in that one, two, or all three expression cassettes comprise the human CMV promoter.

8. The expression vector according to any one of claims 1, 2 and 7, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human CMV promoter.

9. The expression vector according to any one of claims 1 to 4 and 7 to 8, characterized in that one, two, or three expression cassettes comprise the bovine growth hormone polyA signal sequence.

10. The expression vector according to any one of claims 1 to 4 and 7 to 9, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the bovine growth hormone polyA signal sequence.

11. The expression vector according to any one of claims 1 to 4 and 7 to 10, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other a polyA signal sequence selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

12. The expression vector according to any one of claims 1 and 7 to 11, characterized in that one, two, or all three expression cassettes comprise the human gastrin terminator sequence after the polyA signal sequence.

13. The expression vector according to any one of claims 1 and 7 to 12, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human gastrin terminator sequence after the polyA signal sequence.

14. The expression vector according to any one of claims 1 and 7 to 13, characterized in that the antibody light chain expression cassette and/or the

antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other in 5' to 3' direction the bovine growth hormone polyA signal sequence and the human gastrin terminator sequence.

15. The expression vector according to any one of claims 1 to 14, characterized in that the promoter of one, two, or all three expression cassettes comprises an Intron A.

16. The expression vector according to any one of claims 1 to 8 and 12 to 13, characterized in that one, two, or all three expression cassettes comprise the SV40 polyA signal sequence.

17. The expression vector according to claim 16, characterized in that one, two, or all three expression cassettes comprise the SV40 promoter.

18. The expression vector according to any one of claims 1 to 17, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain comprises at least one intron.

19. The expression vector according to any one of claims 1 to 18, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

20. Use of an expression vector according to any one of claims 1 to 19 for the recombinant production of an antibody.

21. Use of an expression vector according to any one of claims 1 to 19 for the generation of a stable cell line.

22. Use of an expression vector according to any one of claims 1 to 19 for the generation of a production cell line.

23. Use of an expression vector according to any one of claims 1 to 19 comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the generation of a stable cell line.

24. Use of an expression vector according to any one of claims 1 to 19 comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the generation of a production cell line.

25. Use of an expression vector according to any one of claims 1 to 19 comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the recombinant production of an antibody.

26. Use of an expression vector according to any one of claims 1 to 19 comprising at least one expression cassette comprising a human CMV promoter for the generation of a production cell line.

27. Use of an expression vector according to any one of claims 1 to 19 comprising at least one expression cassette comprising a human CMV promoter for the generation of a stable cell line.

28. Use of an expression vector according to any one of claims 1 to 19 comprising at least one expression cassette comprising a human CMV promoter for the production of an antibody.

29. A method for the transfection of a eukaryotic cell with an expression vector, characterized in that the expression vector is linearized prior to the transfection by cleavage in the prokaryotic origin of replication.

30. The method according to claim 29, characterized in that the prokaryotic origin of replication is between an antibody light chain expression cassette and an antibody heavy chain expression cassette.

31. The method according to any one of claims 29 to 30, characterized in that the expression vector is an expression vector according to any one of claims 1 to 19.

32. A method for the selection of a eukaryotic cell comprising a nucleic acid encoding an antibody, characterized in that the selection agent is added to the cultivation medium for the first time about 24 hours after transfection.

33. The method according to claim 32, characterized in that the nucleic acid encoding an antibody is comprised in an expression vector according to any one of claims 1 to 19.

34. Use of a method according to any one of claims 32 to 33 for the generation of a eukaryotic cell for the recombinant production of an antibody.

~~35. Use of a cell selected with a method according to any one of claims 32 to 33 for the recombinant production of an antibody.~~

~~36. A method for the production of an antibody comprising the following steps:~~

~~cultivating a eukaryotic cell that has been transfected with an expression vector according to any one of claims 1 to 19, and~~

~~recovering the antibody from the cell or the cultivation medium.~~

~~37. A method for the production of an antibody comprising the following step:~~

~~cultivating a eukaryotic cell, which has been obtained by the transfection with an expression vector encoding an antibody that has been linearized prior to the transfection by cleavage in the prokaryotic origin of replication, and~~

~~recovering the antibody from the cell or the cultivation medium.~~

~~38. A method for the production of an antibody comprising the following step:~~

~~cultivating a eukaryotic cell that has been transfected with an expression vector encoding an antibody, which has been selected by the addition of a selection agent about 24 hours after transfection to the cultivation, and~~

~~recovering the antibody from the cell or the cultivation medium.~~

Patent Claims

~~39~~1. A method for the transfection of a eukaryotic cell with an expression vector encoding an antibody whereby the expression vector comprises prokaryotic and eukaryotic nucleic acid sequences, characterized in that the prokaryotic nucleic acid sequences are removed from the expression vector prior to the transfection of the eukaryotic cell with the expression vector.

~~40~~2. The method according to ~~any one of~~ claims ~~1~~37 to ~~39~~, characterized in that the expression vector encoding an antibody is an expression vector comprising ~~according to any one of claims 1 to 19.~~

- an antibody light chain expression cassette,

- an antibody heavy chain expression cassette, and

- a selection marker expression cassette,

wherein the expression cassettes are arranged unidirectional, and

wherein the expression cassettes are arranged in the 5' to 3' sequence of antibody heavy chain expression cassette, antibody light chain expression cassette and selection marker expression cassette.

3. The method according to claim 2, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other comprise a promoter selected from the human elongation factor 1 alpha promoter, the human CMV promoter, and the SV40 promoter.

4. The method according to claim 2 or 3, characterized in that one, two, or all three expression cassettes comprise the human elongation factor 1 alpha promoter.

5. The method according to any one of claims 2 to 4, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human elongation factor 1 alpha promoter.

6. The method according to any one of claims 2 to 5, characterized in that the expression cassettes do not comprise a terminator sequence.

7. The method according to claim 6, characterized in that the terminator sequence is the human gastrin gene transcription terminator sequence (hGT).

8. The method according to claims 2 or 3, characterized in that one, two, or all three expression cassettes comprise the human CMV promoter.

9. The method according to any one of claims 2, 3 and 8, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human CMV promoter.

10. The method according to any one of claims 2 to 5 and 8 to 9, characterized in that one, two, or three expression cassettes comprise the bovine growth hormone polyA signal sequence.

11. The method according to any one of claims 2 to 5 and 8 to 10, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the bovine growth hormone polyA signal sequence.

12. The method according to any one of claims 2 to 5 and 8 to 11, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other a polyA signal sequence selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

13. The method according to any one of claims 2 and 8 to 12, characterized in that one, two, or all three expression cassettes comprise the human gastrin terminator sequence after the polyA signal sequence.

14. The method according to any one of claims 2 and 8 to 13, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human gastrin terminator sequence after the polyA signal sequence.

15. The method according to any one of claims 2 and 8 to 14, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other in 5' to 3' direction the bovine growth hormone polyA signal sequence and the human gastrin terminator sequence.

16. The method according to any one of claims 2 to 15, characterized in that the promoter of one, two, or all three expression cassettes comprises an Intron A.

17. The method according to any one of claims 2 to 8 and 13 to 14, characterized in that one, two, or all three expression cassettes comprise the SV40 polyA signal sequence.

18. The method according to claim 17, characterized in that one, two, or all three expression cassettes comprise the SV40 promoter.

19. The method according to any one of claims 2 to 18, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain comprises at least one intron.

20. The method according to any one of claims 2 to 19, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~44~~21. Use of a linearized expression vector encoding an antibody comprising no prokaryotic nucleic acid sequences for the transfection of a eukaryotic cell.

~~42~~22. Use of an expression vector encoding an antibody comprising only eukaryotic nucleic acid sequences for the generation of a eukaryotic cell for the recombinant production of an antibody.

~~43~~23. The use according to any one of claims ~~35, 41~~21 or 22~~,~~ ~~42~~, characterized in that the expression vector encoding an antibody is an expression vector comprising~~according to any one of claims 1 to 19.~~

- an antibody light chain expression cassette,

- an antibody heavy chain expression cassette, and

- a selection marker expression cassette,

wherein the expression cassettes are arranged unidirectional, and

wherein the expression cassettes are arranged in the 5' to 3' sequence of antibody heavy chain expression cassette, antibody light chain expression cassette and selection marker expression cassette.

24. The use according to claim 23, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other comprise a promoter selected from the human elongation factor 1 alpha promoter, the human CMV promoter, and the SV40 promoter.

25. The use according to claim 23 or 24, characterized in that one, two, or all three expression cassettes comprise the human elongation factor 1 alpha promoter.

26. The use according to any one of claims 23 to 25, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human elongation factor 1 alpha promoter.

27. The use according to any one of claims 23 to 26, characterized in that the expression cassettes do not comprise a terminator sequence.

28. The use according to claim 27, characterized in that the terminator sequence is the human gastrin gene transcription terminator sequence (hGT).

29. The use according to claims 23 or 24, characterized in that one, two, or all three expression cassettes comprise the human CMV promoter.

30. The use according to any one of claims 23, 24 and 29, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human CMV promoter.

31. The use according to any one of claims 23 to 26 and 29 to 30, characterized in that one, two, or three expression cassettes comprise the bovine growth hormone polyA signal sequence.

32. The use according to any one of claims 23 to 26 and 29 to 31, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the bovine growth hormone polyA signal sequence.

33. The use according to any one of claims 23 to 26 and 29 to 32, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other a polyA signal sequence selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

34. The use according to any one of claims 23 and 29 to 33, characterized in that one, two, or all three expression cassettes comprise the human gastrin terminator sequence after the polyA signal sequence.

35. The use according to any one of claims 23 and 29 to 34, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other the human gastrin terminator sequence after the polyA signal sequence.

36. The use according to any one of claims 23 and 29 to 35, characterized in that the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker expression cassette comprise independently of each other in 5' to 3' direction the bovine growth hormone polyA signal sequence and the human gastrin terminator sequence.

37. The use according to any one of claims 23 to 36, characterized in that the promoter of one, two, or all three expression cassettes comprises an Intron A.

38. The use according to any one of claims 23 to 29 and 34 to 35, characterized in that one, two, or all three expression cassettes comprise the SV40 polyA signal sequence.

39. The use according to claim 38, characterized in that one, two, or all three expression cassettes comprise the SV40 promoter.

40. The use according to any one of claims 23 to 39, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain comprises at least one intron.

41. The use according to any one of claims 23 to 40, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~44. The expression vector according to any one of claims 1 to 19, characterized in that the expression vector is encoding a bispecific antibody.~~

~~45. The expression vector according to claim 44, characterized in that the bispecific antibody has a first binding specificity or binding site that specifically binds to a first antigen or a first epitope on a first antigen and the bispecific antibody has a second binding specificity or binding site that specifically binds to a second antigen or second epitope on a second antigen.~~

~~46. The expression vector according to any one of claims 1 to 19 and 44 to 45, characterized in that the expression vector comprises either~~

- ~~a first expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a first antibody light chain, a polyA signal sequence, and optionally a terminator sequence,~~
- ~~a second expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a second antibody light chain, a polyA signal sequence, and optionally a terminator sequence,~~
- ~~a third expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a first antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence,~~
- ~~a fourth expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a second antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence,~~

~~or~~

- ~~a first expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding an antibody light chain, a polyA signal sequence, and optionally a terminator sequence,~~

- a second expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a first antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence, and
- a third expression cassette comprising in 5' to 3' direction a promoter, a nucleic acid encoding a second antibody heavy chain, a polyA signal sequence, and optionally a terminator sequence.

whereby the antibody light chain is a common light chain for both antibody heavy chains.

47. The expression vector according to any one of claims 1 to 19 and 44 to 46, characterized in that the expression vector comprises

- an antibody light chain expression cassette,
- a first antibody heavy chain expression cassette,
- a second antibody heavy chain expression cassette, and
- a selection marker expression cassette,

wherein at least one of the antibody heavy chain expression cassettes and the antibody light chain expression cassette and the selection marker expression cassette are arranged unidirectional, and

wherein the unidirectional expression cassettes are arranged in the 5' to 3' sequence of antibody heavy chain expression cassette, antibody light chain expression cassette and selection marker expression cassette.

48. The expression vector according to any one of claims 1 to 19 and 44 to 46, characterized in that the expression vector comprises

- a first antibody light chain expression cassette,
- a second antibody light chain expression cassette,
- a first antibody heavy chain expression cassette,
- a second antibody heavy chain expression cassette, and
- a selection marker expression cassette.

wherein one of the antibody heavy chain expression cassettes and one of the antibody light chain expression cassette and the selection marker expression cassette are arranged unidirectional. and

wherein the unidirectional expression cassettes are arranged in the 5' to 3' sequence of antibody heavy chain expression cassette, antibody light chain expression cassette and selection marker expression cassette.

49. The expression vector according to any one of claims 44 to 48, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a hole mutation.

50. The expression vector according to any one of claims 44 to 49, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a knob mutation.

51. The expression vector according to any one of claims 44 to 50, characterized in that one of the antibody light chain expression cassettes encodes an antibody light chain variant comprising an antibody light chain variable domain and an antibody heavy chain CH1 domain as constant domain and/or one of the antibody light chain expression cassettes encodes an antibody light chain comprising an antibody light chain variable domain and an antibody light chain CL domain as constant domain.

52. The expression vector according to any one of claims 44 to 51, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain variant comprising as first constant domain an antibody light chain constant domain (CL), and/or one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising as first constant domain an antibody heavy chain CH1 domain.