It is desirable to provide improved recombinant eukaryotic cell lines to produce fermentation products that can be isolated with high yields. Therefore, it is the object of the present invention to provide for alternative regulatory elements suitable for recombinant production methods, which are simple and efficient.

SUMMARY

The object is solved by the subject matter as claimed.

According to the disclosure there is provided an isolated nucleic acid sequence comprising a promoter, which is a native sequence of *Pichia pastoris* comprising or consisting of the pCS1 nucleic acid sequence of SEQ ID 1, or a functionally active variant thereof which is a size variant, a mutant or hybrid of SEQ ID 1, or a combination thereof.

Disclosed is an isolated nucleic acid sequence comprising a promoter, which is a native sequence of Pichia pastoris comprising or consisting of the pCS1 nucleic acid sequence of SEQ ID 1, or a functionally active variant thereof which is a size variant, a mutant or hybrid of SEQ ID 1, or a combination thereof, wherein said functionally active variant exhibits substantially the same activity as pCS1, specifically the pCS1 nucleic acid sequence of SEQ ID 1.

In a first aspect, there is provided an isolated pCS1 promoter nucleic acid sequence, which is ~~a native sequence of *Pichia pastoris* comprising the pCS1 nucleic acid sequence (SEQ ID 1), or~~ a functionally active variant of a native sequence of *Pichia pastoris* comprising the pCS1 nucleic acid sequence (SEQ ID 1),~~thereof~~ which is a size variant, a mutant or hybrid of SEQ ID 1, or a combination thereof, which functionally active variant has a length of 200-1500 bp, at least 60% sequence identity to the sequence of SEQ ID 1, and wherein said functionally active variant exhibits the same expression or transcriptional strength as the pCS1 nucleic acid sequence SEQ ID 1 +/- 20%, and/or at least an 1.1 fold increase compared to the native *P. pastoris* pGAP promoter set forth in SEQ ID 13.

Specifically, the pCS1 nucleotide sequence is identical with the sequence of SEQ ID 1.

Preferably, the nucleic acid sequence of the disclosure is not identical to the nucleic acid of SEQ ID 87 as listed in WO2007/015178 A2 (i.e. SEQ ID 24 of this application).

Specifically, the functionally active variant is

a) a size variant of pCS1 of SEQ ID 1, preferably consisting of or comprising the nucleic acid sequence selected from the group consisting of SEQ ID 2, 3, 4, 5, 6, 7 and 8;

b) a mutant of the pCS1 of SEQ ID 1, or a mutant of the size variant of a), which mutant has at least 60% homology to the sequence SEQ ID 1 or to the size variant;

c) a hybrid comprising

- a sequence selected from the group consisting of pCS1 of SEQ ID 1, a size variant of a), and a mutant of b); and

CLAIMS

1. An isolated pCS1 promoter nucleic acid sequence, which is a ~~native sequence of *Pichia pastoris* comprising the pCS1 nucleic acid sequence (SEQ ID 1), or a~~ functionally active variant of a native sequence of *Pichia pastoris* comprising the pCS1 nucleic acid sequence (SEQ ID 1),~~thereof~~ which is a size variant, a mutant or hybrid of SEQ ID 1, or a combination thereof, which functionally active variant has a length of 200-1500 bp, at least 60% sequence identity to the sequence of SEQ ID 1, and wherein said functionally active variant exhibits the same expression or transcriptional strength as the pCS1 nucleic acid sequence SEQ ID 1 +/- 20%, and/or at least an 1.1 fold increase compared to the native *P. pastoris* pGAP promoter set forth in SEQ ID 13.

~~2. The nucleic acid sequence according to claim 1, which consists of the pCS1 nucleic acid sequence (SEQ ID 1).~~

~~3~~2. The nucleic acid sequence according to claim 1~~ or 2~~, wherein the functionally active variant is

a) a size variant of SEQ ID 1, preferably comprising or consisting of the nucleic acid sequence selected from the group consisting of SEQ ID 2, 3, 4, 5, and 6, most preferably the nucleic acid sequence consists of SEQ ID 2, 3, 4, 5, or 6;

b) a mutant of SEQ ID 1, or a mutant of the size variant of a), which mutant has at least 60% homology to the sequence SEQ ID 1 or to the size variant;

c) a hybrid comprising

- a sequence selected from the group consisting of SEQ ID 1, a size variant of a), and a mutant of b); and

- at least one further sequence selected from the group consisting of SEQ ID 1, a size variant of a), a mutant of b), and a heterologous sequence; or

d) a sequence which hybridizes under stringent conditions to any of the size variant, or the mutant nucleic acid sequences of a), or b).

43. The nucleic acid sequence according to ~~any of claims 1 to 3~~claim 1 or 2, wherein the functionally active variant is selected from the group consisting of

i) homologs obtainable by modifying the nucleotide sequence of SEQ ID 1 or size variants thereof, by insertion, deletion or substitution of one or more nucleotides within the sequence or at either or both of the distal ends of the sequence; and

ii) analogs derived from species other than *Pichia pastoris*.

54. The nucleic acid sequence according to any of claims 1 to 43, which is operably linked to a nucleotide sequence encoding a protein of interest (POI), which nucleic acid is not natively associated with the nucleotide sequence encoding the POI.

65. The nucleic acid sequence according to claim 54, which further comprises a nucleic acid sequence encoding a signal peptide enabling the secretion of the POI, preferably wherein nucleic acid sequence encoding the signal peptide is located adjacent to the 5' end of the nucleotide sequence encoding the POI.

76. An expression construct comprising the nucleic acid sequence according to any of claims 1 to 65, preferably an autonomously replicating vector or plasmid, or one which integrates into the chromosomal DNA of a host cell.

87. A recombinant host cell which comprises an expression construct according to claim 76, preferably a eukaryotic cell, more preferably a yeast or filamentous fungal cell, more preferably a yeast cell of the Saccharomyces or Pichia genus.

98. The recombinant host cell according to claim 87, comprising multiple copies of the nucleic acid sequence, and/or multiple copies of the expression construct.

109. The recombinant host cell according to claim 87 or 98, which is selected from the group consisting of mammalian, insect, yeast, filamentous fungi and plant cells, preferably a yeast, preferably any of the *P. pastoris* strains CBS 704, CBS 2612, CBS 7435, CBS 9173-9189, DSMZ 70877, X-33, GS115, KM71 and SMD1168.

1110. A stable culture of a plurality of the cell according to any of claims 87 to 109.

~~12~~11. A method of producing a POI by culturing a recombinant host cell line comprising the promoter according to any of claims 1 to ~~6~~5 or the expression construct according to claim ~~7~~6, and a nucleic acid encoding the POI under the transcriptional control of said promoter, or the recombinant host cell according to any of claims ~~8~~7 to ~~10~~9, comprising the steps of

a) cultivating the cell line under conditions to express said POI, and

b) recovering the POI.

~~13~~12. The method according to claim ~~12~~11, wherein the POI is expressed under growth-limiting conditions.

~~14~~13. The method according to claim ~~12~~11 or ~~13~~12, wherein the cell line is cultivated under batch, fed-batch or continuous cultivation conditions, and/or in media containing limited carbon substrate.

~~15~~14. The method according to claim ~~14~~13, wherein the cultivation is performed in a bioreactor starting with a batch phase followed by a fed-batch phase or a continuous cultivation phase.

~~16~~15. The method according to any of claims ~~12~~11 to ~~15~~14, wherein the POI is a heterologous protein, preferably selected from therapeutic proteins, including antibodies or fragments thereof, enzymes and peptides, protein antibiotics, toxin fusion proteins, carbohydrate - protein conjugates, structural proteins, regulatory proteins, vaccines and vaccine like proteins or particles, process enzymes, growth factors, hormones and cytokines, or a metabolite of a POI.

~~17~~16. Use of the isolated nucleic acid sequence of any of claims 1 to ~~6~~5 or the expression construct of claim ~~7~~6 in a method of producing a POI by cultivating a host cell transformed with the nucleic acid sequence and/or the expression construct, preferably wherein the cultivation is performed in a bioreactor starting with a batch phase followed by a fed-batch phase or a continuous cultivation phase.