In WO 2010/056898 rapid expression and cloning of human monoclonal antibodies from memory B-cells is reported. A rapid and efficient single-cell manipulation method for screening antigen-specific antibody-producing cells from human peripheral blood is reported by Jin et al. (Jin, A., et al., Nature Medicine 15 (2009) 1088-1093). Lightwood et al. (Lightwood, D.J., et al., J. Immunol. Meth. 316 (2006) 133-143) report antibody generation through B-cell panning on antigen followed by in situ culture and direct RT-PCR on cells harvested en masse from antigen-positive wells.

Summary ~~of the Invention~~

In the method as reported herein the isolation of nucleic acid fragments or segments encoding antibody variable domains (light and heavy chain) and the insertion of the isolated nucleic acid fragments or segments in eukaryotic expression cassettes (one cassette each for the light and heavy chain, respectively) is performed without the intermediate isolation and analysis of clonal intermediate plasmids/cassettes. Thus, in the method as reported herein the intermediate cloning, isolation and analysis of intermediate plasmids/cassettes is not required, e.g. by analysis of isolated transformed E.coli cells, thus, resulting in a faster method.

The disclosure relates to ~~One aspect as reported herein is~~ a method for the isolation of nucleic acids encoding cognate variable domains of an antibody comprising the following steps:

- 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 acids in a PCR and thereby isolating the nucleic acid fragments encoding the cognate variable domains of an antibody,

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

- cultivating a eukaryotic cell comprising a nucleic acid encoding an antibody, and
- recovering the antibody from the cell or the cultivation medium,

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 ~~One aspect as reported herein is~~ 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 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.

In one embodiment the method comprises as first step: