

Patent Claims

1. A method for producing a multivalent, bispecific antibody comprising the steps of
 - a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the multivalent, bispecific antibody, and
 - b) recovering the multivalent, bispecific antibody from the cell or the cultivation medium,wherein the deoxyribonucleic acid encoding the multivalent, bispecific antibody is stably integrated into the genome of the mammalian cell and comprises in 5'- to 3'-direction either (1)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the first light chain, and
 - a sixth expression cassette encoding the first light chain,or (2)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the first light chain,
 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the first light chain,
 - a seventh expression cassette encoding the first light chain, and
 - an eighth expression cassette encoding the first light chain.
2. A deoxyribonucleic acid encoding a multivalent, bispecific antibody comprising in 5'- to 3'-direction either (1)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the first light chain, and

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- a sixth expression cassette encoding the first light chain,
or (2)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - 5 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the first light chain,
 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the first light chain,
 - a seventh expression cassette encoding the first light chain, and
 - 10 - an eighth expression cassette encoding the first light chain.
 - 3. Use of a deoxyribonucleic acid comprising in 5' - to 3'-direction
either (1)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - 15 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the first light chain, and
 - a sixth expression cassette encoding the first light chain,
 - or (2)
 - 20 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the first light chain,
 - a fifth expression cassette encoding the second heavy chain,
 - 25 - a sixth expression cassette encoding the first light chain,
 - a seventh expression cassette encoding the first light chain, and
 - an eighth expression cassette encoding the first light chain,
- for the expression of the multivalent, bispecific antibody in a mammalian cell.
4. A recombinant mammalian cell comprising a deoxyribonucleic acid encoding
30 a multivalent, bispecific antibody integrated in the genome of the cell, wherein
the deoxyribonucleic acid encoding the multivalent, bispecific antibody
comprises in 5' - to 3'-direction
either (1)
 - a first expression cassette encoding the first heavy chain,
 - 35 - a second expression cassette encoding the first light chain,

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- a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the first light chain, and
 - a sixth expression cassette encoding the first light chain,
 - 5 or (2)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the first light chain,
 - 10 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the first light chain,
 - a seventh expression cassette encoding the first light chain, and
 - an eighth expression cassette encoding the first light chain.
5. A composition comprising two deoxyribonucleic acids, which comprise in turn
- 15 three different recombination recognition sequences and six or eight expression cassettes, wherein
- the first deoxyribonucleic acid comprises in 5'- to 3'-direction,
 - either (1)
 - a first recombination recognition sequence,
 - 20 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain, and
 - a first copy of a third recombination recognition sequence,
 - or (2)
 - 25 - a first recombination recognition sequence,
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the first light chain, and
 - 30 - a first copy of a third recombination recognition sequence,
- and
- the second deoxyribonucleic acid comprises in 5'- to 3'-direction
 - either (1)
 - a second copy of the third recombination recognition sequence,
 - 35 - a fourth expression cassette encoding the second heavy chain,

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- a fifth expression cassette encoding the first light chain,
 - a sixth expression cassette encoding the first light chain, and
 - a second recombination recognition sequence,
- or (2)
- 5 - a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the first light chain,
- a seventh expression cassette encoding the first light chain,
- an eighth expression cassette encoding the first light chain, and
- 10 - a second recombination recognition sequence.
6. A method for producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a multivalent, bispecific antibody and secreting the multivalent, bispecific antibody, comprising the following steps:
- a) providing a mammalian cell comprising an exogenous nucleotide sequence
- 15 integrated at a single site within a locus of the genome of the mammalian cell, wherein the exogenous nucleotide sequence comprises a first and a second recombination recognition sequence flanking at least one first selection marker, and a third recombination recognition sequence located
- between the first and the second recombination recognition sequence, and
- 20 all the recombination recognition sequences are different;
- b) introducing into the cell provided in a) a composition of two deoxyribonucleic acids comprising three different recombination recognition sequences and six or eight expression cassettes, wherein
- the first deoxyribonucleic acid comprises in 5'- to 3'-direction,
- 25 either (1)
- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- a third expression cassette encoding the first light chain, and
- 30 - a first copy of a third recombination recognition sequence,
- or (2)
- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first light chain,
- 35 - a third expression cassette encoding the first light chain,

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- a fourth expression cassette encoding the first light chain, and
 - a first copy of a third recombination recognition sequence,
- and

- the second deoxyribonucleic acid comprises in 5'- to 3'-direction
either (1)

- a second copy of the third recombination recognition sequence,
- a fourth expression cassette encoding the second heavy chain,
- a fifth expression cassette encoding the first light chain,
- a sixth expression cassette encoding the first light chain, and
- a second recombination recognition sequence,

or (2)

- a second copy of the third recombination recognition sequence,
- a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding the first light chain,
- a seventh expression cassette encoding the first light chain,
- an eighth expression cassette encoding the first light chain, and
- a second recombination recognition sequence,

wherein the first to third recombination recognition sequences of the first and second deoxyribonucleic acids are matching the first to third recombination recognition sequence on the integrated exogenous nucleotide sequence,

wherein the 5'-terminal part and the 3'-terminal part of the expression cassette encoding the one second selection marker when taken together form a functional expression cassette of the one second selection marker;

c) introducing

- i) either simultaneously with the first and second deoxyribonucleic acid of b);

or

- ii) sequentially thereafter

one or more recombinases,

wherein the one or more recombinases recognize the recombination recognition sequences of the first and the second deoxyribonucleic acid; (and optionally wherein the one or more recombinases perform two recombinase mediated cassette exchanges;)

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and

d) selecting for cells expressing the second selection marker and secreting the multivalent, bispecific antibody,

5 thereby producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding the multivalent, bispecific antibody and secreting the multivalent, bispecific antibody.

7. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell
10 according to any one of claims 1 to 6, wherein the first heavy chain comprises in the CH3 domain the mutation T366W (numbering according to Kabat) and the second heavy chain comprises in the CH3 domain the mutations T366S, L368A, and Y407V (numbering according to Kabat), or vice versa.

8. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell
15 according to claim 7, wherein one of the heavy chains further comprises the mutation S354C and the respective other heavy chain comprises the mutation Y349C (numbering according to Kabat).

9. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell
20 according to any one of claims 1 to 8, wherein the first light chain is a domain exchanged light chain VH-VL or CH1-CL.

10. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell
25 according to any one of claims 1 to 9, wherein

30 - the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain, a CH3 domain and a first light chain variable domain,

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- the second heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain, a CH3 domain and a second heavy chain variable domain, and

- 5 - the first light chain comprises from N- to C-terminus a second light chain variable domain and a CL domain,

wherein the first heavy chain variable domain and the second light chain variable domain form a first binding site and the second heavy chain variable domain and the first light chain variable domain form a second binding site.

- 10 11. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell according to any one of claims 1, 3, 4 and 6 to 10, wherein exactly one copy of the deoxyribonucleic acid is stably integrated into the genome of the
- 15 mammalian cell at a single site or locus.

12. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell according to any one of claims 1 to 5 and 7 to 11, wherein the deoxyribonucleic acid encoding the multivalent, bispecific antibody comprises a further expression cassette encoding for a selection marker, wherein the expression cassette encoding for the selection marker is located partly 5' and partly 3' to the third recombination recognition sequence, wherein the 5'-located part of said expression cassette comprises the promoter and the start-codon and the 3'-
- 20 located part of said expression cassette comprises the coding sequence without a start-codon and a polyA signal, wherein the start-codon is operably linked to the coding sequence, wherein the 5'-located part of the expression cassette encoding the selection marker comprises a promoter sequence operably linked to a start-codon, whereby the promoter sequence is flanked upstream by the
- 25 third or fourth expression cassette, respectively, and the start-codon is flanked downstream by the third recombination recognition sequence; and the 3'-located part of the expression cassette encoding the selection marker comprises a nucleic acid encoding the selection marker lacking a start-codon and is flanked upstream by the third recombination recognition sequence and
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downstream by the fourth or fifth expression cassette, respectively, wherein the start-codon is operably linked to the coding sequence.

- 5 13. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell according to any one of claims 1 to 12, wherein

each expression cassette for an antibody chain comprises in 5'-to-3' direction a promoter, a nucleic acid encoding an antibody chain, and a polyadenylation signal sequence and optionally a terminator sequence

- 10 and

each expression cassette encoding the selection marker comprises in 5'-to-3' direction a promoter, a nucleic acid encoding the selection marker, and a polyadenylation signal sequence and optionally a terminator sequence,

- 15 wherein the promoter is the human CMV promoter with intron A, the polyadenylation signal sequence is the bGH polyadenylation signal sequence and the terminator is the hGT terminator except for the expression cassette of the selection marker, wherein the promoter is the SV40 promoter and the polyadenylation signal sequence is the SV40 polyadenylation signal sequence and a terminator is absent.

- 20 14. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell according to any one of claims 1, 3, 4 and 6 to 13, wherein the mammalian cell is a CHO cell.

- 25 15. The method for producing a multivalent, bispecific antibody or the deoxyribonucleic acid or the use or the recombinant mammalian cell or the composition or the method for producing a recombinant mammalian cell according to any one of claims 1 to 14, wherein all expression cassettes are arranged unidirectional.

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