CLAIMS

1. An isolated and/or artificial pG1-x promoter, which is a functional variant of the carbon source regulatable pG1 promoter of *Pichia pastoris* identified by SEQ ID 1, where the pG1-x promoter consists of or comprises at least a part of SEQ ID 1, the part of SEQ ID 1 having a length of at least 293 bp, the pG1-x promoter comprising the following promoter regions

a) at least two copies of a core regulatory promoter region; and

b) a non-core regulatory promoter region, which is any region within the pG1-x promoter sequence other than the core regulatory region;

wherein the core regulatory promoter region has a sequence identity which is at least 80% to SEQ ID 2 and SEQ ID 3, the non-core regulatory promoter region has a sequence identity of at least 50% with a corresponding region in SEQ ID 1; and further

wherein the pG1-x promoter is characterized by an increased promoter strength and/or induction ratio as compared to the pG1 promoter, wherein

- the promoter strength is at least 1.1-fold increased in the induced state as compared to the pG1 promoter, and/or

- the induction ratio is at least 1.1-fold increased as compared to the pG1 promoter.

2. The pG1-x promoter of claim 1, wherein SEQ ID 2 and/or SEQ ID 3 comprises one or more transcription factor binding sites (TFBS).

3. The pG1-x promoter of claim 1 or 2, wherein the core regulatory region comprises the nucleotide sequence SEQ ID 4, or a functional variant of SEQ ID 4 with at least 80% sequence identity, which comprises one or more TFBS.

4. The pG1-x promoter of any of claims 1 to 3, wherein the core regulatory region is incorporated into a main regulatory region represented by SEQ ID 5, or a functional variant of SEQ ID 5 with at least 80% sequence identity, which comprises one or more TFBS.

5. The pG1-x promoter of any of claims 1 to 4, which comprises one or more TFBS for any of the transcription factors selected from the group consisting of Rgt1, Cat8-1 and Cat8-2.

6. The pG1-x promoter of any of claims 1 to 5, wherein said at least one mutation is a deletion of one or more nucleotides between the nucleotide sequences SEQ ID 2 and SEQ ID 3.

7. The pG1-x promoter of any of claims 1 to 6, which comprises at least one or at least two T motifs identified by any of SEQ ID 12-29.

8. An isolated and/or artificial pG1-x promoter, comprising or consisting of the nucleotide sequence selected from the group consisting of any of

a) SEQ ID 37–44, preferably any of SEQ ID 45-76;
b) SEQ ID 77–80, preferably any of SEQ ID 81-112;
c) SEQ ID 113-114, preferably any of SEQ ID 115-130;
d) SEQ ID 131-132, preferably any of SEQ ID 133-148; or
e) SEQ ID 185-186, preferably any of SEQ ID 187-202;

or a nucleotide sequence which has at least 80% sequence identity to any of the foregoing.

9. The pG1-x promoter of claim 8, which comprises or consists of the nucleotide sequence selected from the group consisting of any of SEQ ID 37–44, preferably any of SEQ ID 45-76.

10. The pG1-x promoter of any of claims 1 to 9, 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.

11. The nucleic acid of claim 10, which further comprises a nucleotide sequence encoding a signal peptide enabling the secretion of the POI, preferably wherein nucleotide sequence encoding the signal peptide is located adjacent to the 5'-end of the nucleotide sequence encoding the POI.

12. An expression construct comprising the nucleic acid of claim 10 or 11, preferably an autonomously replicating vector or plasmid, or a vector or plasmid which integrates into the chromosomal DNA of a host cell.

13. A recombinant host cell which comprises the expression construct of claim 12, preferably a eukaryotic cell, more preferably a yeast or filamentous fungal cell, more preferably a yeast cell of the Saccharomyces or Pichia genus.

14. A method of producing a protein of interest (POI) by culturing a recombinant host cell line of claim 13, comprising the steps of

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

b) recovering the POI.

15. The method of claim 14, wherein the cultivation comprises

a) a first step using a basal carbon source repressing the pG1-x promoter, preferably wherein the basal carbon source is selected from the group consisting of glucose, glycerol, ethanol, a mixture thereof, and complex nutrient material, followed by

b) a second step using no or a limited amount of a supplemental carbon source de-repressing the pG1-x promoter to induce production of the POI, preferably wherein the supplemental carbon source is a hexose such as glucose, fructose, galactose or mannose, a disaccharide, such as saccharose, an alcohol, such as glycerol or ethanol, or a mixture of any of the foregoing.

16. The method of claim 15, wherein the second step b) employs a feed medium that provides for the supplemental carbon source in a growth limiting amount to keep the specific growth rate within the range of 0.001 h^{-1} to 0.2 h^{-1}, preferably 0.005 h^{-1} to 0.15 h^{-1}.

17. The method of claims 15 or 16, wherein the cultivation is performed in a bioreactor starting with a batch phase as the first step, followed by a fed-batch phase or a continuous cultivation phase as the second step, wherein the batch phase is performed for around 20 to 36h; and the cultivation in the fed-batch phase is performed for any of around 15 to 80h, around 15 to 70h, around 15 to 60h, around 15 to 50h, around 15 to 45h, around 15 to 40h, around 15 to 35h, around 15 to 30h, around 15 to 35h, around 15 to 25h, or around 15 to 20h; preferably around 20 to 40h.

18. The method of any of claims 14 to 17, wherein the recombinant host cell is yeast of any of the Saccharomyces genus or Pichia genus or *Komagataella* genus, preferably Pichia pastoris or Komagataella pastoris.

19. The method of any of claims 14 to 18, wherein the pG1-x promoter is any of SEQ ID 37–44, preferably any of SEQ ID 45-76, preferably wherein the pG1-x promoter comprises SEQ ID 39, more preferably SEQ ID 49.

20. The method of any of claims 14 to 19, wherein the POI is produced at a transcription rate of at least 15% as compared to the native pGAP promoter of the cell.

21. The method of any of claims 14 to 20, 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.