tem™, Lonza) cell lines produced >=1 g/L mAb in a fed-batch shake flask process. CHOK1SV expresses the GS enzyme endogenously, thus, positive transfectants can be obtained using glutamine-free media and selection of methionine sulfoximine (MSX). Despite such efficient selection systems, a rigorous and laborious screening regime is still required. Thus, the construction of manufacturing cell lines is a laborious and lengthy process. Not only do these cell lines need to be selected for positive growth and productivity characteristics, they also need to be cloned, and essentially need to produce the product of the correct quality for the duration of the manufacturing process. Furthermore, for a process to be economic, the cell lines generated need to exhibit consistent productivity over many cell generations. What would be desirable is to provide high-producing cell lines with positive growth characteristics with a minimum of screening activity each time a new protein of interest, e. g. a new monoclonal antibody, is to be expressed.

The technical problem underlying the present invention is to overcome the above-identified disadvantages, in particular to provide, preferably in a simple and efficient manner, high producing cell lines with a high stability and positive growth and productivity characteristics, in particular cell lines which provide a consistent productivity over a long cultivation and production period.

Summary

In one aspect, there is provided a site-specific integration (SSI) host cell comprising an endogenous Fer1L4 gene, wherein an exogenous nucleotide sequence is integrated in said Fer1L4 gene

and wherein the exogenous nucleotide sequence comprises at least two recombination target sites.

In one aspect, there is provided a method for producing an SSI host cell comprising the steps of

a) providing a cell comprising an endogenous Fer1L4 gene, wherein an exogenous nucleotide sequence is integrated in said Fer1L4 gene, and wherein the exogenous nucleotide sequence comprises at least two recombination target sites, flanking at least one first gene coding sequence of interest; subsequently

b) transfecting the cells provided in step a) with a vector comprising a first exchangeable cassette, the cassette comprising at least two matching recombinant target sites, flanking at least one second gene coding sequence of interest, namely a selection marker gene, subsequently

c) effecting a site-directed recombination-mediated cassette exchange and subsequently

d) selecting transfected cells expressing second gene coding sequence of interest, preferably the selection marker gene, so as to obtain the SSI host cell comprising the first exchangeable cassette stably integrated in its genome.

In one aspect, there is provided the use of an isolated polynucleotide molecule comprising at least one nucleotide sequence se-

lected from the group consisting of nucleotide sequences as given in SEQ ID No. 7, 8, 9 and homologous sequences thereof with a homology of at least 40 % for producing cell lines showing enhanced expression.

In one aspect, there is provided a host cell, wherein the host cell comprises a polynucleotide molecule comprising at least one nucleotide sequence selected from the group consisting of nucleotide sequences as given in SEQ ID No. 7, 8, 9 and homologous sequences thereof with a homology of at least 40 %, or a vector containing said polynucleotide molecule and an exogenous gene coding sequence of interest within or adjacent to the polynucleotide molecule or the vector containing said polynucleotide.

In one aspect, there is provided a method for producing a cell or cell line, in particular a high producer cell or cell line with a high productivity stability, wherein a Fer1L4 nucleotide sequence is integrated in a vector comprising at least one gene coding sequence of interest, wherein subsequently the vector is stably transfected into a cell or cell line and wherein subsequently stably transfected cells or cell lines are selected and obtained.

In one aspect, there is provided a method for the production of a product of a gene coding sequence of interest comprising cultivating a host cell or cell line produced according to the method as described herein in a suitable medium and recovering the product therefrom.

In one aspect, there is provided a method for producing an SSI host cell, the method comprising introducing an exogenous nucleotide sequence into an endogenous Fer1L4 gene in a cell, where-

in the exogenous nucleotide sequence comprises at least one gene coding sequence of interest and/or at least two recombination target sites.

Detailed description

The present invention solves its underlying technical problem by the provision of the teaching according to the independent claims.

In particular, the present invention solves its technical problem by the provision of a site-specific integration (SSI) host cell comprising an endogenous Fer1L4 gene, wherein an exogenous nucleotide se-