Summary

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, 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 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 example 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 example the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

In one example the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

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

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

In one example the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell. In one example the mammalian cell is a CHO cell for the selection of a stable transfected cell. In one example 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 example 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 example the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

In one example the first expression cassette and the second expression cassette are arranged unidirectional for the stable production of an antibody.

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

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

In one example the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell. In one example the mammalian cell is a CHO cell for the stable production of an antibody. In one example the mammalian cell is a HEK cell for the transient production of an antibody.

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

In one example the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

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

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

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

In one example the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

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

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

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

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

In one example the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell. In one example the mammalian cell is a CHO cell for the selection of a stable transfected cell. In one example 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 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 one example 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 example the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

In one example the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

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

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

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

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

In one example the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell. In one example the mammalian cell is a CHO cell for the stable production of an antibody. In one example the mammalian cell is a HEK cell for the transient production of an antibody.

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

In one example the first expression cassette and the second expression cassette are arranged unidirectional for the selection of a stable transfected cell.

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

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

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

In one example the expression vector is free of any transcription terminator sequences. In one example 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, there is provided 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 example 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 example the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

In one example 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 example 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 example the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell. In one example the mammalian cell is a CHO 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 three expression cassettes are organized unidirectional and in the sequence first expression cassette-second expression cassette-third expression cassette.

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

In one example 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 example 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 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

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

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

In one example 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 example 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 example the mammalian cell is selected from CHO cell, HEK cell, BHK cell, NS0 cell, and SP2/0 cell. In one example 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 example 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 example the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

In one example 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 example 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.

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 example 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 example 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 example 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 example the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

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

In one example the expression plasmid further comprises a selection marker. In one example 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 example the eukaryotic cell is a mammalian cell. In one example 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

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

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

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

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

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

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

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

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

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

In one example the detectable polypeptide is green fluorescent protein.

In one example the selectable marker is neomycin.

There is provided 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.

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 example the nucleic acid encodes an antibody heavy chain.

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

In one example the detectable polypeptide is green fluorescent protein.

In one example the selectable marker is neomycin.

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 disclosed herein and ii) a nucleic acid encoding the respective other antibody chain not encoded by the expression plasmid as disclosed herien,
- selecting a cell expressing the detectable polypeptide.

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 example 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 example the polyA signal sequence is selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

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

In another aspect, 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 example 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 example the polyA signal sequence is selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

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

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

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

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

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

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

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

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

In one example the GFP sequence is encoded by the sequence of SEQ ID NO: 13.

In one example the neomycin selection marker has the sequence of SEQ ID NO: 14.

In one example the GFP-PEST-NEO fusion polypeptide is encoded by the sequence of SEQ ID NO: 15.

In one example the EMCV-IRES has the sequence of SEQ ID NO: 16.

In one example the EV71-IRES has the sequence of SEQ ID NO: 17.

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

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

If one example the expression vector comprises either

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

or

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

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

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

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

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

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

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

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

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

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

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

In one example encodes one of the antibody heavy chain expression cassettes an antibody heavy chain comprising a knob mutation.

In one example encodes one of the antibody light chain expression cassettes an antibody light chain 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 an antibody light chain comprising an antibody light chain variable domain and an antibody light chain CL domain as constant domain.

In one example one of the antibody heavy chain expression cassettes encodes an antibody heavy chain 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.

Detailed Description of the Invention

I. General aspects

As known to a person skilled in the art enables the use of recombinant DNA technology the production of numerous derivatives of a nucleic acid and/or polypeptide. Such derivatives can, for example, be modified in one individual or several positions by substitution, alteration, exchange, deletion, or insertion. The modification or derivatization can, for example, be carried out by means of site directed mutagenesis. Such modifications can easily be carried out by a person