expression of recombinant lgG antibodies is reported by Li et al. (J. Immunol. Meth. 318 (2007) 113-124). Ho, S.C.L., et al. report "IRES-mediated tricistronic vectors for enhancing generation of high monoclonal antibody expressing CHO cell lines (J. Biotechnol. 157 (2011) 130-139). Production of anti-CD2 chimeric antibody by recombinant animal cells is reported by Hotta, A., et al. (J. Biosci. Bioeng. 98 (2004) 298-303). Lee, J-C., et al. report "High-efficiency protein expression mediated by enterovirus 71 internal ribosome entry (Biotechnol. Bioeng. 90 (2005) 656-662). In WO 2008/142124 recombinant protein production in Avian EBX® cells is reported.

Summary

It has been found that for the recombinant production of antibodies the position of the heavy chain expression cassette in front of the light chain expression cassette (HC-LC (5'-3')) provides for better expression results compared to the inverse order (LC-HC (5'-3')). Additionally it has been found that the position of the selection marker after both antibody chain expression cassettes provides for better expression results (HC-LC-SM (5'-3')) compared to the bidirectional position in front of the first antibody chain (SM (3'-5')-HC-LC (5'-3')).

It has been found that for stable transfections the in row arrangement of 1) antibody heavy chain, 2) antibody light chain and 3) selection marker has shown to be optimal. But whereas the hEF1α promoter is clearly superior to the hCMV promoter in stable pools, we have seen the clear opposite effect on single clone level. Here, the human cytomegalovirus immediate early promoter/enhancer (hCMV) generated clones with highest productivity. Moreover, its 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 1) 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 (EF1alpha), 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.

As reported herein is an expression vector comprising

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

wherein the expression cassettes are arranged unidirectional, and

wherein the 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.

One aspect as reported herein is an expression vector comprising

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

wherein the expression cassettes are arranged unidirectional,

wherein the 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,

wherein all of the expression cassettes comprise a human CMV promoter,

wherein one, two, or all three of the expression cassettes comprise a bovine growth hormone polyA signal sequence, and

wherein one, two, or all three of the expression cassettes comprise a human gastrin terminator sequence after the polyA signal sequence.

In one example the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker cassette comprise independently of each other comprise a promoter selected from the human elongation factor 1 alpha promoter, the human CMV promoter, and the SV40 promoter.

In one example one, two, or all three expression cassettes comprise the human elongation factor 1 alpha promoter. In one example the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker cassette comprise independently of each other the human elongation factor 1 alpha promoter. In one example the expression cassette does not comprise a terminator sequence, i.e. the expression cassette is free of a terminator sequence. In one example the terminator sequence is the human gastrin gene transcription terminator sequence (hGT).

In one example one, two, or all three expression cassettes comprise the human CMV promoter. In one example the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker cassette comprise independently of each other the human CMV promoter.

In one example one, two, or three expression cassettes comprise the bovine growth hormone polyA signal sequence. In one example the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker cassette comprise independently of each other the bovine growth hormone polyA signal sequence.

In one example the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker cassette comprise independently of each other a polyA signal sequence selected from the bovine growth hormone polyA signal sequence and the SV40 polyA signal sequence.

In one example one, two, or all three expression cassettes comprise the human gastrin terminator sequence after the polyA signal sequence with the proviso that the expression cassettes do not comprise the human elongation factor 1 alpha promoter. In one example the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker cassette comprise independently of each other the human gastrin terminator sequence after the polyA signal sequence.

In one example the antibody light chain expression cassette and/or the antibody heavy chain expression cassette and/or the selection marker cassette comprise independently of each other in 5’ to 3’ direction the bovine growth hormone polyA signal sequence and the human gastrin terminator sequence with the proviso that the expression cassettes do not comprise the human elongation factor 1 alpha promoter.

In one example the promoter of one, two, or all three expression cassettes comprises an Intron A.

In one example one, two, or all three expression cassettes comprise the SV40 polyA signal sequence.

In one example one, two, or all three expression cassettes comprise the SV40 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.

One aspect as reported herein is the use of an expression vector as reported herein for the recombinant production of an antibody.

One aspect as reported herein is the use of an expression vector as reported herein for the generation of a stable cell line.

One aspect as reported herein is the use of an expression vector as reported herein for the generation of a production cell line.

As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the generation of a stable cell line.

As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the generation of a production cell line.

As reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human elongation factor 1 alpha promoter for the recombinant production of an antibody.

One aspect as reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human CMV promoter for the generation of a production cell line.

One aspect as reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human CMV promoter for the generation of a stable cell line.

One aspect as reported herein is the use of an expression vector as reported herein comprising at least one expression cassette comprising a human CMV promoter for the production of an antibody.

As reported herein is a method for the transfection of a eukaryotic cell with an expression vector, characterized in that the expression vector is linearized prior to the transfection by cleavage in the prokaryotic origin of replication.

In one example the prokaryotic origin of replication is between one antibody light chain expression cassette and one antibody heavy chain expression cassette.

As reported herein is the use of a method as reported herein for the generation of a eukaryotic cell for the recombinant production of an antibody.

As reported herein is a method for the selection of a eukaryotic cell comprising a nucleic acid encoding an antibody, characterized in that the selection agent is added to the cultivation medium for the first time about 24 hours after transfection.

As reported herein is the use of a method as reported herein for the generation of a eukaryotic cell for the recombinant production of an antibody.

As reported herein is the use of a cell selected with a method as reported herein for the recombinant production of an antibody.

Another aspect as reported herein is a method for the production of an antibody comprising the following steps:

- cultivating a eukaryotic cell that has been transfected with an expression vector according to the disclosure, and
- recovering the antibody from the cell or the cultivation medium.

As reported herein is a method for the production of an antibody comprising the following steps:

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

As reported herein is a method for the production of an antibody comprising the following step:

- cultivating a eukaryotic cell, which has been obtained by the transfection with an expression vector that has been linearized prior to the transfection by cleavage in the prokaryotic origin of replication, and
- recovering the antibody from the cell or the cultivation medium.

As reported herein is a method for the production of an antibody comprising the following step:

- cultivating a eukaryotic cell, which has been selected by the addition of a selection agent about 24 hours after transfection to the cultivation, and
- recovering the antibody from the cell or the cultivation medium.

As reported herein is a method for the transfection of a eukaryotic cell with an expression vector comprising prokaryotic and eukaryotic nucleic acid sequences, characterized in that the prokaryotic nucleic acid sequences are removed from the expression vector prior to the transfection of the eukaryotic cell with the expression vector.

As reported herein is the use of a linearized expression vector comprising no prokaryotic nucleic acid sequences for the transfection of a eukaryotic cell.

As reported herein is the use of an expression vector comprising only eukaryotic nucleic acid sequences for the generation of a eukaryotic cell for the recombinant production of an antibody.

In one example 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.

In 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 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.

In one example 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.

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.

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.

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 skilled in the art (see e.g. Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, USA (1999)). The use of recombinant technology enables a person skilled in the art to transform various host cells with heterologous nucleic acid(s). Although the transcription and translation, i.e. expression, machinery of different cells use the same elements, cells belonging to different species may have among other things a different so-called codon usage. Thereby identical polypeptides (with respect to amino acid