expression over time (Wurm, F.M. Nature Biotechnology 22, 1393-1398 (2004)). This makes the selection of suitable clones a tedious and time consuming procedure.

Several strategies have been developed to overcome the positional effects of the adjacent chromatin. For example, "anti-repressor" elements flanking the vectors have been used or vectors have been integrated specifically into chromosomal loci with open chromatin (Kwaks, T.H. et al. Nature Biotechnology 21, 553-558 (2003), Huang, Y. et al. Journal of Immunological Methods 322, 28-39 (2007)).

Artificial chromosomes have been used in the art to provide for transgenic hosts, such as animals or plants.

WO2002/097059A2 describes artificial chromosomes that have been engineered to contain available sites for site-specific, recombination-directed integration of DNA, particularly useful for engineering transgenic animals.

US2008/0060093A1 discloses methods of generating plants transformed with autonomous mini-chromosomes.

Moralli et al (EMBO reports 2006 Vol 7(9) 911-918) disclose human artificial chromosome (HAC) vectors to be used as a gene transfer system for expression and complementation studies.

The development of appropriate protein expression vectors constitutes one critical step in recombinant protein production in eukaryotic cell lines. For recombinant protein production in cell culture, generally a vector is sought that displays the following features:

1) expression should be independent of the integration site in the genome,

2) expression should correlate with the number of integrated transgene copies,

3) expression should be maintained over time, and

4) the expression level should be high.

Summary

In one aspect, there is provided a method of producing a protein in a eukaryotic production cell line, comprising the steps of a) providing a backbone of an artificial chromosome, b) recombining the nucleic acid encoding said protein into said backbone to generate an expression vector, c) introducing said expression vector into a eukaryotic host cell line to obtain a eukaryotic expression cell line, wherein at least 5 copies of the artificial chromosome are stably integrated into the chromosome of the host cell, d) cultivating said expression cell line to produce said protein, and e) isolating said protein.

Detailed Description

It is the object of the present invention to provide a method and a vector that allows the generation of stable cell clones to improve recombinant protein production in eukaryotic production cell lines.

The object has been solved by the subject matter of the present invention.

According to the present invention there is provided a method of producing a protein in a eukaryotic cell line, comprising the steps of

a) providing a backbone of an artificial chromosome,

b) recombining the nucleic acid encoding said protein into said backbone to generate an expression vector,

11. Method according to any of claims 1 to 9, wherein said vector contains a locus of an abundant protein.

12. Method according to any of claims 1 to 9, wherein said vector contains a locus of mammalian or insect origin.

~~13. Vector for use in a method according to any of claims 1 to 12, based on an~~ artificial chromosome including a gene encoding a secretion protein.

14. Chromosome of a host cell containing the stably integrated vector according to claim 13.

~~15. Transgenic production cell line harbouring the vector according to claim 13.~~