whereby the nucleic acid encoding the antibody is obtained by

- synthesizing single stranded cDNA using the RNA obtained from an antibody secreting B-cell as template in an RT-PCR,
- amplifying the variable domain encoding nucleic acid(s) in a PCR, and
- inserting the variable domain encoding nucleic acid(s) in one or more eukaryotic expression plasmids.

In one embodiment the PCR primer are removed after the PCR.

In one embodiment the method is performed without the isolation and analysis of intermediate nucleic acids.

The disclosure relates to a method for producing an antibody comprising the following step:

- cultivating a eukaryotic cell transfected with one or more expression plasmids encoding the antibody heavy and light chains whereby the one or more expression plasmids have been prepared from a pool of plasmid transformed E.coli cells,
- recovering the antibody from the cell or the cultivation medium.

~~One aspect as reported herein~~ The disclosure relates to ~~is~~ a method for producing an antibody comprising the following step:

- recovering the antibody from the cultivation medium of a eukaryotic cell comprising a nucleic acid encoding the antibody,

whereby the nucleic acid encoding the antibody is obtained by

- amplifying cognate variable domain encoding nucleic acids from single stranded cDNA obtained from the RNA of an antibody secreting B-cell as template in a PCR, and
- inserting the variable domain encoding nucleic acids in a eukaryotic expression plasmid by ligation independent cloning,

wherein a pool of nucleic acids encoding the antibody light and heavy chain variable domain, respectively, is used for the insertion.

One aspect as reported herein is a method for producing an antibody comprising the following step:

- recovering the antibody from the cultivation medium of a eukaryotic cell comprising a nucleic acid encoding the antibody,

whereby the nucleic acid encoding the antibody is obtained by

- extracting total RNA from an antibody producing B-cell of an immunized experimental animal,

extracted polyA$^+$ mRNA,

- conducting PCR using a species specific primer,

- removing the species specific PCR primer to thereby purify the PCR product,

- incubating the PCR product with a T4 polymerase,

- linearizing and amplifying plasmid-DNA,

- inserting the variable domain encoding nucleic acids into the amplified plasmid by sequence and ligation independent cloning, whereby the insertion does not require an enzymatic ligation,

- preparing plasmid from a pool of plasmid transformed E.coli cells, and

- transfecting eukaryotic cells with the prepared plasmid,

wherein a pool of nucleic acids encoding the antibody light and heavy chain variable domain, respectively, is used for the insertion.

In one embodiment the method comprises as first step:

Patent Claims

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

- recovering the antibody from the cultivation medium of a eukaryotic cell comprising a nucleic acid encoding the antibody,

whereby the nucleic acid encoding the antibody is obtained by

- extracting ~~amplifying cognate variable domain encoding nucleic acids from single stranded cDNA obtained from the~~ total RNA ~~of~~ from an antibody ~~secreting~~ producing B-cell of an immunized experimental animal,
- synthesizing single stranded cDNA or conducting reverse transcription of extracted polyA$^+$ mRNA,
- conducting ~~as template in a~~ PCR using a species specific primer,
- removing the species specific PCR primer to thereby purify the PCR product,
- incubating the PCR product with a T4 polymerase,
- linearizing and amplifying plasmid-DNA,

~~and~~

- inserting the variable domain encoding nucleic acids into the amplified plasmid by sequence and ~~a eukaryotic expression plasmid by~~ ligation independent cloning, whereby the insertion does not require an enzymatic ligation,
- preparing plasmid from a pool of plasmid transformed E.coli cells, and
- transfecting eukaryotic cells with the prepared plasmid,

wherein a pool of nucleic acids encoding the antibody light and heavy chain variable domain, respectively, is used for the insertion.

~~2. A method for producing an antibody comprising the following step:~~

~~cultivating a eukaryotic cell transfected with an expression plasmid encoding the antibody, whereby the eukaryotic cell has been transfected with a pool of expression plasmids that has been prepared from a pool of plasmid transformed E.coli cells,~~

~~recovering the antibody from the cell or the cultivation medium.~~

~~3~~2. The method according to claim 1, characterized in that the B-cell is a rabbit B-cell.

~~4~~3. The method according to ~~any one of~~ claims 1 or ~~3~~2, characterized in that the B-cell is a single deposited B-cell.

~~5~~4. The method according to any one of claims 1 ~~or~~ to 3 ~~or 4~~, characterized in that the B-cell is cultivated for about 7 days.

~~6~~5. The method according to any one of claims 1 ~~and 3~~ to ~~5~~4, characterized in that the B-cell and its progeny produces more than 20 ng/ml antibody in 7 days of co-cultivation with feeder cells starting from a single cell.

~~7~~6. The method according to any one of claims 1 ~~and 3~~ to ~~6~~5, characterized in that

the species specific PCR primer have the nucleic acid sequences of SEQ ID NO: 5 and 6 or SEQ ID NO: 7 or 8.

~~8. The method according to any one of claims 1 and 3 to 7, characterized in that the PCR primer are removed after the PCR.~~

97. The method according to any one of claims 1 to 86, characterized in that the nucleic acid fragments are inserted into the expression plasmid by sequence and ligation independent cloning.

108. The method according to any one of claims 1 ~~and 3~~ to 97, characterized in that about 300 ng nucleic acid is used in the insertion reaction.

119. The method according to any one of claims 1 to 118, characterized in that the expression plasmid is obtained by sequence and ligation independent cloning of the variable domain encoding nucleic acid into a variable domain free amplified expression plasmid.

1210. The method according to claim 119, characterized in that the plasmid is linearized prior to the amplification.