

Patent Claims

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

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- a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding either the first light chain or the second heavy chain or the second light chain, and
 - a seventh expression cassette encoding the second light chain,
- 5 for the expression of the trivalent, bispecific antibody in a mammalian cell.
4. A recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent, bispecific antibody integrated in the genome of the cell, wherein the deoxyribonucleic acid encoding the trivalent, bispecific antibody comprises in 5'- to 3'-direction
- 10
- a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first heavy 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,
- 15
- a sixth expression cassette encoding either the first light chain or the second heavy chain or the second light chain, and
 - a seventh expression cassette encoding the second light chain.
5. A composition comprising two deoxyribonucleic acids, which comprise in turn three different recombination recognition sequences and four expression cassettes, wherein
- 20
- the first deoxyribonucleic acid comprises in 5'- to 3'-direction,
 - a first recombination recognition sequence,
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the first heavy chain,
- 25
- a third expression cassette encoding the first light chain,
 - 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
- 30
- a second copy of the third recombination recognition sequence,
 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding either the first light chain or the second heavy chain or the second light chain,
 - a seventh expression cassette encoding the second light chain, and

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- a second recombination recognition sequence.

6. A method for producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent, bispecific antibody and secreting the trivalent, bispecific antibody, comprising the following steps:

5 a) providing a mammalian cell comprising an exogenous nucleotide sequence 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
10 between the first and the second recombination recognition sequence, and 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 at least seven expression cassettes, wherein

15 - the first deoxyribonucleic acid comprises in 5' - to 3' -direction,
- a first recombination recognition sequence,
- a first expression cassette encoding the first heavy chain,
- a second expression cassette encoding the first heavy chain,
- a third expression cassette encoding the first light chain,
20 - 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
- a second copy of the third recombination recognition sequence,
25 - a fifth expression cassette encoding the second heavy chain,
- a sixth expression cassette encoding either the first light chain or the second heavy chain or the second light chain,
- a seventh expression cassette encoding the second light chain, and
- a second recombination recognition sequence,

30 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,

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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

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

or

ii) sequentially thereafter
one or more recombinases,

10 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;)

and

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

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

20 7. The method for producing a trivalent, 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 6, wherein the deoxyribonucleic acid comprises after the seven expression cassette an eighth expression cassette
25 encoding the second light chain

8. The method for producing a trivalent, 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 7, wherein the first heavy chain comprises
30 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).

9. The method for producing a trivalent, 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 claim 8, 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).
10. The method for producing a trivalent, 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 9, wherein the first heavy chain is an extended heavy chain comprising an additional domain exchanged Fab fragment VH-VL or CH1-CL.
11. The method for producing a trivalent, 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 10, wherein the first light chain is a domain exchanged light chain VH-VL or CH1-CL.
12. The method for producing a trivalent, 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 11, wherein
- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain, a CH3 domain, a peptidic linker, a second heavy chain variable domain and a CL domain,
 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
 - the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and
 - the second light chain comprises from N- to C-terminus a second light chain variable domain and a CL domain,

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

5 13. The method for producing a trivalent, 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 12, wherein exactly one copy of the deoxyribonucleic acid is stably integrated into the genome of the mammalian cell at a single site or locus.

10 14. The method for producing a trivalent, 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 13, wherein the deoxyribonucleic acid encoding the trivalent, bispecific antibody comprises a further expression
15 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'-located part of said expression cassette
20 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 fourth expression
25 cassette 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 downstream by the fifth expression
30 cassette, wherein the start-codon is operably linked to the coding sequence.

15. The method for producing a trivalent, 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

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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

and

5 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,

wherein the promoter is the human CMV promoter with intron A, the polyadenylation signal sequence is the bGH polyadenylation signal sequence
10 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.

16. The method for producing a trivalent, bispecific antibody or the
15 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 15, wherein the mammalian cell is a CHO cell.

17. The method for producing a trivalent, bispecific antibody or the
20 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 16, wherein all cassettes are arranged unidirectional.

18. A method for producing a trivalent antibody comprising the steps of
25 a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the trivalent antibody, and

b) recovering the trivalent antibody from the cell or the cultivation medium,

wherein the deoxyribonucleic acid encoding the trivalent antibody is stably integrated into the genome of the mammalian cell and comprises in 5'- to 3'-
30 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, and
 - 5 - a fifth expression cassette encoding the second 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,
 - 10 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain,
- or (3)
- a first expression cassette encoding the first heavy chain,
 - 15 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the second light chain,
- or (4)
- 20 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the first heavy chain,
 - 25 - a sixth expression cassette encoding the second light chain,
- or (5)
- a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - 30 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the second light chain,
 - a seventh expression cassette encoding the second light chain,
- or (6)
- 35 - a first expression cassette encoding the first light chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first heavy chain,

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- a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain.
19. A deoxyribonucleic acid encoding a trivalent antibody comprising in 5'- to 3'-
5 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,
 - 10 - a fourth expression cassette encoding the second heavy chain, and
 - a fifth expression cassette encoding the second light chain,
- or (2)
- 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 second light chain,
 - a sixth expression cassette encoding the second light chain,
- or (3)
- 20 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the second light chain,
- 25 or (4)
- a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - 30 - a fifth expression cassette encoding the first heavy chain,
 - a sixth expression cassette encoding the second light chain,
- or (5)
- a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - 35 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,

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- a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the second light chain,
 - a seventh expression cassette encoding the second light chain,
 - or (6)
 - 5 - a first expression cassette encoding the first light chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first heavy chain,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
 - 10 - a sixth expression cassette encoding the second light chain.
20. 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, and
 - a fifth expression cassette encoding the second light chain,
 - or (2)
 - a first expression cassette encoding the first heavy chain,
 - 20 - 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 second light chain,
 - a sixth expression cassette encoding the second light chain,
 - 25 or (3)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - 30 - a fifth expression cassette encoding the second light chain,
 - or (4)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - 35 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the first heavy chain,

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- a sixth expression cassette encoding the second light chain,
or (5)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - 5 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the second light chain,
 - a seventh expression cassette encoding the second light chain,
 - 10 or (6)
 - a first expression cassette encoding the first light chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first heavy chain,
 - a fourth expression cassette encoding the second heavy chain,
 - 15 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain,
- for the expression of the trivalent antibody in a mammalian cell.
21. A recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent antibody integrated in the genome of the cell, wherein the
- 20 the deoxyribonucleic acid encoding the trivalent antibody 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,
 - 25 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second heavy chain, and
 - a fifth expression cassette encoding the second light chain,
- or (2)
- a first expression cassette encoding the first heavy chain,
 - 30 - 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 second light chain,
 - a sixth expression cassette encoding the second light chain,
- 35 or (3)
- a first expression cassette encoding the first heavy chain,

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- a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the second light chain,
 - 5 or (4)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - 10 - a fifth expression cassette encoding the first heavy chain,
 - a sixth expression cassette encoding the second light chain,
 - or (5)
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - 15 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain,
 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the second light chain,
 - a seventh expression cassette encoding the second light chain,
 - 20 or (6)
 - a first expression cassette encoding the first light chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first heavy chain,
 - a fourth expression cassette encoding the second heavy chain,
 - 25 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain.
22. A composition comprising two deoxyribonucleic acids, which comprise in turn three different recombination recognition sequences and five to seven expression cassettes, wherein
- 30 - the first deoxyribonucleic acid comprises in 5'- to 3'-direction, 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,
 - 35 - a third expression cassette encoding the first light chain, and
 - a first copy of a third recombination recognition sequence,

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

or (3)

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

or (4)

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

or (5)

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

or (6)

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

and

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

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- a second copy of the third recombination recognition sequence,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain, and
 - a second recombination recognition sequence,
- 5 or (2)
- a second copy of the third recombination recognition sequence,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain, and
- 10 - a second recombination recognition sequence,
- or (3)
- a second copy of the third recombination recognition sequence,
 - a fifth expression cassette encoding the second light chain, and
 - a second recombination recognition sequence,
- 15 or (4)
- a second copy of the third recombination recognition sequence,
 - a fifth expression cassette encoding the first heavy chain,
 - a sixth expression cassette encoding the second light chain, and
 - a second recombination recognition sequence,
- 20 or (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 second light chain,
 - a seventh expression cassette encoding the second light chain, and
- 25 - a second recombination recognition sequence,
- or (6)
- a second copy of the third recombination recognition sequence,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
- 30 - a sixth expression cassette encoding the second light chain, and
- a second recombination recognition sequence.
23. A method for producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a trivalent antibody and secreting the trivalent antibody, comprising the following steps:
- 35 a) providing a mammalian cell comprising an exogenous nucleotide sequence 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 all the recombination recognition sequences are different;

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b) introducing into the cell provided in a) a composition of two deoxyribonucleic acids comprising three different recombination recognition sequences and five to seven expression cassettes, wherein

- the first deoxyribonucleic acid comprises in 5'- to 3'-direction,

10

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
- a first copy of a third recombination recognition sequence,

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

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or (3)

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

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or (4)

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

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or (5)

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- 5
- a first recombination recognition sequence,
 - a first expression cassette encoding the first heavy chain,
 - a second expression cassette encoding the second heavy chain,
 - a third expression cassette encoding the first light chain,
 - a fourth expression cassette encoding the second light chain, and
 - a first copy of a third recombination recognition sequence,
- or (6)
- 10
- a first recombination recognition sequence,
 - a first expression cassette encoding the first light chain,
 - a second expression cassette encoding the first light chain,
 - a third expression cassette encoding the first heavy chain, and
 - a first copy of a third recombination recognition sequence,
- and
- 15
- the second deoxyribonucleic acid comprises in 5' - to 3' -direction either (1)
- 20
- a second copy of the third recombination recognition sequence,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain, and
 - a second recombination recognition sequence,
- or (2)
- 25
- a second copy of the third recombination recognition sequence,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain, and
 - a second recombination recognition sequence,
- or (3)
- 30
- a second copy of the third recombination recognition sequence,
 - a fifth expression cassette encoding the first heavy chain,
 - a sixth expression cassette encoding the second light chain, and
 - a second recombination recognition sequence,
- or (4)
- 35
- a second copy of the third recombination recognition sequence,
 - a fifth expression cassette encoding the second heavy chain,

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- 5
- a sixth expression cassette encoding the second light chain,
 - a seventh expression cassette encoding the second light chain,
 - and
 - a second recombination recognition sequence,
- or (6)
- a second copy of the third recombination recognition sequence,
 - a fourth expression cassette encoding the second heavy chain,
 - a fifth expression cassette encoding the second light chain,
 - a sixth expression cassette encoding the second light chain, and
 - 10 - 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,

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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
- 20
- i) either simultaneously with the first and second deoxyribonucleic acid of b);
 - or
 - ii) sequentially thereafter
- one or more recombinases,

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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;)

and

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- d) selecting for cells expressing the second selection marker and secreting the trivalent antibody,

thereby producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding the trivalent antibody and secreting the trivalent antibody.

24. The method for producing a trivalent 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 18 to 23, 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.
25. The method for producing a trivalent 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 claim 24, 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).
26. The method for producing a trivalent 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 18 to 25, wherein the first heavy chain is an extended heavy chain comprising an additional domain exchanged Fab fragment.
27. The method for producing a trivalent 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 18 to 26, wherein the first light chain is a domain exchanged light chain VH-VL or CH1-CL.
28. The method for producing a trivalent 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 18 to 27, wherein
- the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a first light chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
 - the second heavy chain comprises from N- to C-terminus the first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,

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

5 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.

29. The method for producing a trivalent antibody or the deoxyribonucleic acid or
the use or the recombinant mammalian cell or the composition or the method
10 for producing a recombinant mammalian cell according to any one of claims
18, 20, 21 and 23 to 28, wherein the deoxyribonucleic acid is stably integrated
into the genome of the mammalian cell at a single site or locus.

30. The method for producing a trivalent antibody or the deoxyribonucleic acid or
the use or the recombinant mammalian cell or the composition or the method
15 for producing a recombinant mammalian cell according to any one of claims
18 to 22 and 24 to 29, wherein the deoxyribonucleic acid encoding the trivalent
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,
20 wherein the 5'-located part of said expression cassette comprises the promoter
and the start-codon and the 3'-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.

31. The method for producing a trivalent antibody or the deoxyribonucleic acid or
25 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
18 to 22 and 24 to 30, 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
30 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.

32. The method for producing a trivalent 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 18, 20, 21 and 23 to 31, wherein the mammalian cell is a CHO cell.
- 5 33. The method for producing a trivalent 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 18 to 32, wherein in case the organization in 5' to 3'-direction has as first expression cassette an expression cassette encoding the first heavy chain all
10 cassettes are arranged unidirectional.
34. The method for producing a trivalent 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
15 17 to 32, wherein in case the organization in 5' to 3'-direction is first expression cassette encoding the first light chain, second expression cassette encoding the first light chain, third expression cassette encoding the first heavy chain, fourth expression cassette encoding the second heavy chain, fifth expression cassette encoding the second light chain, sixth expression cassette encoding the second light chain, the first to third expression cassettes are
20 arranged unidirectional and the fourth to sixth expression cassette are arranged unidirectional whereby the first to third expression cassettes are arranged in opposite direction of the fourth to sixth expression cassette.
35. A method for producing a bivalent, bispecific antibody comprising the steps of
- 25 a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the bivalent, bispecific antibody, and
- b) recovering the bivalent, bispecific antibody from the cell or the cultivation medium,
- wherein the deoxyribonucleic acid encoding the bivalent, bispecific antibody is stably integrated into the genome of the mammalian cell and comprises in
30 5' - to 3'-direction
- a first expression cassette encoding the first light chain,
 - a second expression cassette encoding the first heavy chain,
 - a third expression cassette encoding the second light chain, and

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- a fourth expression cassette encoding the second heavy chain,

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).

5

36. A deoxyribonucleic acid encoding a bivalent, bispecific antibody comprising in 5'- to 3'-direction

- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first heavy chain,
- 10 - a third expression cassette encoding the second light chain, and
- a fourth expression cassette encoding the second heavy chain,

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).

15

37. Use of a deoxyribonucleic acid comprising in 5'- to 3'-direction

- a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first heavy chain,
- a third expression cassette encoding the second light chain, and
- 20 - a fourth expression cassette encoding the second heavy chain,

for the expression of the bivalent, bispecific antibody in a mammalian cell,

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).

25

38. A recombinant mammalian cell comprising a deoxyribonucleic acid encoding a bivalent, bispecific antibody integrated in the genome of the cell, wherein the deoxyribonucleic acid encoding the bivalent, bispecific antibody comprises in 5'- to 3'-direction

- 30 - a first expression cassette encoding the first light chain,
- a second expression cassette encoding the first heavy chain,
- a third expression cassette encoding the second light chain, and

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- a fourth expression cassette encoding the second heavy chain,

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).

39. A composition comprising two deoxyribonucleic acids, which comprise in turn three different recombination recognition sequences and four expression cassettes, wherein

- the first deoxyribonucleic acid comprises in 5' - to 3' -direction,

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

and

- the second deoxyribonucleic acid comprises in 5' - to 3' -direction

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

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).

40. A method for producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a bivalent, bispecific antibody and secreting the bivalent, bispecific antibody, comprising the following steps:

- a) providing a mammalian cell comprising an exogenous nucleotide sequence 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 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 four expression cassettes, wherein

- the first deoxyribonucleic acid comprises in 5'- to 3'-direction,

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

and

- the second deoxyribonucleic acid comprises in 5'- to 3'-direction

- a second copy of the third recombination recognition sequence,
- a third expression cassette encoding the second light chain,
- a fourth expression cassette encoding the second heavy 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;

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);

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;)

5 and

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

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

10

41. The method for producing a bivalent, 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 35 to 40, 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).

15

42. The method for producing a bivalent, 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 35 to 41, wherein the second light chain is a domain exchanged light chain VH-CH1 after VH-VL exchange or a domain exchanged light chain VL-CH1 after CH1-CL exchange.

20

43. The method for producing a bivalent, 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 35 to 42, wherein

25

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

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

5 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 44. The method for producing a bivalent, 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 35, 37, 38 and 40 to 43, wherein exactly one copy of the deoxyribonucleic acid is stably integrated into the genome of the mammalian cell at a single site or locus.

15 45. The method for producing a bivalent, 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 35 to 39 and 40 to 44, wherein the deoxyribonucleic acid encoding the bivalent, bispecific antibody comprises a further expression cassette encoding for a selection marker, wherein the
20 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'-located part of said expression cassette comprises the coding sequence without a start-codon and a polyA signal, wherein the start-codon is
25 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 second expression cassette and the start-codon is flanked downstream by the third recombination recognition sequence; and the
30 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 downstream by the third expression cassette, wherein the start-codon is operably linked to the coding sequence.

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46. The method for producing a bivalent, 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 35 to 45, wherein
- 5 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
- 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,
- 10 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.
- 15
47. The method for producing a bivalent, 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 35, 37, 38 and 40 to 46, wherein the mammalian cell is a CHO cell.
- 20
48. The method for producing a bivalent, 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 35 to 47, wherein all cassettes are arranged unidirectional.
- 25
49. A method for producing a multivalent, bispecific antibody comprising the steps of
- 30 a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the multivalent, bispecific antibody, and

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- 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

5

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,
- 10 - 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,
- 15 - 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,
- 20 - a seventh expression cassette encoding the first light chain, and
- an eighth expression cassette encoding the first light chain.

50. A deoxyribonucleic acid encoding a multivalent, bispecific antibody comprising in 5'- to 3'-direction

either (1)

- 25 - 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
- 30 - 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,
- 35 - a fourth expression cassette encoding the first light chain,
- a fifth expression cassette encoding the second heavy chain,

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- 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.
51. Use of a deoxyribonucleic acid comprising in 5'- to 3'-direction
- 5 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,
 - 10 - 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,
 - 15 - 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
 - 20 - an eighth expression cassette encoding the first light chain,
- for the expression of the multivalent, bispecific antibody in a mammalian cell.
52. A recombinant mammalian cell comprising a deoxyribonucleic acid encoding a multivalent, bispecific antibody integrated in the genome of the cell, wherein the deoxyribonucleic acid encoding the multivalent, bispecific antibody
- 25 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,
 - 30 - 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,
 - 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 first light chain,
 - a fifth expression cassette encoding the second heavy chain,
 - a sixth expression cassette encoding the first light chain,
 - 5 - a seventh expression cassette encoding the first light chain, and
 - an eighth expression cassette encoding the first light chain.
53. A composition comprising two deoxyribonucleic acids, which comprise in turn three different recombination recognition sequences and six or eight expression cassettes, wherein
- 10 - the first deoxyribonucleic acid comprises in 5' - to 3' -direction, 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,
 - 15 - a third expression cassette encoding the first light chain, and
 - 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,
 - 20 - 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
 - a first copy of a third recombination recognition sequence,
- and
- 25 - 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,
 - 30 - 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,
 - 35 - a sixth expression cassette encoding the first light chain,

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- a seventh expression cassette encoding the first light chain,
- an eighth expression cassette encoding the first light chain, and
- a second recombination recognition sequence.

5 54. 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:

10 a) providing a mammalian cell comprising an exogenous nucleotide sequence 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 all the recombination recognition sequences are different;

15 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,
either (1)

- 20
- 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
 - 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,

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- 5
- 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,
 - 10 - an eighth expression cassette encoding the first light chain, and
 - a second recombination recognition sequence,

15 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;

- 20 c) introducing
- i) either simultaneously with the first and second deoxyribonucleic acid of b);
 - or
 - ii) sequentially thereafter
- one or more recombinases,

25 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;)

and

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

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

55. 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 49 to 54, 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.
56. 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 claim 55, 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).
57. 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 49 to 56, wherein the first light chain is a domain exchanged light chain VH-VL or CH1-CL.
58. 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 49 to 57, wherein
- 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,
 - 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

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- 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.

59. 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 49, 51, 52 and 54 to 58, wherein exactly one copy of the deoxyribonucleic acid is stably integrated into the genome of the mammalian cell at a single site or locus.
60. 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 49 to 53 and 55 to 59, 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'-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 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 downstream by the fourth or fifth expression cassette, respectively, wherein the start-codon is operably linked to the coding sequence.

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61. 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 49 to 60, wherein
- 5 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
- 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,
- 10 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.
- 15
62. 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 49, 51, 52 and 54 to 61, wherein the mammalian cell is a CHO cell.
- 20
63. 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 49 to 62, wherein all expression cassettes are arranged unidirectional.
- 25
64. A method for producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a polypeptide and secreting the polypeptide comprising the following steps:
- 30
- a) providing a mammalian cell comprising an exogenous nucleotide sequence integrated at a single site within a locus of the genome of the

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5 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 all the recombination recognition sequences are different, wherein the mammalian cell is free of Cre-recombinase encoding DNA;

10 b) introducing into the cell provided in a) a composition of two deoxyribonucleic acids comprising three different recombination recognition sequences and one to eight expression cassettes, wherein

15 the first deoxyribonucleic acid comprises in 5'- to 3'-direction,
- a first recombination recognition sequence,
- one or more expression cassette(s),
- a 5'-terminal part of an expression cassette encoding one second selection marker, and
- a first copy of a third recombination recognition sequence,
and

20 the second deoxyribonucleic acid comprises in 5'- to 3'-direction
- a second copy of the third recombination recognition sequence,
- a 3'-terminal part of an expression cassette encoding the one second selection marker,
- one or more expression cassette(s), and
- a second recombination recognition sequence,

25 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,

30 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,

wherein the deoxyribonucleic acids are free of Cre-recombinase encoding DNA;

c) introducing

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

ii) sequentially thereafter

Cre-recombinase mRNA as sole source of Cre-recombinase,

5 wherein the Cre-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;)

and

10 d) selecting for cells expressing the second selection marker and secreting the polypeptide,

thereby producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding the polypeptide and secreting the polypeptide.

65. The method according to claim 64, wherein the Cre mRNA encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 20.

66. The method according to claim 64, wherein the Cre mRNA comprises the nucleotide sequence of SEQ ID NO: 21 or a codon usage optimized variant thereof.

67. The method according to any one of claims 64 to 66, wherein exactly one copy of the deoxyribonucleic acid is stably integrated into the genome of the mammalian cell at a single site or locus.

68. The method according to any one of claims 64 to 67, wherein the deoxyribonucleic acid encoding the polypeptide comprises at least 4 expression cassettes wherein

- 25 - a first recombination recognition sequence is located 5' to the most 5' (i.e. first) expression cassette,
- a second recombination recognition sequence is located 3' to the most 3' expression cassette, and
- a third recombination recognition sequence is located
- 30 - between the first and the second recombination recognition sequence, and
- between two of the expression cassettes,

and

wherein all recombination recognition sequences are different.

- 5 69. The method according to any one of claims 64 to 68, wherein the deoxyribonucleic acid encoding the polypeptide comprises a further expression cassette encoding for a selection marker.
- 10 70. The method according to any one of claims 64 to 69, wherein the deoxyribonucleic acid encoding the polypeptide comprises a further expression cassette encoding for a selection marker and 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'-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.
- 15 71. The method according to any one of claims 64 to 70, wherein the ratio by weight between Cre mRNA and mixture of first and second vector is in the range of from 1:3 to 2:1.
- 20 72. The method according to any one of claims 64 to 71, wherein the ratio by weight between Cre mRNA and mixture of first and second vector is about 1:5.
- 25 73. The method according to any one of claims 64 to 72, wherein each of the expression cassettes comprise in 5'-to-3' direction a promoter, a coding sequence and a polyadenylation signal sequence optionally followed by a terminator sequence, 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.
- 30 74. The method according to any one of claims 64 to 73, wherein the mammalian cell is a CHO cell.
75. The method according to any one of claims 64 to 74, wherein the polypeptide is a heterotetramer comprising a first antibody heavy chain, a second antibody

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heavy chain, a first antibody light chain and a second antibody light chain, the deoxyribonucleic acid comprises four expression cassettes and

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

15 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.

76. The method according to any one of claims 64 to 74, wherein the polypeptide is a heterotetramer comprising a first antibody heavy chain, a second antibody heavy chain, a first antibody light chain and a second antibody light chain, the deoxyribonucleic acid comprises four expression cassettes and

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

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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.

- 5 77. The method according to any one of claims 64 to 74, wherein the polypeptide is a heterotetramer comprising a first antibody heavy chain, a second antibody heavy chain, a first antibody light chain and a second antibody light chain, the deoxyribonucleic acid comprises four expression cassettes and
- 10 - the first heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain, a CH3 domain, a peptidic linker, a second heavy chain variable domain and a CL domain,
- 15 - the second heavy chain comprises from N- to C-terminus a first heavy chain variable domain, a CH1 domain, a hinge region, a CH2 domain and a CH3 domain,
- 20 - the first light chain comprises from N- to C-terminus a first light chain variable domain and a CH1 domain, and
- the second light chain comprises from N- to C-terminus a second light chain variable domain and a CL domain,
- 20 wherein the second heavy chain variable domain and the first light chain variable domain form a first binding site and the first heavy chain variable domain and the second light chain variable domain form a second binding site.
- 25 78. The method according to any one of claims 64 to 77, wherein the first recombinase recognition sequence is L3, the second recombinase recognition sequence is 2L and the third recombinase recognition sequence is LoxFas.
- 30 79. Use of Cre-recombinase mRNA for increasing the number of recombinant mammalian cells comprising a deoxyribonucleic acid encoding a polypeptide or protein of interest stably integrated at a single site in the genome of said cell by targeted integration,

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80. The use according to claim 79, wherein the recombinant cell further secretes the polypeptide of interest into the cultivation medium upon cultivation therein.