Summary of the Invention

It has been found that the performance of an expression vector largely depends on its intended use and that best vectors for transient transfections, stable pools and single clone selection differ.

To highlight the main findings: For transient transfection the bidirectional expression of the antibody light and heavy chain and the use of the full length hCMV promoter including Intron A were advantageous. For stable transfection, however, the in row arrangement of 1) antibody light chain, 2) antibody heavy chain and 3) selection marker has shown to be advantageous.

But whereas the hEF1α promoter is clearly superior to the hCMV promoter in stable pools, the clear opposite effect on single clone level has been found. Here, with the human cytomegalovirus immediate early promoter/enhancer (hCMV) clones with highest productivity were obtained.

Moreover, the hCMV promoter 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 i) 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) the promoter is the human elongation factor 1 alpha promoter (hEF1alpha), the polyA signal sequence is the bovine growth hormone polyA signal sequence (bGH polyA) and the terminator sequence is absent.

By using an expression vector as outlined above a higher number of antibody producing/secreting cells can be obtained after transfection and, thus, the required efforts to identify a high producer cell suitable for large scale recombinant antibody production are reduced.

Therefore, ~~one aspect as~~ 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 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 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.

~~One aspect as reported herein is~~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 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 bidirectional for the transient production of the antibody.

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.

In one embodiment the first expression cassette and the second expression cassette are arranged bidirectional for the transient production of an antibody.

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 stable production of an antibody. In one embodiment the mammalian cell is a HEK cell for the transient production of an antibody.

~~One aspect as reported herein is~~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.

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,

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

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.

~~One aspect as reported herein is~~There is provided 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.

In another aspect, there is provided a method for selecting a recombinant transient transfected 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

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

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 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.

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.

~~One aspect as reported herein is~~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 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.

In another aspect, there is provided a method for producing an antibody comprising the following step:

a) cultivating a transient transfected 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,

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

b) recovering the antibody from the transient transfected 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

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.

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 stable production of an antibody. In one embodiment the mammalian cell is a HEK cell for the transient production of an antibody.

~~One aspect as reported herein is~~There is provided 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.

In another aspect, there is provided 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

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

It has been found that for the stable recombinant production of an antibody the 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,

results in an improved expression yield in a stably transfected recombinant mammalian cell.

In contrast to the above it has been found that for the transient recombinant production of an antibody the 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,

results in an improved expression yield in a transiently transfected recombinant mammalian cell.

The term in opposite direction denotes that one expression cassette is transcribed in the 5' -> 3' direction and one expression cassette is transcribed in 3'-> 5' direction.

Thus, ~~one aspect as~~ reported herein is the 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.

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 CHO cell.

~~One aspect as reported herein is~~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 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.

~~One aspect as reported herein is the~~In another aspect, 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.

~~One aspect as reported herein is~~In another aspect, 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 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.

Further, it has been found among other things that expression vectors comprising the hCMV promoter and the human Ef1α promoter with Intron A instead of the short human CMV promoter without Intron A enhance transient and pool gene expression.

~~One aspect as reported herein is~~There is provided 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 one or both of the expression cassettes comprise in addition after the polyA signal sequence the human gastrin terminator sequence.

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.

~~One aspect as reported herein is~~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.

~~One aspect as reported herein is~~There is provided a eukaryotic cell comprising an expression plasmid as reported herein.

~~One aspect as reported herein is~~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.

~~One aspect as reported herein is~~There is provided 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.

~~One aspect as reported herein is~~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.

~~One aspect as reported herein is~~There is provided a eukaryotic cell comprising an expression plasmid as reported herein.

~~One aspect as reported herein is~~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.

~~One aspect as reported herein is~~In another aspect, 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.

~~One aspect as reported herein~~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.

~~One aspect as reported herein is~~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.

~~One aspect as reported herein is the~~In another aspect, 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.

~~One aspect as reported herein is~~In another aspect, 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.

~~One aspect as reported herein is~~In another aspect, 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.

~~One aspect as reported herein is~~In another aspect, there is provided ~~the~~ 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.

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

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

In one embodiment the hCMV promoter has the sequence of SEQ ID NO: 01. This is the hCMV promoter without Intron A and without 5'UTR.

In one embodiment the hCMV promoter has the sequence of SEQ ID NO: 02. This is the hCMV promoter without Intron A and with 5'UTR.

In one embodiment the hCMV promoter has the sequence of SEQ ID NO: 03. This is the full length hCMV promoter with Intron A.

In one embodiment the human elongation factor 1 alpha promoter has the sequence of SEQ ID NO: 04. This is the hEF1alpha promoter without Intron A.

In one embodiment the human elongation factor 1 alpha promoter has the sequence of SEQ ID NO: 05. This is the hEF1alpha promoter with Intron A.

In one embodiment the human elongation factor 1 alpha promoter has the sequence of SEQ ID NO: 06. This is a short hEF1alpha promoter with Intron A and with 5'UTR.

In one embodiment the rat CMV promoter has the sequence of SEQ ID NO: 07.

In one embodiment the SV40 polyA signal sequence has the sequence of SEQ ID NO: 08.

In one embodiment the bovine growth hormone polyA signal sequence has the sequence SEQ ID NO: 09.

In one embodiment the human gastrin terminator has the sequence of SEQ ID NO: 10.

In one embodiment the SV40 promoter has the sequence of SEQ ID NO: 11.

In one embodiment the PEST sequence of ornithine decarboxylase is encoded by the sequence of SEQ ID NO: 12.

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 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 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.

~~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.~~

64. The method according to any one of claims 1 to 53, characterized in that the expression plasmid further comprises a selection marker.

~~7. 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.~~

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

96. The method according to any one of claims 1 to 85, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

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

117. The method according to any one of claims 1 to ~~3 and 5 to~~ 86, 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,

- 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 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 a hGT terminator sequence,

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

9. The method according to any one of claims 1 to 8, characterized in that expression vector is encoding a bispecific antibody.

10. The method according to any one of claims 8 to 9, 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.

11. The method according to any one of claims 8 to 10, 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.

12. The method according to any one of claims 8 to 11, 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.

13. The method according to any one of claims 8 to 12, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a hole mutation.

14. The method according to any one of claims 8 to 13, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a knob mutation.

15. The method according to any one of claims 8 to 14, 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.

16. The method according to any one of claims 8 to 15, 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.

~~12~~17. 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 bidirectional for the transient production of the antibody.

~~13~~18. The method according to claim ~~12~~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.

~~14~~19. The method according to any one of claims ~~12~~17 to ~~13~~18, 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~~20. The method according to any one of claims ~~12~~ 17 to ~~16~~19, characterized in that the expression plasmid further comprises a selection marker.

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

~~19~~21. The method according to any one of claims ~~12~~ 17 to ~~15 and 17 to 18~~20, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

~~20~~22. The method according to any one of claims ~~12~~ 17 to ~~19~~21, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

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

~~22~~23. The method according to any one of claims ~~12~~ 17 to ~~14 and 16 to 20~~22, characterized in that the mammalian cell is a HEK cell for the transient production of an antibody.

~~23~~24. 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 bidirectional for the selection of a transient transfected cell.

2425. The expression vector according to claim 2324, 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.

2526. The expression vector according to any one of claims 23–24 to 2425, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~26. 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 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.~~

2827. The expression vector according to any one of claims 23–24 to 2726, characterized in that the expression plasmid further comprises a selection marker.

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

3028. The expression vector according to any one of claims 23–24 to 26 and 28 to 2927, characterized in that the expression cassettes are arranged in the sequence 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,

- 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,
- 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 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 a hGT terminator sequence,

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

30. The expression vector according to any one of claims 24 to 29, characterized in that expression vector is encoding a bispecific antibody.

31. The expression vector according to any one of claims 24 to 30, 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.

32. The expression vector according to any one of claims 24 to 31, 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.

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.

~~31~~38. A method for selecting a recombinant transient transfected 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

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

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.

3239. The method according to claim 3438, 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.

3340. The method according to any one of claims 31-38 to 3239, 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.~~

3641. The method according to any one of claims 31-38 to 3540, 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.~~

3842. The method according to any one of claims 31-38 to 34 and 36 to 3741, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

3943. The method according to any one of claims 31-38 to 3843, characterized in that the human elongation factor 1 alpha promoter comprises an Intron A.

4044. The method according to any one of claims 31-38 to 3943, characterized in that the expression vector is free of any transcription terminator sequences.

4145. The method according to claim 4044, characterized in that the terminator sequence is the hGT sequence.

4246. The method according to any one of claims ~~31~~ 38 to ~~41~~45, 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.~~

4447. The method according to claim 4246, characterized in that the mammalian cell is a HEK cell for the selection of a transient transfected cell.

4548. A method for producing an antibody comprising the following step:

a) cultivating a transient transfected 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,

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

b) recovering the antibody from the transient transfected cell or the cultivation medium.

4649. The method according to claim 4548, 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.

4750. The method according to any one of claims ~~45~~ 48 to 4649, 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.~~

5051. The method according to any one of claims ~~45~~ 48 to 4950, 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~~ 48 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~~ 48 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~~ 48 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~~ 48 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.~~

5857. The method according to claim 56, characterized in that the mammalian cell is a HEK cell for the transient production of an antibody.

5958. 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

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

6059. The expression vector according to claim 5958, 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.

6160. The expression vector according to any one of claims 59–58 to 6059, 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.~~

6461. The expression vector according to any one of claims 59–58 to 6460, 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.~~

6662. The expression vector according to any one of claims 59–58 to ~~62 and 64 to~~ 6561, characterized in that the expression cassettes are arranged in the sequence LC-HC-SM.

6763. The expression vector according to any one of claims 59–58 to 6662, characterized in that the human elongation factor 1 alpha promoter comprises an Intron A.

6864. The expression vector according to any one of claims 59–58 to 6763, characterized in that the expression vector is free of any transcription terminator sequences.

6965. The expression vector according to claim 6864, 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~~66. 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~~67. The use according to claim ~~82~~66, 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~~68. The use according to any one of claims ~~82-~~66 to ~~83~~67, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~85~~69. The use according to any one of claims ~~82-~~66 to ~~84~~68, 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~~70. The use according to any one of claims ~~82-~~66 to ~~84~~68, 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~~71. The use according to any one of claims ~~82-~~66 to ~~86~~70, characterized in that the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell.

~~88~~72. The use according to any one of claims ~~82-~~66 to ~~87~~71, characterized in that the mammalian cell is a HEK cell.

73. The use according to any one of claims 66 to 72, 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,
- 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 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 a hGT terminator sequence,

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

74. The use according to any one of claims 66 to 73, characterized in that expression vector is encoding a bispecific antibody.

75. The use according to any one of claims 66 to 74, 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.

76. The use according to any one of claims 66 to 75, 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.

77. The use according to any one of claims 66 to 76, 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.

78. The use according to any one of claims 66 to 77, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a hole mutation.

79. The use according to any one of claims 66 to 78, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a knob mutation.

80. The use according to any one of claims 66 to 79, 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.

81. The use according to any one of claims 66 to 80, 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.

~~89~~82. 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~~83. The expression vector according claim ~~89~~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.

~~91~~84. The expression vector according to any one of claims ~~89~~–82 to ~~90~~83, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

~~92~~85. The expression vector according to any one of claims ~~89~~ 82 to ~~91~~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.

9386. The expression vector according to any one of claims ~~89~~-82 to ~~91~~84, 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.

9487. 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.

9588. 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.

9689. 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~~-87 and ii) a nucleic acid encoding the respective other antibody chain not encoded by the expression plasmid according to claim ~~94~~87,
- selecting a cell expressing the detectable polypeptide.

9790. 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.

9891. 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.

9992. 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~~90 and ii) a nucleic acid encoding the respective other antibody chain not encoded by the expression plasmid according to claim ~~97~~90,
- selecting a cell expressing the detectable polypeptide.

~~100. The expression plasmid or the use or the method according to any one of claims 1 to 99, 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.~~

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

~~102. The expression plasmid or the use or the method according to any one of claims 100 to 101, 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. The expression plasmid or the use or the method according to any one of claims 100 to 102, 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. The expression plasmid or the use or the method according to any one of claims 100 to 103, 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. The expression plasmid or the use or the method according to any one of claims 100 to 104, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a hole mutation.~~

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

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