Therefore, reported herein is 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 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

and thereby obtaining a multitude of recombinant mammalian cells,

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

In one aspect, there is provided a method for selecting a recombinant transient 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 bovine growth hormone (bGH) polyA signal sequence, and a human gastrin gene transcription terminator (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 bidirectional,

and thereby obtaining a multitude of recombinant mammalian cells,

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

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 for the selection of a stable transfected cell.

In one embodiment the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

In one embodiment the expression plasmid further comprises a selection marker. In one embodiment the expression cassettes and the selection marker are arranged unidirectional. In one embodiment the expression cassettes are arranged in the sequence LC-HC-SM.

In one embodiment the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell. In one embodiment the mammalian cell is a CHO cell for the selection of a stable transfected cell. In one embodiment the mammalian cell is a HEK cell for the selection of a transient transfected cell.

There is provided 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 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.

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 for the stable production of an antibody.

- 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.

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 for the selection of a stable transfected cell.

In one embodiment the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

In one embodiment the expression plasmid further comprises a selection marker. In one embodiment the expression cassettes and the selection marker are arranged unidirectional. In one embodiment the expression cassettes are arranged in the sequence LC-HC-SM.

It has been found that for the generation of stable recombinant antibody expressing/secreting cells lines when using a human elongation factor 1 alpha promoter (hEF1alpha) in combination with bGH polyA signal sequence the presence of an hGT terminator sequence reduces obtainable expression yield.

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

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 for the selection of a stable transfected cell.

In one embodiment the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

In one embodiment the expression plasmid further comprises a selection marker. In one embodiment the expression cassettes and the selection marker are arranged unidirectional. In one embodiment the expression cassettes are arranged in the sequence LC-HC-SM.

- 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.

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 for the selection of a stable transfected cell.

In one embodiment the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

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

- 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.

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 for the selection of a stable transfected cell.

In one embodiment the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

In one embodiment the expression plasmid further comprises a selection marker. In one embodiment the expression cassettes and the selection marker are arranged unidirectional. In one embodiment the expression cassettes are arranged in the sequence LC-HC-SM.

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

In one embodiment the expression vector is free of any transcription terminator sequences. In one embodiment the terminator sequence is the hGT sequence.

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

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 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.

In one embodiment 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.

There is provided 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.

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 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.

In one embodiment 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.

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

There is provided 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.

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 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 provided 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 provided a eukaryotic cell comprising an expression plasmid as reported herein.

There is provided 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 provided 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 provided is 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 provided 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 provided 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.

In one embodiment the selectable marker is neomycin.

There is provided a method for the selection of eukaryotic cell expressing an antibody comprising the following steps:

- cultivating a eukaryotic cell comprising i) an expression plasmid as reported in this aspect and ii) a nucleic acid encoding the respective other antibody chain not encoded by the expression plasmid as reported in this aspect,
- selecting a cell expressing the detectable polypeptide.

There is provided 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.

In one embodiment the promoter sequence is selected from the human CMV promoter sequence with or without Intron A, the SV40 promoter sequence, and the human elongation factor 1 alpha promoter sequence with or without Intron A.

In one embodiment the polyA signal sequence is selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

In one embodiment the plasmid comprises 3' to the polyA signal sequence the human gastrin terminator sequence.

There is provided 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.

In one embodiment the promoter sequence is selected from the human CMV promoter sequence with or without Intron A, the SV40 promoter sequence, and the human elongation factor 1 alpha promoter sequence with or without Intron A.

In one embodiment the polyA signal sequence is selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

In one embodiment the plasmid comprises 3' to the polyA signal sequence the human gastrin terminator sequence.

Patent Claims

1. A method for selecting a recombinant transient 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 bovine growth hormone (bGH) polyA signal sequence, and a human gastrin gene transcription terminator (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 bidirectional,

   and thereby obtaining a multitude of recombinant mammalian cells,

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

5. The method according to any one of claims 1 to 4, characterized in that the expression cassettes are arranged in the sequence light chain-heavy chain-selection marker (LC-HC-SM).

6. The method according to any one of claims 1 to 5, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

7. The method according to any one of claims 1 to 6, characterized in that the mammalian cell is a HEK cell for the selection of a transient transfected cell.

8. The method according to any one of claims 1 to 7, 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 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 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 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 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 a hGT terminator sequence,

whereby the first expression cassette and the second expression cassette are arranged bidirectional for the selection of a transient transfected cell.

25. The expression vector according to claim 24, 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.

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

27. The expression vector according to any one of claims 24 to 26, characterized in that the expression plasmid further comprises a selection marker.

28. The expression vector according to any one of claims 24 to 27, characterized in that the expression cassettes are arranged in the sequence light chain-heavy chain-selection marker (LC-HC-SM).

29. The expression vector according to any one of claims 24 to 28, 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 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 a hGT terminator sequence,

33. The expression vector according to any one of claims 24 to 32, 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.

34. The expression vector according to any one of claims 24 to 33, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a hole mutation.

35. The expression vector according to any one of claims 24 to 34, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a knob mutation.

36. The expression vector according to any one of claims 24 to 35, 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.

37. The expression vector according to any one of claims 24 to 36, 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.