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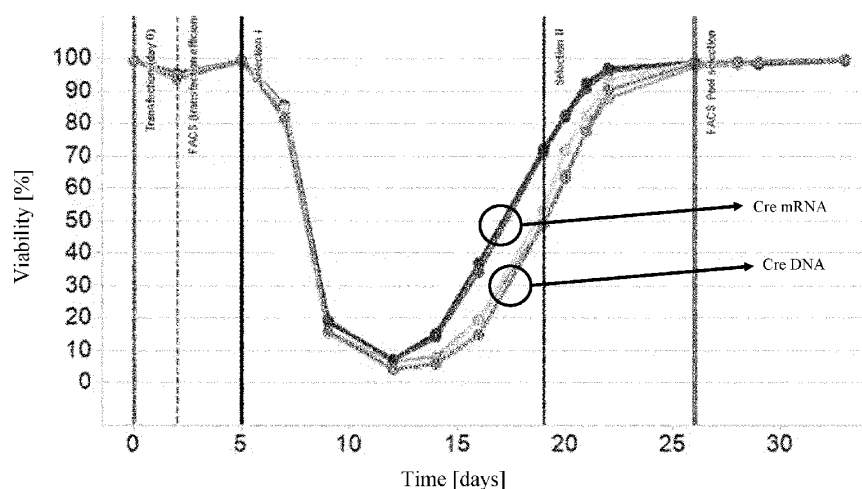
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(54) Title: METHOD FOR THE GENERATION OF A PROTEIN EXPRESSING CELL BY TARGETED INTEGRATION USING CRE MRNA

Figure 2



(57) Abstract: Herein is reported a method for producing a recombinant mammalian cell comprising a deoxyribonucleic acid encoding a polypeptide and secreting the polypeptide comprising the steps of 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 one to eight expression cassettes, wherein 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 expres-

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sion cassette encoding one second selection marker, 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 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, 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 Cre-recombinase mRNA, and 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, wherein the Cre-recombinase mRNA is the only source of Cre-recombinase in the method.