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.

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.

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

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

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

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

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

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

There is also ~~One aspect as~~ reported herein ~~is~~ an expression plasmid comprising

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In one embodiment the detectable polypeptide is green fluorescent protein.

In one embodiment the selectable marker is neomycin.

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

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

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