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.

In one 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 thereof which is a size variant and/or a mutant of SEQ ID 1, which functionally active variant has a length of 200-1500 bp and at least 90% sequence identity to the sequence of SEQ ID 1.

According to a specific embodiment, 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, wherein said functionally active variant exhibits substantially the same activity as pCS1, specifically the pCS1 nucleic acid sequence of SEQ ID 1.

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;

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 thereof which is a size variant and/or a mutant of SEQ ID 1, which functionally active variant has a length of 200-1500 bp and at least 90% sequence identity to the sequence of SEQ ID 1.

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

3. The nucleic acid according to claim 1 or 2, wherein said functionally active variant exhibits substantially the same activity as the pCS1 nucleic acid sequence (SEQ ID 1).

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

a) a size variant of SEQ ID 1; or

b) a mutant of SEQ ID 1, or a mutant of the size variant of a), which mutant has at least 90% sequence identity to the sequence SEQ ID 1 or to the size variant.

5. The nucleic acid sequence according to claim 4 a), wherein the size variant of SEQ ID 1 comprises or consists of the nucleic acid sequence selected from the group consisting of SEQ ID 2, 3, 4, 5, and 6.

6. The nucleic acid sequence according to claim 5, wherein the nucleic acid sequence consists of SEQ ID 2, 3, 4, 5, or 6.

7. The nucleic acid sequence according to any of claims 1 to 6, wherein the functionally active variant is 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.

8. The nucleic acid sequence according to any of claims 1 to 7, 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.

9. The nucleic acid sequence according to claim 8, which further comprises a nucleic acid sequence encoding a signal peptide enabling the secretion of the POI.

10. The nucleic acid sequence according to claim 9, wherein the nucleic acid sequence encoding the signal peptide is located adjacent to the 5' end of the nucleotide sequence encoding the POI.

11. An expression construct comprising the nucleic acid sequence according to any of claims 1 to 10.

12. The expression construct according to claim 11, wherein the expression construct is an autonomously replicating vector or plasmid, or one which integrates into the chromosomal DNA of a host cell.

13. A recombinant host cell which comprises a nucleic acid sequence according to any of claims 1 to 10 or an expression construct according to claims 11 or 12.

14. The recombinant host cell according to claim 13, wherein the recombinant host cell is a eukaryotic cell.

15. The recombinant host cell according to claim 14, wherein the eukaryotic host cell is a yeast or filamentous fungal cell.

16. The recombinant host cell according to claim 15, wherein the yeast cell is a yeast cell of the Saccharomyces or Pichia genus.

17. The recombinant host cell according to any of claims 13 to 16, comprising multiple copies of the nucleic acid sequence, and/or multiple copies of the expression construct.

18. The recombinant host cell according to any of claims 13 to 17, which is selected from the group consisting of mammalian, insect, yeast, filamentous fungi and plant cells.