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 antibody light chain and the antibody heavy chain expression cassettes comprise a human CMV promoter,

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

wherein ~~one, two, or all three of~~ the antibody light chain and the antibody heavy chain expression cassettes comprise a human gastrin terminator sequence after the polyA signal sequence.

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~~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~~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~~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.

Patent Claims

1. 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 antibody light chain and the antibody heavy chain expression cassettes comprise a human CMV promoter,

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

wherein ~~one, two, or all three of~~ the antibody light chain and the antibody heavy chain expression cassettes comprise a human gastrin terminator sequence after the polyA signal sequence.

2. The expression vector according to claim 1, characterized in that ~~one, two, or all three of~~ the selection marker expression cassette~~s~~ comprise~~s~~ ~~the~~ an SV40 promoter or/and an SV40 polyA signal sequence.

3. The expression vector 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 comprises at least one intron.

4. The expression vector according to any one of claims 1 to 3, characterized in that the nucleic acid encoding the antibody light chain and/or the nucleic acid encoding the antibody heavy chain is cDNA.

5. Use of an expression vector according to any one of claims 1 to 4 for the recombinant production of an antibody.

6. Use of an expression vector according to any one of claims 1 to 4 for the generation of a stable cell line.

7. Use of an expression vector according to any one of claims 1 to 4 for the generation of a production cell line.

~~8. Use of an expression vector according to any one of claims 1 to 4 comprising at least one expression cassette comprising a human CMV promoter for the generation of a production cell line.~~

~~9. Use of an expression vector according to any one of claims 1 to 4 comprising at least one expression cassette comprising a human CMV promoter for the generation of a stable cell line.~~

~~10. Use of an expression vector according to any one of claims 1 to 4 comprising at least one expression cassette comprising a human CMV promoter for the production of an antibody.~~

~~11~~8. 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 any one of claims 1 to 4, and
- recovering the antibody from the cell or the cultivation medium.

~~12~~9. The expression vector according to any one of claims 1 to 4, characterized in that the expression vector is encoding a bispecific antibody.

~~13~~10. The expression vector according to claim ~~12~~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 a first antigen and the bispecific antibody has a second binding specificity or binding site that specifically binds to a second antigen or second epitope on a second antigen.

~~14~~11. The expression vector according to any one of claims 1 to 4 and ~~12 to 13~~9 to 10, characterized in that the expression vector comprises either

- a first expression cassette comprising in 5' to 3' direction ~~a~~ the human CMV promoter, a nucleic acid encoding a first antibody light chain, ~~a~~ the bovine growth hormone polyA signal sequence, and ~~optionally a~~the human gastrin terminator sequence,
- a second expression cassette comprising in 5' to 3' direction the human CMV~~a~~ promoter, a nucleic acid encoding a second antibody light chain, ~~a~~ the bovine growth hormone polyA signal sequence, and the human gastrin~~optionally a~~ terminator sequence,
- a third expression cassette comprising in 5' to 3' direction the human CMV~~a~~ promoter, a nucleic acid encoding a first antibody heavy chain, ~~a~~ the bovine growth hormone polyA signal sequence, and the human gastrin~~optionally a~~ terminator sequence,
- a fourth expression cassette comprising in 5' to 3' direction the human CMV~~a~~ promoter, a nucleic acid encoding a second antibody heavy chain, ~~a~~ the bovine growth hormone polyA signal sequence, and the human gastrin~~optionally a~~ terminator sequence,

or

- a first expression cassette comprising in 5' to 3' direction the human CMV~~a~~ promoter, a nucleic acid encoding an antibody light chain, ~~a~~ the bovine growth hormone polyA signal sequence, and the human gastrin~~optionally a~~ terminator sequence,

- a second expression cassette comprising in 5’ to 3’ direction the human CMV~~a~~ promoter, a nucleic acid encoding a first antibody heavy chain, ~~a~~ the bovine growth hormone polyA signal sequence, and the human gastrin~~optionally a~~ terminator sequence, and
- a third expression cassette comprising in 5’ to 3’ direction the human CMV~~a~~ promoter, a nucleic acid encoding a second antibody heavy chain, ~~a~~ the bovine growth hormone polyA signal sequence, and the human gastrin~~optionally a~~ terminator sequence,

whereby the antibody light chain ~~is a common light chain for both~~can form a pair with each of the antibody heavy chains.

~~15~~12. The expression vector according to any one of claims 1 to 4 and ~~12 to 14~~9 to 11, 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,

wherein at least one of the antibody heavy chain expression cassettes and 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.

~~16~~13. The expression vector according to any one of claims 1 to 4 and ~~12 to 14~~9 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,

wherein one of the antibody heavy chain expression cassettes and 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.

~~17~~14. The expression vector according to any one of claims ~~12 to 16~~9 to 13, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a hole mutation.

~~18~~15. The expression vector according to any one of claims ~~12 to 17~~9 to 14, characterized in that one of the antibody heavy chain expression cassettes encodes an antibody heavy chain comprising a knob mutation.

~~19~~16. The expression vector according to any one of claims ~~12 to 18~~9 to 15, 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.

~~20~~17. The expression vector according to any one of claims ~~12 to 19~~9 to 16, 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.