In one embodiment the expression plasmid further comprises a selection marker. In one embodiment the expression cassettes and the selection marker are arranged bidirectional.

There is also reported herein the use of an expression plasmid as reported herein for the transient expression of an antibody or the stable expression of an antibody.

There is also reported herein a eukaryotic cell comprising an expression plasmid as reported herein.

In one aspect, there is provided a method for selecting a recombinant stable transfected mammalian cell comprising the following steps: a) transfecting a mammalian cell with an expression vector comprising - a first expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody light chain, a bGH polyA signal sequence, and a hGT terminator sequence, - a second expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody heavy chain, a bGH polyA signal sequence, and a hGT terminator sequence, whereby the first expression cassette and the second expression cassette are arranged unidirectional, and thereby obtaining a multitude of recombinant mammalian cells, b) selecting from the multitude of recombinant mammalian cells a (single) stable transfected recombinant mammalian cell.

There is also reported herein a method for the production of an antibody comprising the steps of

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

In one embodiment the eukaryotic cell is a mammalian cell. Hence, in another aspect, there is provided a method for producing an antibody comprising the following steps: a) cultivating a mammalian cell comprising - a first expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody light chain, a bGH polyA signal sequence, and a hGT terminator sequence, - a second expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody heavy chain, a bGH polyA signal sequence, and a hGT terminator sequence, and b) recovering the antibody from the cell or the cultivation medium, whereby the first expression cassette and the second expression cassette are arranged unidirectional for the stable production of the antibody. In one embodiment the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

In another aspect, there is provided an expression vector comprising - a first expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody light chain, a bGH polyA signal sequence, and a hGT terminator sequence, - a second expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody heavy chain, a bGH polyA signal sequence, and a hGT terminator sequence.

There is also reported herein an expression plasmid comprising

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

wherein the first and/or the second promoter is the human elongation factor 1 alpha promoter.

In one embodiment one or both of the expression cassettes do not comprise in addition after the polyA signal sequence the human gastrin terminator sequence.

In one embodiment one or both of the expression cassettes are free of the human gastrin terminator sequence.

In one embodiment the human elongation factor 1 alpha promoter comprises an Intron A.

In one embodiment the first and the second polyA signal sequence is selected independently of each other from the SV40 polyA signal sequence and the bovine growth hormone polyA signal sequence.

In one embodiment the first and the second promoter are selected independently of each other from the human CMV promoter, the SV40 promoter, and the human elongation factor 1 alpha promoter.

In one embodiment the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain comprises at least one intron.

In one embodiment the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

In one embodiment the first expression cassette and the second expression cassette are arranged unidirectional.

In one embodiment the expression plasmid further comprises a selection marker.

In one embodiment the expression cassettes and the selection marker are arranged bidirectional.

There is also reported herein the use of an expression plasmid as reported herein for the transient expression of an antibody or the stable expression of an antibody.

There is also reported herein a eukaryotic cell comprising an expression plasmid as reported herein.

There is also reported herein a method for the production of an antibody comprising the steps of

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

In one embodiment the eukaryotic cell is a mammalian cell. In one embodiment the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

There is also ~~One aspect as~~ reported herein ~~is~~ an expression plasmid comprising in 5' to 3' direction a promoter sequence, a nucleic acid encoding an antibody heavy chain or an antibody light chain, an IRES element, a nucleic acid sequence encoding a

selection marker, and a polyA signal sequence, whereby the IRES element is the EMCV-IRES element.

In one embodiment the nucleic acid encodes an antibody heavy chain.

In one embodiment the selection marker is a fusion protein of the formula A-C-S, whereby A is a detectable polypeptide, C is a proteolytic signal sequence and S is a selectable marker.

In one embodiment the proteolytic signal sequence is the PEST sequence of the ornithine decarboxylase.

In one embodiment the detectable polypeptide is green fluorescent protein.

In one embodiment the selectable marker is neomycin.

There is also reported herein a nucleic acid encoding a polypeptide that comprises in N-terminal to C-terminal direction a green fluorescent protein, the PEST sequence of ornithine decarboxylase, and neomycin.

There is also reported herein the use of a nucleic acid encoding a polypeptide that comprises in N-terminal to C-terminal direction a green fluorescent protein, the PEST sequence of ornithine decarboxylase, and neomycin for the selection of antibody secreting cells.

There is also ~~One aspect as~~ reported herein ~~is~~ the use of an expression cassette comprising in 5' to 3' direction a promoter sequence, a nucleic acid encoding an antibody heavy chain or an antibody light chain, an IRES element, a nucleic acid sequence encoding a selection marker, and a polyA signal sequence for the selection of antibody producing cells, whereby the IRES element is the EMCV-IRES element.

In one embodiment the nucleic acid encodes an antibody heavy chain.

In one embodiment the selection marker is a fusion protein of the formula A-C-S, whereby A is a detectable polypeptide, C is a proteolytic signal sequence and S is a selectable marker.

In one embodiment the proteolytic signal sequence is the PEST sequence of the ornithine decarboxylase.

In one embodiment the detectable polypeptide is green fluorescent protein.

Patent Claims

1. A method for selecting a recombinant stable transfected mammalian cell comprising the following steps:

   a) transfecting a mammalian cell with an expression vector comprising

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

   - a second expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody heavy chain, a bGH polyA signal sequence, and a hGT terminator sequence, ~~and~~

   whereby the first expression cassette and the second expression cassette are arranged unidirectional,

   and thereby obtaining a multitude of recombinant mammalian cells,

   b) selecting from the multitude of recombinant mammalian cells a (single) stable transfected recombinant mammalian cell.

2. The method according to claim 1, 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.

3. The method according to any one of claims 1 to 2, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~4. The method according to any one of claims 1 to 3, characterized in that the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.~~

~~5. The method according to any one of claims 1 to 3, characterized in that the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.~~

~~6~~4. The method according to any one of claims 1 to ~~5~~3, characterized in that the expression plasmid further comprises a selection marker.

~~7~~5. The method according to any one of claims 1 to 4 ~~and 6~~, characterized in that the expression cassettes and the selection marker are arranged unidirectional.

~~8~~6. The method according to any one of claims 1 to ~~4 and 6 to 7~~5, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

~~9~~7. The method according to any one of claims 1 to ~~8~~6, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

~~10~~8. The method according to any one of claims 1 to ~~4 and 6 to 8~~7, characterized in that the mammalian cell is a CHO cell for the selection of a stable transfected cell.

~~11. The method according to any one of claims 1 to 3 and 5 to 8, characterized in that the mammalian cell is a HEK cell for the selection of a transient transfected cell.~~

~~12~~9. A method for producing an antibody comprising the following steps:

a) cultivating a mammalian cell comprising

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

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

b) recovering the antibody from the cell or the cultivation medium,

whereby the first expression cassette and the second expression cassette are arranged unidirectional for the stable production of the antibody.

~~13~~10. The method according to claim ~~12~~9, 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.

~~14~~11. The method according to any one of claims ~~12~~9 to ~~13~~10, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~15. The method according to any one of claims 12 to 14, characterized in that the first expression cassette and the second expression cassette are arranged unidirectional for the stable production of an antibody.~~

~~16. The method according to any one of claims 12 to 14, characterized in that the first expression cassette and the second expression cassette are arranged bidirectional for the transient production of an antibody.~~

~~17~~12. The method according to any one of claims ~~12~~ 9 to ~~16~~11, characterized in that the expression plasmid further comprises a selection marker.

~~18~~13. The method according to any one of claims ~~12~~ 9 to ~~15 and 17~~12, characterized in that the expression cassettes and the selection marker are arranged unidirectional.

~~19~~14. The method according to any one of claims ~~12~~ 9 to ~~15 and 17 to 18~~13, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

~~20~~15. The method according to any one of claims ~~12~~ 9 to ~~19~~14, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

~~21~~16. The method according to any one of claims ~~12~~ 9 to ~~15 and 17 to 20~~15, characterized in that the mammalian cell is a CHO cell for the stable production of an antibody.

~~22. The method according to any one of claims 12 to 14 and 16 to 20, characterized in that the mammalian cell is a HEK cell for the transient production of an antibody.~~

~~23~~17. An expression vector comprising

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

- a second expression cassette comprising in 5' to 3' direction a hCMV promoter, a nucleic acid encoding an antibody heavy chain, a bGH polyA signal sequence, and a hGT terminator sequence.

~~24~~18. The expression vector according to claim ~~23~~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.

~~25~~19. The expression vector according to any one of claims ~~23~~ 17 to ~~24~~18, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~26~~20. The expression vector according to any one of claims ~~23~~ 17 to ~~25~~19, characterized in that the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

~~27. The expression vector according to any one of claims 23 to 25, characterized in that the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.~~

~~28~~21. The expression vector according to any one of claims ~~23~~ 17 to ~~27~~20, characterized in that the expression plasmid further comprises a selection marker.

~~29~~22. The expression vector according to any one of claims ~~23~~ 17 to ~~26 and 28~~21, characterized in that the expression cassettes and the selection marker are arranged unidirectional.

~~30~~23. The expression vector according to any one of claims ~~23~~ 17 to ~~26 and 28 to 29~~22, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

~~31. A method for selecting a recombinant mammalian cell comprising the following step:~~

~~a) transfecting a mammalian cell with an expression vector comprising~~

~~a first expression cassette comprising in 5' to 3' direction a hEF1alpha promoter, a nucleic acid encoding an antibody light chain, and a bGH polyA signal sequence,~~

~~a second expression cassette comprising in 5' to 3' direction a hEF1alpha promoter, a nucleic acid encoding an antibody heavy chain, a bGH polyA signal sequence, and~~

~~and thereby obtaining a multitude of recombinant mammalian cells,~~

b) selecting from the multitude of recombinant mammalian cells a (single) recombinant mammalian cell.

32. The method according to claim 31, 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.

33. The method according to any one of claims 31 to 32, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

34. The method according to any one of claims 31 to 33, characterized in that the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

35. The method according to any one of claims 31 to 33, characterized in that the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

36. The method according to any one of claims 31 to 35, characterized in that the expression plasmid further comprises a selection marker.

37. The method according to any one of claims 31 to 34 and 36, characterized in that the expression cassettes and the selection marker are arranged unidirectional.

38. The method according to any one of claims 31 to 34 and 36 to 37, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

39. The method according to any one of claims 31 to 38, characterized in that the human elongation factor 1 alpha promoter comprises an Intron A.

40. The method according to any one of claims 31 to 39, characterized in that the expression vector is free of any transcription terminator sequences.

41. The method according to claim 40, characterized in that the terminator sequence is the hGT sequence.

~~42. The method according to any one of claims 31 to 41, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.~~

~~43. The method according to claim 42, characterized in that the mammalian cell is a CHO cell for the selection of a stable transfected cell.~~

~~44. The method according to claim 42, characterized in that the mammalian cell is a HEK cell for the selection of a transient transfected cell.~~

~~45. A method for producing an antibody comprising the following step:~~

~~a) cultivating a mammalian cell comprising~~

~~a first expression cassette comprising in 5' to 3' direction a hEF1alpha promoter, a nucleic acid encoding an antibody light chain, and a bGH polyA signal sequence,~~

~~a second expression cassette comprising in 5' to 3' direction a hEF1alpha promoter, a nucleic acid encoding an antibody heavy chain, and a bGH polyA signal sequence, and~~

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

~~46. The method according to claim 45, 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.~~

~~47. The method according to any one of claims 45 to 46, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.~~

~~48. The method according to any one of claims 45 to 47, characterized in that the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.~~

~~49. The method according to any one of claims 45 to 47, characterized in that the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.~~

50. The method according to any one of claims 45 to 49, characterized in that the expression plasmid further comprises a selection marker.

51. The method according to any one of claims 45 to 48 and 50, characterized in that the expression cassettes and the selection marker are arranged unidirectional.

52. The method according to any one of claims 45 to 48 and 50 to 51, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

53. The method according to any one of claims 45 to 52, characterized in that the human elongation factor 1 alpha promoter comprises an Intron A.

54. The method according to any one of claims 45 to 53, characterized in that the expression vector is free of any transcription terminator sequences.

55. The method according to claim 54, characterized in that the terminator sequence is the hGT sequence.

56. The method according to any one of claims 45 to 55, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

57. The method according to claim 56, characterized in that the mammalian cell is a CHO cell for the stable production of an antibody.

58. The method according to claim 56, characterized in that the mammalian cell is a HEK cell for the transient production of an antibody.

59. An expression vector comprising

- a first expression cassette comprising in 5' to 3' direction a hEF1alpha promoter, a nucleic acid encoding an antibody light chain, and a bGH polyA signal sequence, and

- a second expression cassette comprising in 5' to 3' direction a hEF1alpha promoter, a nucleic acid encoding an antibody heavy chain, and a bGH polyA signal sequence.

60. The expression vector according to claim 59, 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.

61. The expression vector according to any one of claims 59 to 60, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

62. The expression vector according to any one of claims 59 to 61, characterized in that the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

63. The expression vector according to any one of claims 59 to 61, characterized in that the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

64. The expression vector according to any one of claims 59 to 64, characterized in that the expression plasmid further comprises a selection marker.

65. The expression vector according to any one of claims 59 to 62 and 64, characterized in that the expression cassettes and the selection marker are arranged unidirectional.

66. The expression vector according to any one of claims 59 to 62 and 64 to 65, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

67. The expression vector according to any one of claims 59 to 66, characterized in that the human elongation factor 1 alpha promoter comprises an Intron A.

68. The expression vector according to any one of claims 59 to 67, characterized in that the expression vector is free of any transcription terminator sequences.

69. The expression vector according to claim 68, characterized in that the terminator sequence is the hGT sequence.

70. Use of an expression vector comprising

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

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

- a third expression cassette comprising in 5' to 3' direction a third promoter, a nucleic acid conferring resistance to a selection agent, a third polyA signal sequence, and optional a third transcription terminator sequence,

whereby the three expression cassettes are organized unidirectional and in the sequence first expression cassette-second expression cassette-third expression cassette,

for the stable recombinant production of an antibody in a mammalian cell.

71. The use according to claim 70, 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.

72. The use according to any one of claims 70 to 71, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

73. The use according to any one of claims 70 to 72, characterized in that the first and the second promoter are the hCMV promoter, the first and second polyA signal sequence are the bGH polyA signal sequence, and the transcription termination sequence is present and is the hGT terminator sequence.

74. The use according to any one of claims 70 to 72, characterized in that the first and the second promoter are the hEF1alpha promoter, the first and second polyA signal sequence are the bGH polyA signal sequence, and the expression cassettes are free of a transcription terminator sequence.

75. The use according to any one of claims 70 to 74, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

76. The use according to any one of claims 70 to 75, characterized in that the mammalian cell is a CHO cell.

~~77. An expression vector comprising~~

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

~~a second expression cassette comprising in 5' to 3' direction a second promoter, a nucleic acid encoding an antibody heavy chain, a second polyA signal sequence, and optional a second transcription terminator sequence, and~~

~~a third expression cassette comprising in 5' to 3' direction a third promoter, a nucleic acid conferring resistance to a selection agent, a third polyA signal sequence, and optional a third transcription terminator sequence,~~

~~whereby the three expression cassettes are organized unidirectional and in the sequence first expression cassette-second expression cassette-third expression cassette.~~

~~78. The expression vector according to claim 77, 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.~~

~~79. The expression vector according to any one of claims 77 to 78, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.~~

~~80. The expression vector according to any one of claims 77 to 79, characterized in that the first and the second promoter are the hCMV promoter, the first and second polyA signal sequence are the bGH polyA signal sequence, and the transcription termination sequence is present and is the hGT terminator sequence.~~

~~81. The expression vector according to any one of claims 77 to 79, characterized in that the first and the second promoter are the hEF1alpha promoter, the first and second polyA signal sequences are the bGH polyA signal sequence, and the expression cassettes are free of a transcription terminator sequence.~~

~~82. Use of an expression vector comprising~~

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

~~a second expression cassette comprising in 5' to 3' direction a second promoter, a nucleic acid encoding an antibody heavy chain, a second polyA signal sequence, and optional a second transcription terminator sequence, and~~

~~a third expression cassette comprising in 5' to 3' direction a third promoter, a nucleic acid conferring resistance to a selection agent, a third polyA signal sequence, and optional a third transcription terminator sequence,~~

~~whereby the expression cassettes are organized bidirectional whereby the first expression cassette and the second expression cassette are arranged in opposite direction~~

~~for the transient recombinant production of an antibody in a mammalian cell.~~

~~83. The use according to claim 82, 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.~~

~~84. The use according to any one of claims 82 to 83, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.~~

~~85. The use according to any one of claims 82 to 84, characterized in that the first and the second promoter are the hCMV promoter, the first and second polyA signal sequence are the bGH polyA signal sequence, and the transcription termination sequence is present and is the hGT terminator sequence.~~

~~86. The use according to any one of claims 82 to 84, characterized in that the first and the second promoter are the hEF1alpha promoter, the first and second polyA signal sequence are the bGH polyA signal sequence, and the expression cassettes are free of a transcription terminator sequence.~~

87. The use according to any one of claims 82 to 86, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

88. The use according to any one of claims 82 to 87, characterized in that the mammalian cell is a HEK cell.

89. An expression vector comprising

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

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

a third expression cassette comprising in 5' to 3' direction a third promoter, a nucleic acid conferring resistance to a selection agent, a third polyA signal sequence, and optional a third transcription terminator sequence,

whereby the expression cassettes are organized bidirectional whereby the first expression cassette and the second expression cassette are arranged in opposite direction.

90. The expression vector according claim 89, 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.

91. The expression vector according to any one of claims 89 to 90, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

92. The expression vector according to any one of claims 89 to 91, characterized in that the first and the second promoter are the hCMV promoter, the first and second polyA signal sequence are the bGH polyA signal sequence, and the transcription termination sequence is present and is the hGT terminator sequence.

93. The expression vector according to any one of claims 89 to 91, characterized in that the first and the second promoter are the hEF1alpha promoter, the first and second polyA signal sequences are the bGH polyA signal sequence, and the expression cassettes are free of a transcription terminator sequence.

94. An expression plasmid comprising in 5' to 3' direction a promoter sequence, a nucleic acid encoding an antibody heavy chain or an antibody light chain, an IRES element, a nucleic acid sequence encoding a selection marker, and a polyA signal sequence, whereby the IRES element is the EMCV-IRES element.

95. Use of an expression cassette comprising in 5' to 3' direction a promoter sequence, a nucleic acid encoding an antibody heavy chain or an antibody light chain, an IRES element, a nucleic acid sequence encoding a selection marker, and a polyA signal sequence for the selection of antibody producing cells, whereby the IRES element is the EMCV-IRES element.

96. A method for the selection of eukaryotic cell expressing an antibody comprising the following steps:

- cultivating a eukaryotic cell comprising i) an expression plasmid according to claim 94 and ii) a nucleic acid encoding the respective other antibody chain not encoded by the expression plasmid according to claim 94,
- selecting a cell expressing the detectable polypeptide.

97. An expression plasmid comprising in 5' to 3' direction a promoter sequence, a nucleic acid encoding an antibody light chain, an IRES element, a nucleic acid sequence encoding an antibody heavy chain, and a polyA signal sequence, whereby the IRES element is the EV71-IRES element.

98. Use of an expression plasmid comprising in 5' to 3' direction a promoter sequence, a nucleic acid encoding an antibody light chain, an IRES element, a nucleic acid sequence encoding an antibody heavy chain, and a polyA signal sequence, for the expression of an antibody, whereby the IRES element is the EV71-IRES element.

99. A method for the selection of eukaryotic cell expressing an antibody comprising the following steps:

~~cultivating a eukaryotic cell comprising i) an expression plasmid according to claim 97 and ii) a nucleic acid encoding the respective other antibody chain not encoded by the expression plasmid according to claim 97,~~

~~selecting a cell expressing the detectable polypeptide.~~

~~100~~24. The expression ~~plasmid~~ vector or ~~the use or~~ the method according to any one of claims 1 to ~~99~~23, characterized in that the expression vector comprises either

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

or

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

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

~~101~~25. The expression ~~plasmid or the use~~vector or the method according to any one of claims 1 to ~~100~~24, characterized in that expression vector is encoding a bispecific antibody.

~~102~~26. The expression ~~plasmid or the use~~vector or the method according to any one of claims ~~100~~24 to ~~101~~25, 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 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.

~~103~~27. The expression ~~plasmid or the use~~vector or the method according to any one of claims ~~100~~24 to ~~102~~26, 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.

~~104~~28. The expression ~~plasmid or the use~~vector or the method according to any one of claims ~~100~~24 to ~~103~~27, 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.

~~105~~29. The expression ~~plasmid or the use~~vector or the method according to any one of claims ~~100~~24 to ~~104~~28, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a hole mutation.

~~106~~30. The expression ~~plasmid or the use~~vector or the method according to any one of claims ~~100~~24 to ~~105~~29, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a knob mutation.

~~107~~31. The expression ~~plasmid or the use~~vector or the method according to any one of claims ~~100~~ 24 to ~~106~~30, 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.

~~108~~32. The expression ~~plasmid or the use~~vector or the method according to any one of claims ~~100~~ 24 to ~~107~~31, 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.