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(54) **YEAST PROMOTER AND USE THEREOF**

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**Related U.S. Patent Documents**

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(51) **Int. Cl.**

**C12N 1/16** (2006.01)  
**C12N 15/81** (2006.01)  
**C07H 21/04** (2006.01)

(52) **U.S. Cl.** ..... **435/254.2**; 435/69.1; 435/320.1; 536/24.1

(58) **Field of Classification Search** ..... None  
See application file for complete search history.

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EP 0361991 4/1990

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(57) **ABSTRACT**

The present invention concerns DNA sequences from the *K. lactis* PGK gene having transcriptional promoter activity. to expression vectors comprising these sequences and to their use for the production of proteins.

**23 Claims, 14 Drawing Sheets**

10	20	30	40	50	60
ICTRSTTTA	GCGGGTCATC	GAAATTTAGT	AGCGAGTCTA	TTAGGGACCA	GAGTTGCAAC
70	80	90	100	110	120
CTGAGCTTTA	ATGCGTCATC	CTGTCGTTGC	TTCAAGTTCC	CCACTTGAAT	CACTTGGACA
130	140	150	160	170	180
AACCGITTTCA	TTGGTTTGAG	GAAGGTGACG	GATCTGGGTA	GAAACTGGAC	TACTGCATCT
190	200	210	220	230	240
GTTGGTAGTC	TTGATGCCAT	GGTGATGAGC	CATTGCCAAT	GGAAAAGAGT	GAATTCAGAT
250	260	270	280	290	300
TCCAAGATTT	GGTCAATGAT	TGATTTTGTA	AGATTGAGAT	CGTAATCCTG	ATACTCTTTG
310	320	330	340	350	360
AGCCCAIGTTT	CCAACAGTTC	TTCGGAATCT	GCCGGTGTGG	AAACGAGTAT	TTCGGAGTAC
370	380	390	400	410	420
AATCTCGGTG	GTTGCGTTAT	CTGAGAGGAT	GGTGTAGTGG	TTTGATGTTG	CTGTGTGAAA
430	440	450	460	470	480
GATGATGCAG	AGCTGATCAA	CGATTCGAAC	TGGGAGATCA	CTTCGTTTAC	TTCTTCCTGG
490	500	510	520	530	540
TTCCCGTTAC	CTGTTTGCGT	TTCCTCATAC	AITGGTACGC	TATCCTCATC	TTCAGATAAC
550	560	570	580	590	600
GAAATATCAA	ACTCATCGGA	ATCGGACGCG	TCGTTCAAAT	CGCCCTCATC	CTTGGTAATG
610	620	630	640	650	660
TTCTTGAACC	GGTCGAGAAG	GTTGAGAATC	TCTGTCCGAA	CACCACCCTG	CGGCGTATAAC
670	680	690	700	710	720
CAGAACCAGA	ATAAATGTA	GCACATCTTA	ACTTTCTCTA	AGGAAACATC	TGAACTCTGA
730	740	750	760	770	780
TCAACGCATT	CCGTAAGTAT	ACTGTTTGCC	TTGTCTCTGG	TGAATTTATG	AGGGTAAGAC
790	800	810	820	830	840
TCTGAGATCA	TAAGTAACTG	TTGAGCATCG	AAGTTGTGT	AGTTTGAAT	TAGGGATCTG
850	860	870	880	890	900
GAAAGATGCG	GTACCACTGC	TTTGATGACA	TTATCTGGCG	GGTCAACGG	TACCAATTCC
910	920	930	940	950	960
TGCAAGATA	GCGAATCCAA	CGGTTTTAAC	TCAGAGTAAT	GGTTGATCAA	CTCGATGAAA
970	980	990	1000	1010	1020
ACGTCCCAAT	GGATGGATTG	CATCAAGTGT	TGATGTTCCA	CCAAATTAAG	ACAATATTTT
1030	1040	1050	1060	1070	1080
GTAACGTTTT	CGAGTGAAAC	TGACACGGGC	CTGCCCTCAG	CACTCGTAGA	CACGAGTAAC
1090	1100	1110	1120	1130	1140
GTTCTGAGAC	CTCTCGTACA	GGGAAGCGAC	ATATCGTTCA	ATAGACTATG	GAACAAAGTG
1150	1160	1170	1180	1190	1200
TACACCGCAG	CGATATCCTT	GCATTTGCAA	AACGATTGAA	TAAGTGACGT	CGATGCTAAA
1210	1220	1230	1240	1250	1260
TCCTGGATAA	GTACGCTGGT	ATCGTGTAAG	CCCATGAGAA	CGACACGTTT	CTCATCACIA

FIG. 1A

[SEQ ID NO: 1]

1270	1280	1290	1300	1310	1320
GAAGCCGAAC	TGTTGTCTTC	AGTGGGGATT	GGTTCGACAT	TTTGCCAATT	GCTGTGCGATG
1330	1340	1350	1360	1370	1380
TACCCCTTTC	AAGCCATGTA	CCTTAAATCT	TCATCCTTGG	CAAGTAGATT	CATCGGGTGT
1390	1400	1410	1420	1430	1440
GTTTGAAGTA	AGAATATTG	CTTGTTTTTA	TGGTATCAAA	GGTATATGTT	GTAGAAGACA
1450	1460	1470	1480	1490	1500
ATTTCCGGTA	ATCCAATTGT	CTGTCTGCTC	AGTTTAGCAC	ATGTATAGTA	CGTTGCACAT
1510	1520	1530	1540	1550	1560
AGTCTACAAT	ATTCAGCATT	CAGCAITCAG	TATACAGCAT	ATGGCTAAAT	GATCACAAAT
1570	1580	1590	1600	1610	1620
GTGATTGATG	ATTTGACACG	ACTAGAAAAG	AGAACGAAAA	AGGGAAATTC	ATGTCACGTC
1630	1640	1650	1660	1670	1680
CGTTGGCACG	TGACATGGAA	TATCGAAGAA	AGAAAAAAA	AAACGATCTC	GTCCTAGTGG
1690	1700	1710	1720	1730	1740
AAGCCCAGAG	TCTGGTCCCC	COGGAGTCTT	CCCAAAACAA	GAAGCTGACA	CATGTTGACA
1750	1760	1770	1780	1790	1800
CAGAACACCC	CACAGCAAT	GCACCACGCT	ACGTAGATCA	GGAAGCTTAA	CTCTAGCGAC
1810	1820	1830	1840	1850	1860
CTGTGCGCTCG	CCCCACAGAA	CCTCACCCGA	GAACCACACA	TTACACGCCG	CCAGCTCCCA
1870	1880	1890	1900	1910	1920
CTATACTCAT	CTTGCTTCCC	TTAAGCGTTC	TCACGATTCC	TTCGCTGCCC	TTCITCAAGA
1930	1940	1950	1960	1970	1980
GTCTTCTGAT	TCTAATTCTC	ATTGCAAATC	CTCTACAGTT	AATGAATTGC	TTGACATGAC
1990	2000	2010	2020	2030	2040
ATTCATTGTC	TCATGGTTTT	GGCTTTTTGG	CTTTTGTCTT	TTAAAGCTAT	ATCAACTTTA
2050	2060	2070	2080	2090	2100
<u>CATATAAATA</u>	<u>TACGTCAAAA</u>	<u>GGGGATTCAT</u>	<u>TAATTAGAAA</u>	<u>ATTCTCTTTT</u>	<u>TCAATAGTTG</u>
2110	2120	2130	2140	2150	2160
CTATTCATTA	TCAATCTATT	CAACTCAATT	GGTATTIATT	TTCATCTTTT	TGTCATCCTA
2170	2180	2190	2200	2210	2220
AACCATCAAC	<u>AATATTTAAA</u>	<u>TATATCTGTT</u>	<u>GCTACATTAA</u>	<u>GAGTTACTTC</u>	<u>AGAAATAACA</u>
2230	2240	2250			
AAAAAATCGA	TCAAGAATTA	ATAAAAATG			

Met

{SEQ ID NO: 1}

FIG. 1B

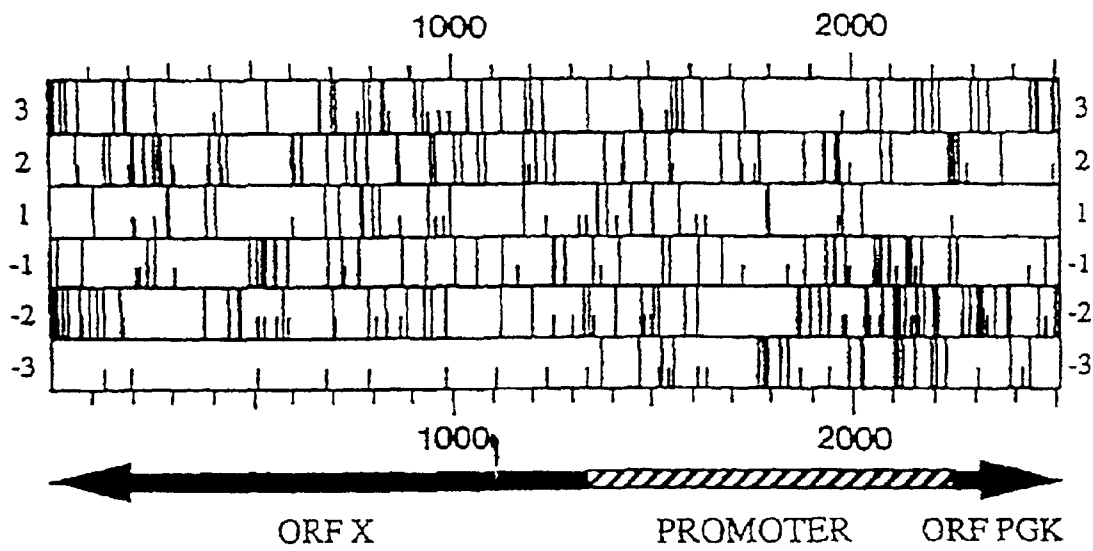


FIG. 2

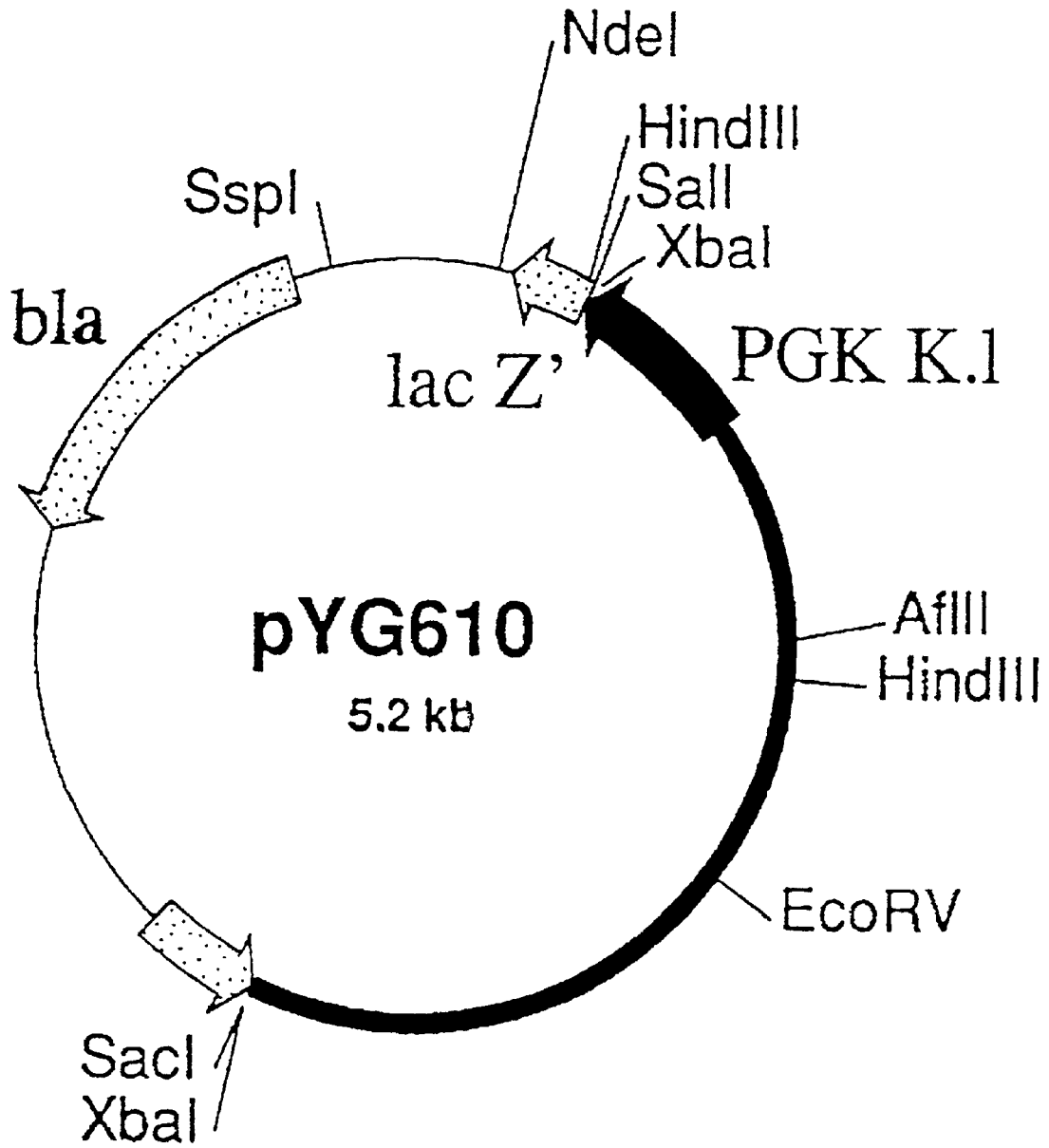


FIG. 3

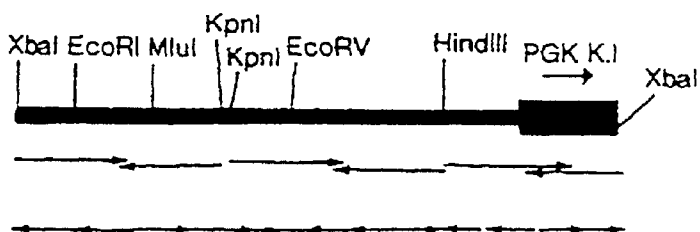


FIG. 4

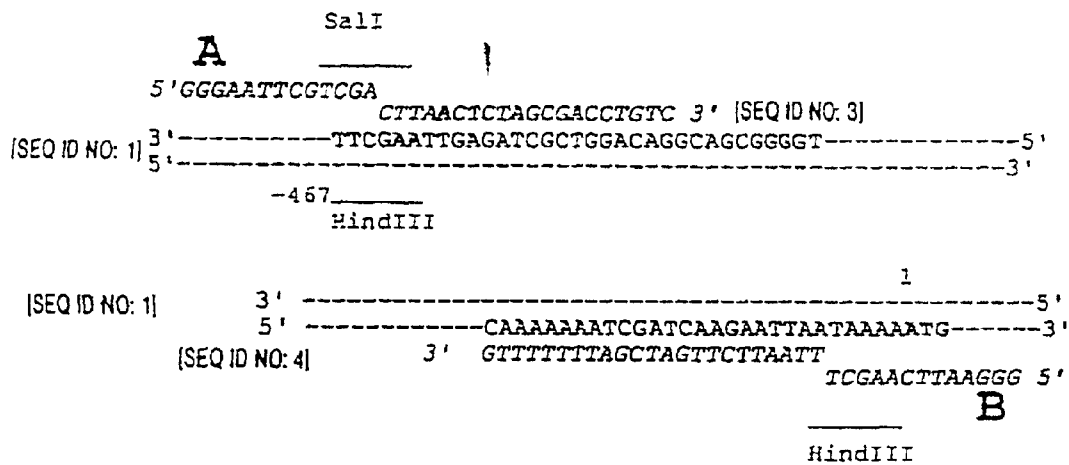


FIG. 5

Oligodeoxynucleotide A

5'CATGTCGACTTTTATTAATTCTTGATCGAT3' [SEQ ID NO: 5]

SalI

Oligodeoxynucleotide B

[SEQ ID NO: 6]

5'ATGAAGCTTAAATCTTCATCCTTGGC3'

HindIII

Oligodeoxynucleotide C

5'GGGTGAGGTTCTGTGGGGCGAGCGACAGGTCGCTAGAGTTAAGCATCCTGATC3'

(Position: 439 to 492)

[SEQ ID NO: 7]

Oligodeoxynucleotide D

5'GATCAGGATGCTTAACTCTAGCGACCTGTCGCTCGCCCCACAGAACCTCACCC3'

(Position: 439 to 492)

[SEQ ID NO: 8]

HindIII	10	20	30	40	50	60
<u>aagctt</u> TTAA	ATCTTCATCC	TGGCAAGTA	GATTCATCGG	GTGTGTTTGA	AGTAAGAATA	60
ttcgaaAATT	TAGAAGTAGG	AACCGTTCAT	CTAAGTAGCC	CACACAAACT	TCATTCTTAT	
TTTGCTTGTT	TTTATGGTAT	CAAAGGTATA	TGTTGTAGAA	GACAATTTCC	GGTAATCCAA	120
AAACGAACAA	AAATACCATA	GTTTCCATAT	ACAACATCTT	CTGTTAAAGG	CCATTAGGTT	
TTGTCTGTCT	GCTCAGTTTA	GCACATGTAT	AGTACGTTGC	ACATAGTCTA	CAATATTCAG	180
AACAGACAGA	CGAGTCAAAT	CGTGACATA	TCATGCAACG	TGTATCAGAT	GTTATAAGTC	
CATTCAGCAT	TCAGTATACA	GCATATGGCT	AAATGATCAC	AAATGTGATT	GATGATTTGA	240
GTAAGTCGTA	AGTCATATGT	CGTATACCGA	TTACTAGTG	TTTACACTAA	CTACTARACA	
CACGACTAGA	AAAGAGAACG	AAAAAGGGAA	ATTCATGTCA	CGTGCGTTGG	CACGTGACAT	300
GTGCTGATCT	TTTCTCTTGC	TTTTTCCCTT	TAAGTACAGT	GCACGCAACC	GTGCACTGTA	
GGAATATCGA	AGAAAGAAAA	AAAAAACGA	TCTCGTCCTA	GTGGAAGCCC	AGAGTCTGGT	360
CCTTATAGCT	TCTTCTTTT	TTTTTTGGCT	AGAGCAGGAT	CACCTTCGGG	TCTCAGACCA	
CCCCCGGAG	TCTTCCAAA	ACAAGAAGCT	GACACATGTT	GACACAGAAC	ACCCACAGC	420
GGGGGGCCTC	AGAAGGGTTT	TGTTCTTCGA	CTGTGTACAA	CTGTGTCTTG	TGGGGTGTCC	
AAATGCACCA	CGCTACGTAG	ATCAGGATGC	<u>TTAACTCTAG</u>	CGACCTGTCC	CTCGCCCCAC	480
TTTACSTGGT	GCGATGCATC	TAGTCCTACG	AATTGAGATC	GCTGGACAGC	GAGCGGGGTG	
AGAACCTCAC	CCGAGAACCA	CACATTACAC	GCCGCCAGCT	CCCACTATAC	TCATCTTGCT	540
TCTTGAGAGT	GGCTCTTGGT	GTGTAATGTG	CGGCGGTCGA	GGGTGATATG	AGTAGAACGA	
TCCCTTAAGC	GTTCTCACGA	TTCGTTCGCT	GCCCTTCTTC	AAGAGTCTTC	TGATTCTAAT	600
AGGGAATTTC	CAAGAGTGCT	AAGCAAGCGA	CGGGAAGAAG	TTCTCAGAAG	ACTAAGATTA	
TCTCATTCGA	AATCCTCTAC	AGTTAATGAA	TTGCTTGACA	TGACATTCAT	TGTCTCATGG	660
AGAGTAAGCT	TTAGGAGATG	TCAATTACTT	AACGAACTGT	ACTGTAAGTA	ACAGAGTACC	
TTTTGGCTTT	TTGGCTTTTG	TCTTTTAAAG	CTATATCAAC	TTTACATATA	AATATACGTC	720
AAAACCGAAA	AACCGAAAAC	AGAAAATTTT	GATATAGTTG	AAATGTATAT	TTATATGCAG	
AAAAGGGGAT	TCATTAATTA	GAAAATTTCT	TTTTTCAATA	GTTGCTATTC	ATTATCAATC	780
TTTTCCCCTA	AGTAATTAAT	CTTTTAAGAG	AAAAGTTTAT	CAACGATAAG	TAATAGTTAG	
TATTCAACTC	AATTGGTTAT	TATTTTCATC	TTTTTGTCAT	CCTAAACCAT	CAACAATATT	840
ATAAGTTGAG	TTAACCAATA	ATAAAAGTAG	AAAACAGTA	GGATTTGGTA	GTTGTTATAA	
TAAATATATC	TGTTGCTACA	TTAAGAGTTA	CTTCAGAAAT	AACAAAAAAA	TCGATCAAGA	900
ATTTATATAG	ACAACGATGT	AATTCTCAAT	GAAGTCTTTA	TTGTTTTTTT	AGCTAGTTCT	
ATTAATAAAA	<u>Agtcagac</u>					917
TAATTATTTT	Tcagctg					
	10 SalI	20	30	40	50	60

[SEQ ID NO: 2]

FIG. 6

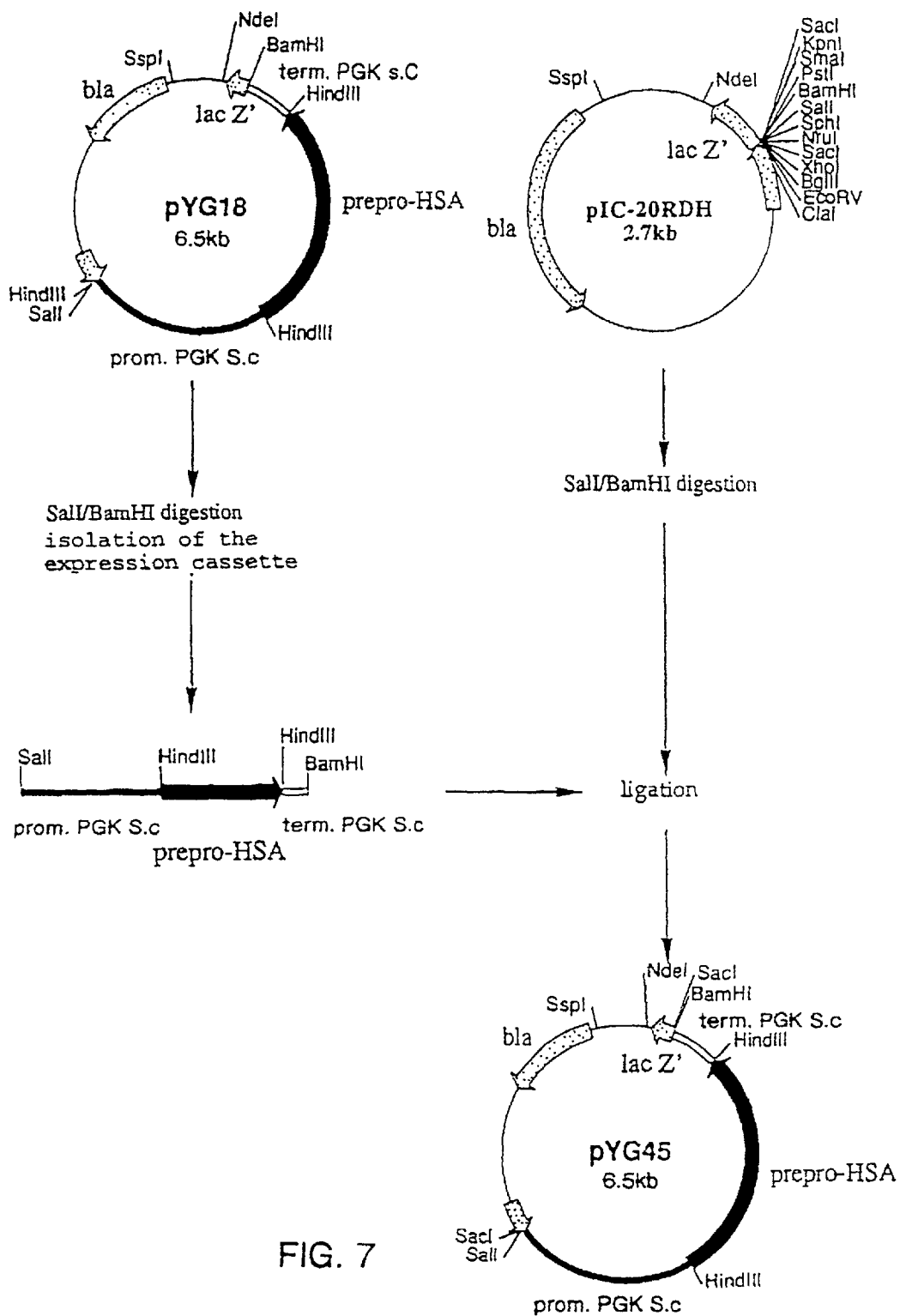
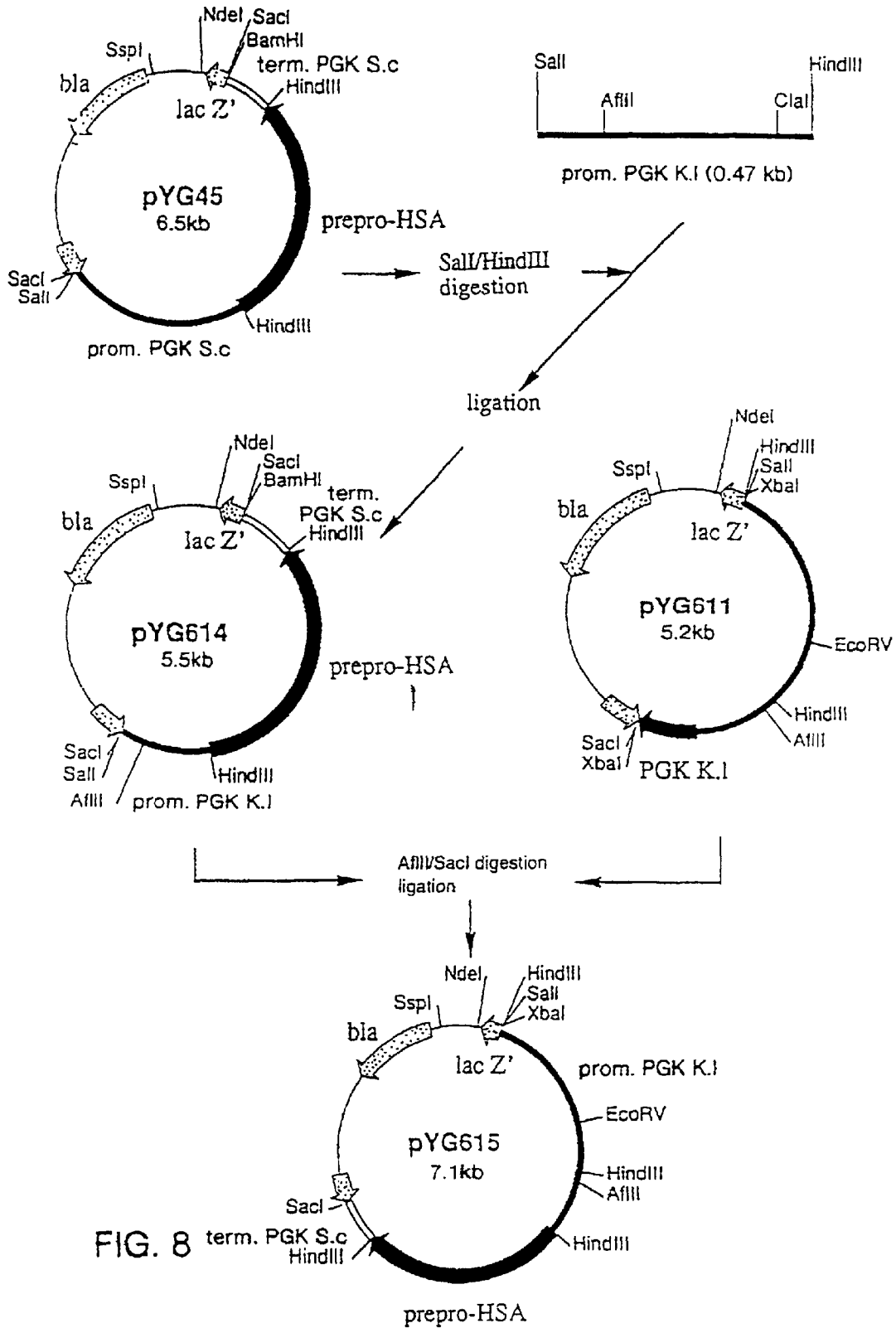


FIG. 7



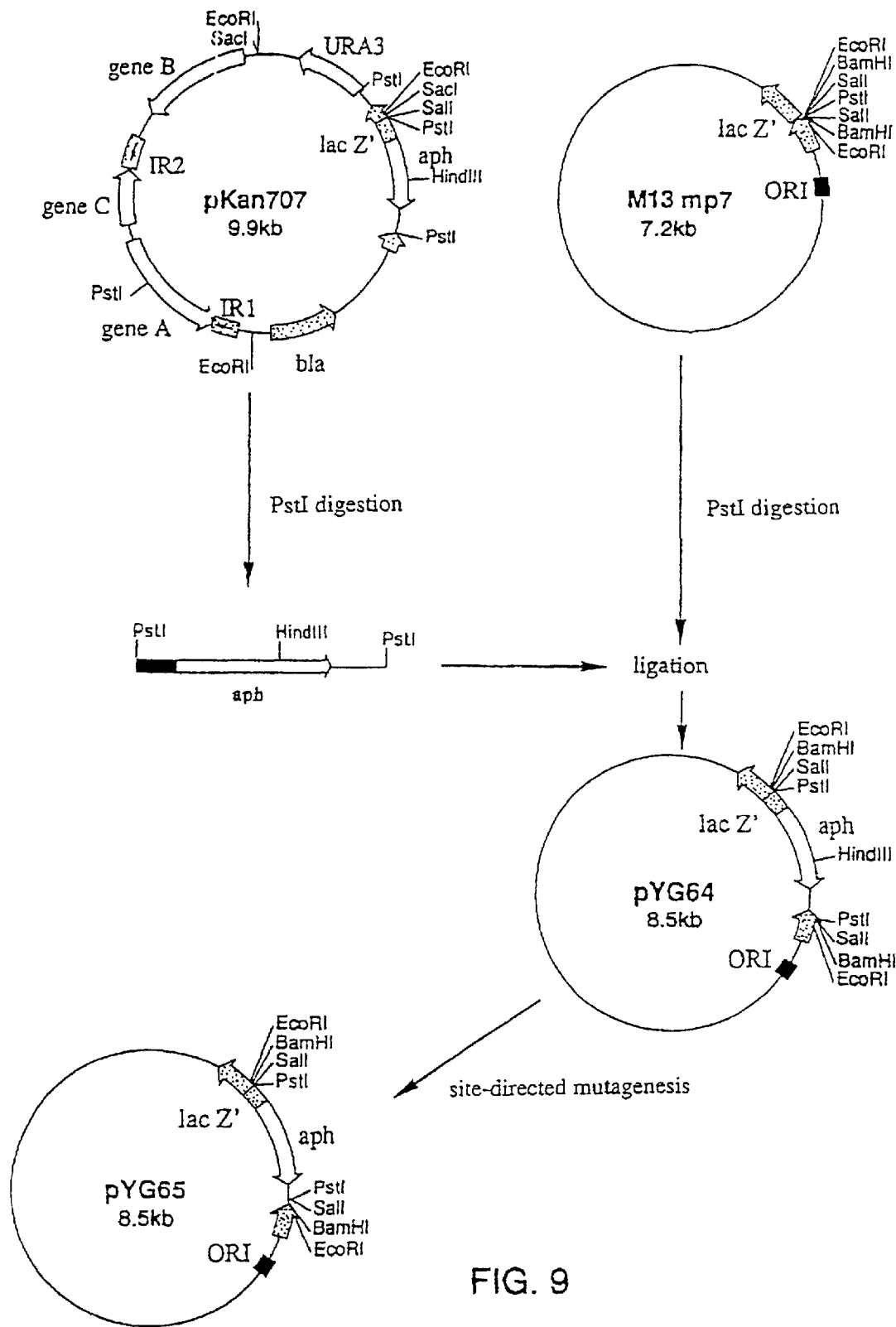


FIG. 9

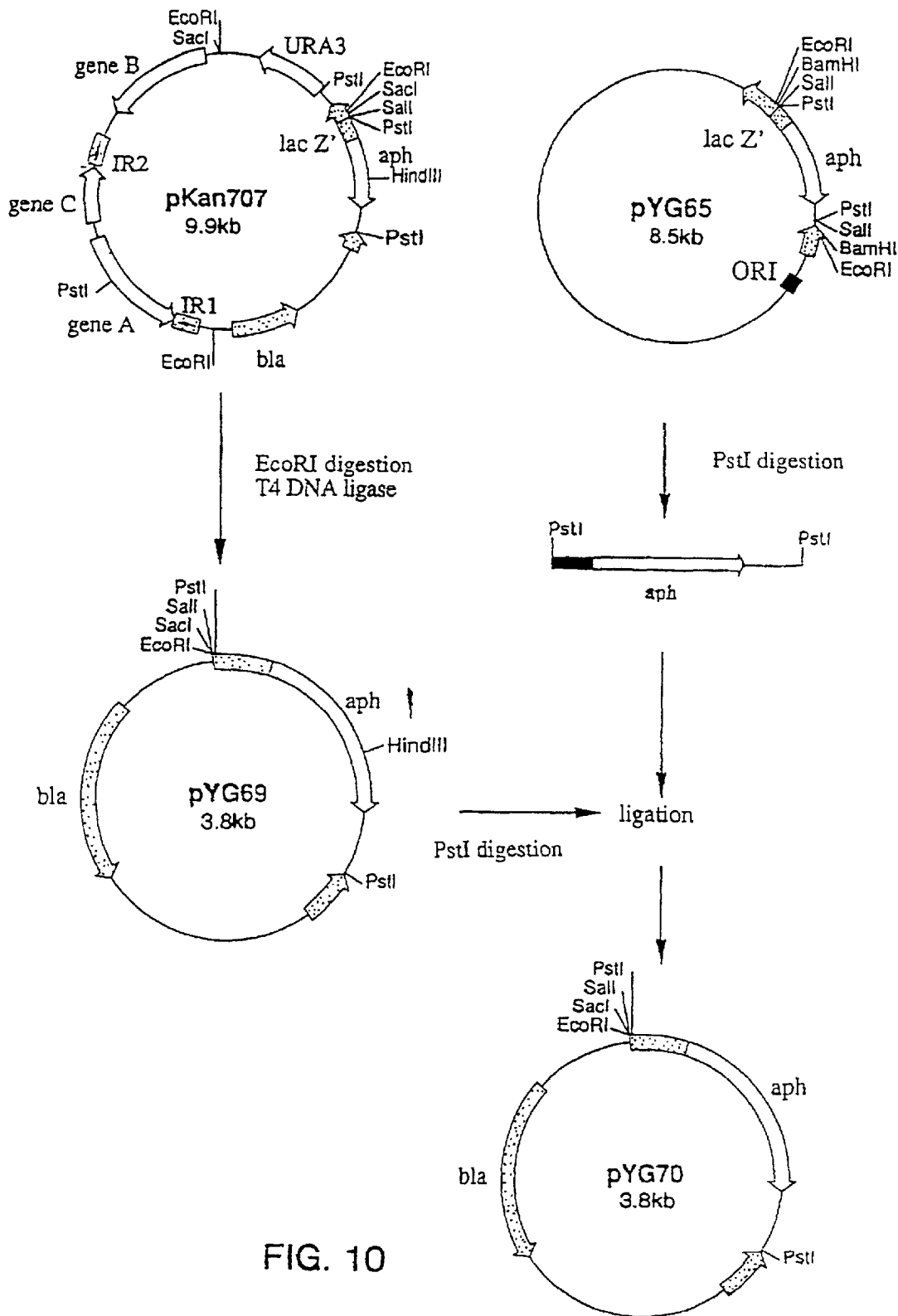


FIG. 10

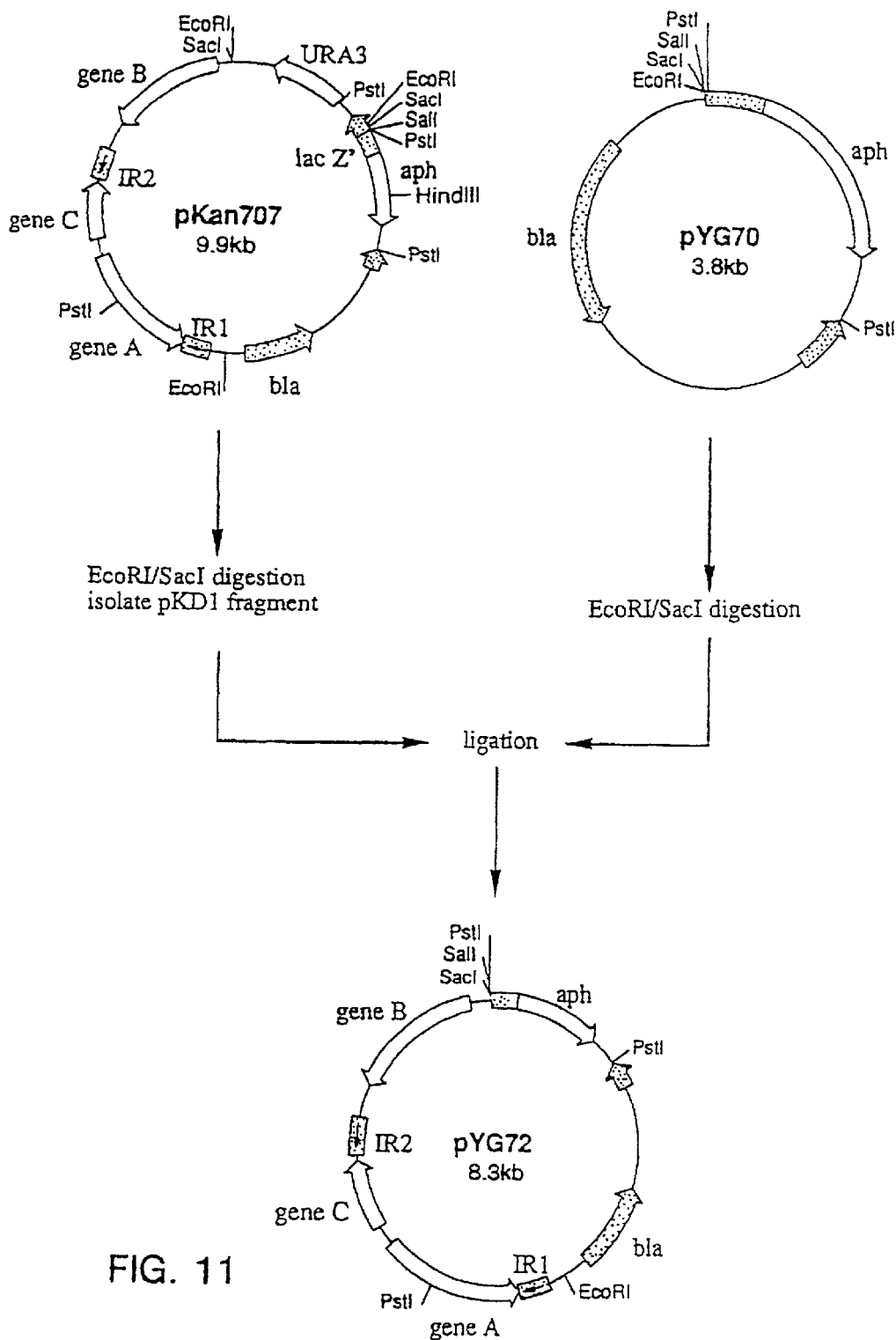


FIG. 11

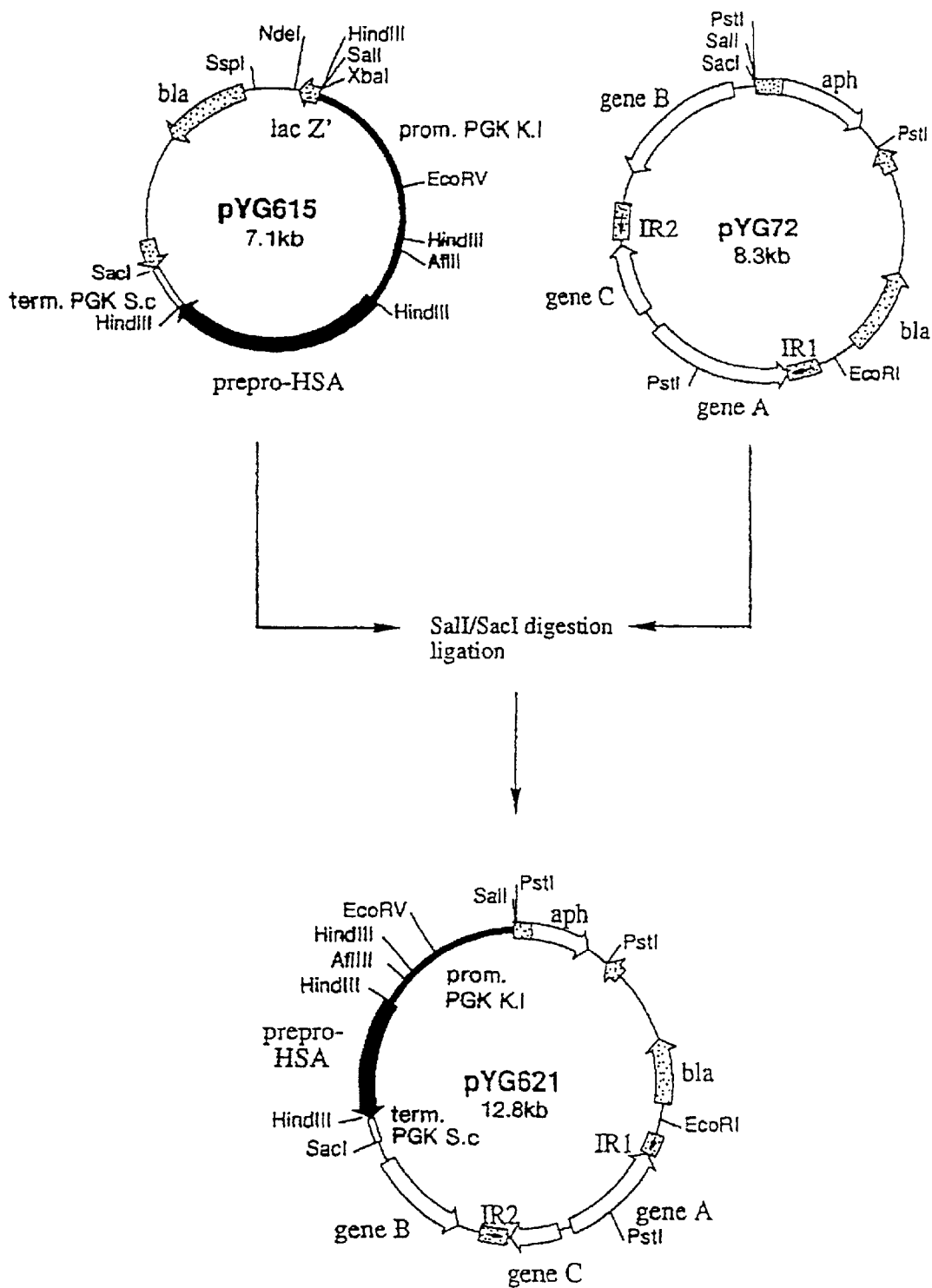


FIG. 12

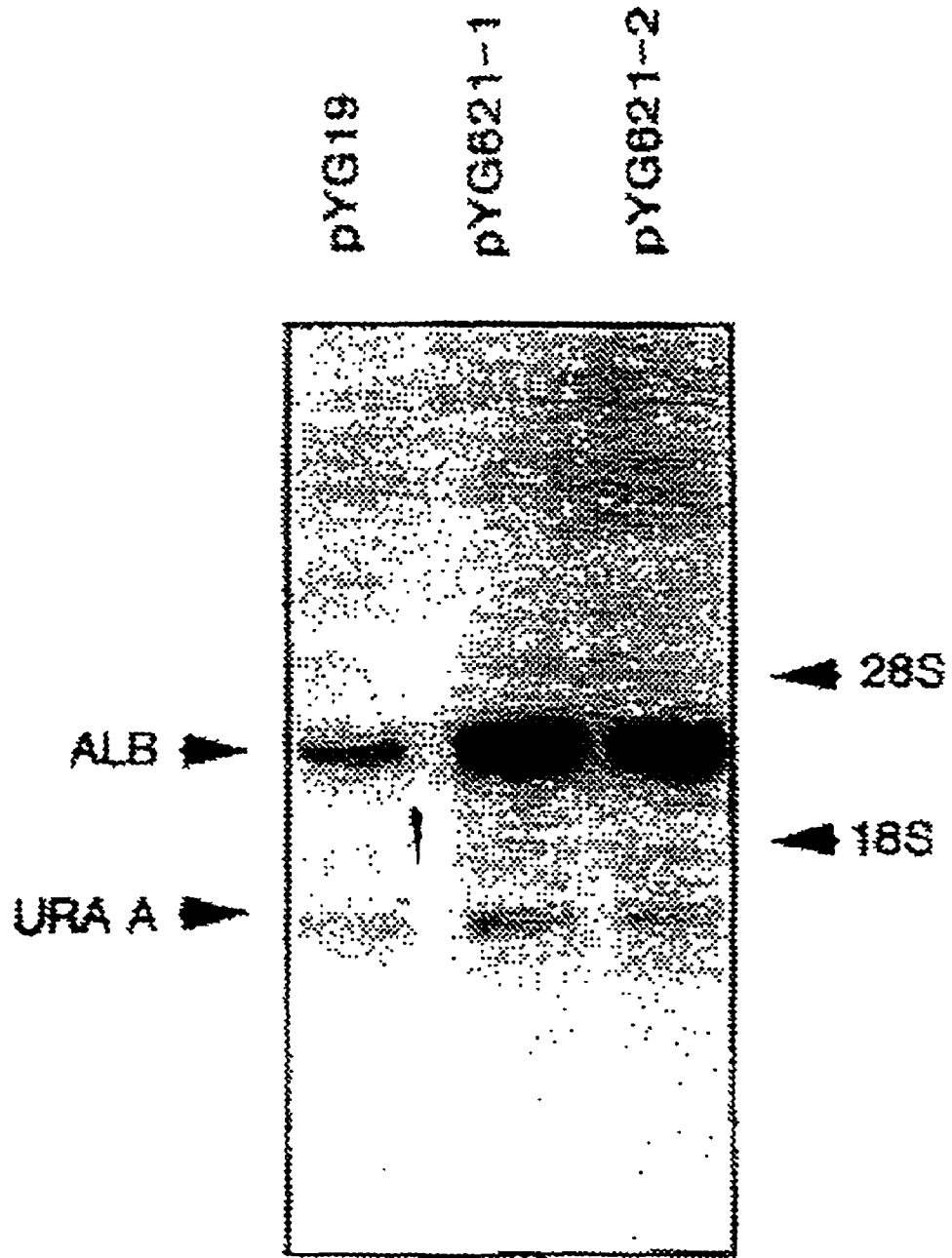


FIG. 13

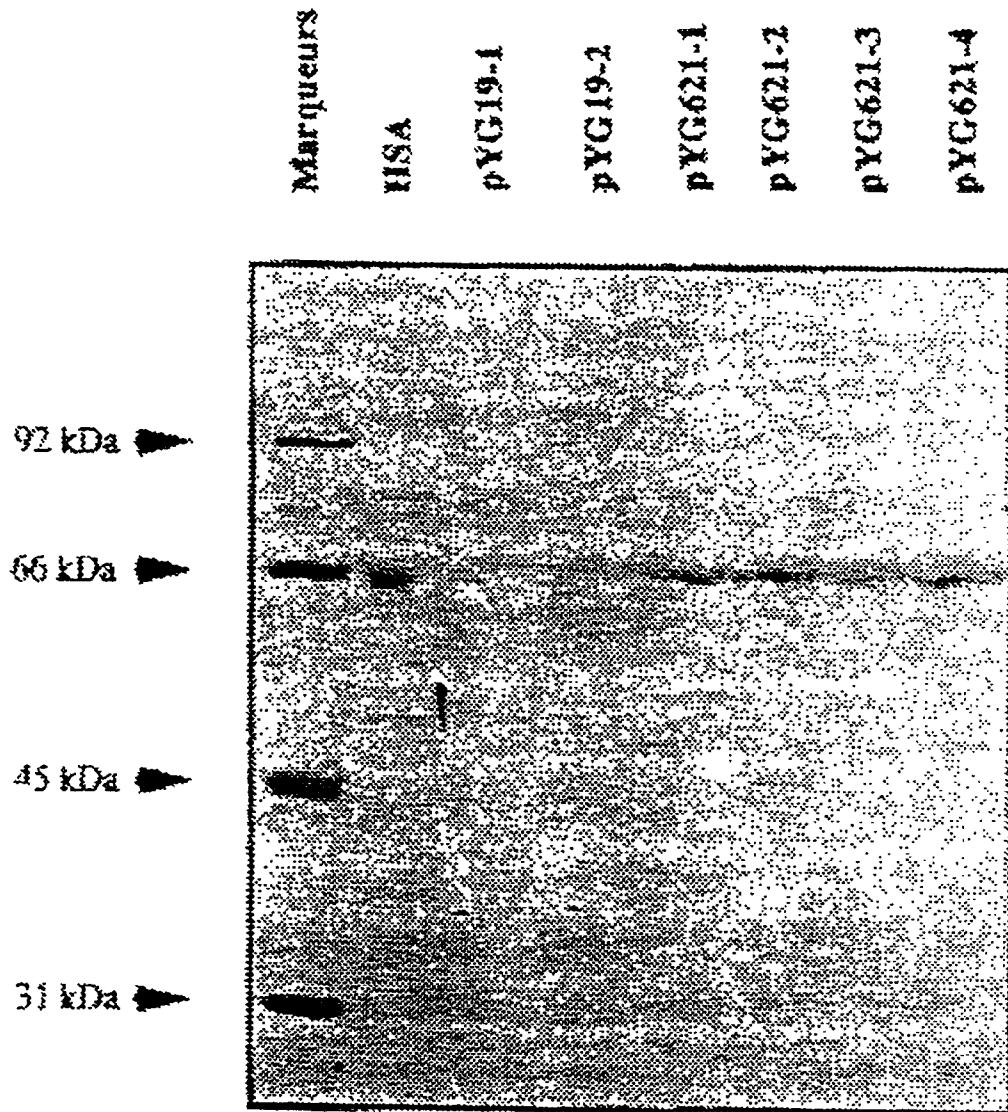


FIG. 14

## YEAST PROMOTER AND USE THEREOF

**Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.**

This a continuation of application Ser. No. 08/140,093 filed on Nov. 1, 1993, abandoned, which is a national stage application filed under 35 U.S.C.371 of PCT/FR92/00375 filed Apr. 28, 1992.

The present invention relates to the field of molecular biology. More particularly, it relates to a novel DNA sequence having a transcriptional promoter activity, to expression vectors containing this sequence and to the use thereof for the production of proteins, for example heterologous proteins. The invention also relates to recombinant cells containing this DNA sequence.

The progress accomplished in the field of molecular biology has enabled microorganisms to be modified in order to make them produce heterologous proteins. In particular, many genetic studies have been carried out on the bacterium *E. coli*. However, the industrial application of these novel modes of production is still limited, in particular by problems of efficiency of gene expression in these recombinant microorganisms. Accordingly, research studies have been carried out with the aim of increasing the efficiency of these production systems in order to isolate strong promoters enabling high levels of expression of heterologous proteins to be obtained. In *E. coli*, the tryptophan operon and the lactose operon promoters may be mentioned in particular.

More recently, in the yeast *S. cerevisiae*, studies have been carried out in promoters derived from genes involved in glycolysis. There may be mentioned in particular the work on the promoter of the 3-phosphoglycerate kinase PGK gene (Dobson et al., *Nucleic Acids Res.* 10:2625, 1982; Hitzeman et al., *Nucleic Acids Research* 10:7791, 1982), the glyceraldehyde-3-phosphate dehydrogenase GAPDH gene (Holland et al., *J. Biol. Chem.* 254:9839, 1979; Musti et al., *Gene* 25:133, 1983), the alcohol dehydrogenase 1 ADH1 gene (Bennetzen et al., *J. Biol. Chem.* 257:3018, 1982; Denis et al., *J. Biol. Chem.* 25:1165, 1983), and the enolase 1 ENO1 gene (Uemura et al., *Gene* 45:65, 1986).

Recently, genetic tools have been developed in order to make use of the yeast *Kluyveromyces* as a host cell for the production of recombinant proteins. The discovery of a 2-micron type plasmid derived from *K. drosophilum* (plasmid pKD1; described in European Patent No. 241,435) has enabled a very efficient host/vector system for the production of recombinant proteins (EP 361,991) to be established. However, the promoters used in this system have never been optimized. In particular, the promoters involved are essentially heterologous promoters, that is to say derived from other microorganisms, such as *S. cerevisiae*. This situation may lead to various disadvantages. The activity of the promoter may be limited because of the absence of certain elements of the transcriptional machinery (for example, trans-activators). A certain toxicity to the host cell may occur due to an absence of regulation, or the stability of the vector may be affected.

Under these conditions, the lack of strong homologous promoters in *Kluyveromyces* constitutes a limiting factor in the industrial exploitation of this expression system.

The Applicant has now identified, cloned and sequenced a region of the *Kluyveromyces lactis* genome having transcriptional promoter activity (see FIG. 1 [SEQ ID NO: 1]). More specifically, this region corresponds to the promoter of

the *K. lactis* PGK gene. This region, or derivatives or fragments thereof, may be used for the efficient production of recombinant proteins in yeasts in the *Kluyveromyces* genus. It is understood that this sequence may also be used in other host organisms.

Moreover, an analysis of the region of the *Kluyveromyces* genome obtained has identified two reading frames in the two opposite directions (see FIG. 2). This observation shows that the complementary strand of the region presented in FIG. 1 also possesses a promoter activity acting in the other direction.

One subject of the present invention therefore lies in a DNA sequence comprising all or part of the sequence presented in FIG. 1 [SEQ ID NO: 1], or a sequence of its complementary strand, or of a derivative of these, and possessing promoter activity.

Within the context of the present invention, derivative is understood to mean any sequence obtained from the sequence given in FIG. 1 [SEQ ID NO: 1], by structural modifications (mutations, deletions, substitutions, additions, restrictions and the like) which conserve promoter activity. In particular, the mutations may involve one or more nucleotides, and the additions are/or substitutions may involve regulatory elements or activator regions such as UASs.

When a derivative is produced, its transcriptional promoter activity may be demonstrated in several ways and in particular by placing a resistance gene or a complementation marker under the control of the sequence studied. Other techniques known to a person skilled in the art may obviously be used to this effect.

A more specific subject of the invention relates to a DNA sequence corresponding to the region between the 2 open reading frames ORF PGF and ORF X, as presented in FIG. 6 [SEQ ID NO: 2].

Another subject of the invention relates to a recombinant DNA comprising a DNA sequence as defined above.

This recombinant DNA may contain, for example, the promoter sequence presented in FIG. 1 [SEQ ID NO: 1] or a derivative thereof, in which a restriction site is inserted, facilitating the use of this sequence as "portable" promoter.

Preferably, this recombinant DNA contains, in addition, one or more structural genes.

Still more preferably, the recombinant DNA also contains signals permitting the secretion of the expression product of said structural gene(s).

In a specific embodiment of the invention, the recombinant DNA is part of an expression plasmid which may be autonomously replicating or integrative in nature.

In particular, autonomously replicating vectors may be obtained by using autonomously replicating sequences (ARS) in the host selected. In yeast in particular, replication origins derived from known plasmids (pKD1, 2 $\mu$ , and the like) may be involved.

Integrative vectors may be obtained in particular by using homologous sequences at certain regions of the host genome which permit integration of the vector by homologous recombination.

The sequence presented in FIG. 1 was obtained by screening a total genomic DNA library from *Kluyveromyces lactis* by means of a heterologous probe derived from the *S. cerevisiae* PGK gene. The Applicant has indeed shown that it is possible to close a promoter region in *Kluyveromyces*, by hybridization using heterologous probes corresponding to a *S. cerevisiae* gene. Details of the cloning of the sequence are given in the examples. The intergenic region may then be isolated from this sequence, in particular by restriction site

insertion using the PCR amplification technique, as indicated in the examples.

Another subject of the invention relates to the recombinant cells which contain a DNA sequence as defined above.

Advantageously, cells are chosen from yeasts, and still more preferably from yeasts of the *Kluyveromyces* genus. It is understood, however, that the invention covers all the recombinant cells in which the promoter regions of the invention are active.

These cells may be obtained by any method enabling a foreign DNA to be introduced into a cell. The methods involved may be in particular transformation, electroporation or any other technique known to a person skilled in the art.

Another object of the invention relates to the use of a sequence as defined above, for the expression of recombinant genes.

As illustrated by the examples, the DNA sequences according to the invention indeed permit high levels of production of recombinant proteins.

Moreover, the bi-directional promoter activity of the sequences of the invention permits a particularly advantageous use. In particular, it is possible to use these sequences in the 2 directions possible, for the simultaneous expression of several structural genes.

Advantageously, the invention relates to the use of a sequence as defined above for the simultaneous expression of recombinant genes in the 2 opposite directions.

Advantageously, the sequences of the invention may be used for the expression of genes encoding proteins of interest in the pharmaceutical or foodstuffs sectors. By way of example, there may be mentioned enzymes (such as in particular superoxide dismutase, catalase, amylases, lipases, amidases, ezymosin and the like), blood derivatives (such as serum albumin, alpha- or beta-globin, factor VIII, factor IX, von Willebrand factor, fibronectin, alpha-1-antitrypsin and the like), insulin and its variants, lymphokines (such as interleukins, interferons, colony stimulating factors (G-CSF, GM-CSF, M-CSF and the like), TNF, TRF and the like), growth factors (such as growth hormones, erythropoietin, FGF, EGF, PDGF, TGF and the like), apolipoproteins, antigenic polypeptides for the production of vaccines (hepatitis, cytomegalovirus, Epstein-Barr, herpes and the like) or alternatively polypeptide fusions such as in particular fusions comprising an active part fused with a stabilizing part (for example fusions between albumin or fragments of albumin and the virus receptor or part of a virus receptor (CD4, and the like)).

The invention will be more completely described by means of the examples which should be considered as illustrative and nonlimiting. Other and further objects, features, and advantages will be apparent from the following description of the preferred embodiments of the invention given for the purpose of disclosure when taken in conjunction with the following drawings.

#### BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: Nucleotide sequence of the 2.2 kb region of the chromosomal fragment situated upstream of the initiation codon for translation of the *K. lactis* PGK gene having the promoter activity [SEQ ID NO: 1].

FIG. 2: Analysis of the open reading frames. The 6 possible reading frames are shown ad -3, -2, -1, 1, 2, 3. The vertical half-lines represent translation initiation codons. The full vertical lines represent stop codons. The clear regions show the open reading frames (ORF X and ORF PGK).

FIG. 3: Restriction map of the plasmid pYG610. The black region corresponds to the region isolated from the *K. lactis* genome.

FIG. 4: Strategy for sequencing the 2.5 kb *Xba*I fragment.

FIG. 5: Sequence and location of the oligodeoxynucleotides used in the PCR reaction for inserting a *Hind*III site at position -6 with respect to the ATG of the sequence in FIG. 1. The oligodeoxynucleotides are represented in italics [SEQ ID NOS: 3 and 4]. ATG corresponds to the initiation codon for translation of the PGK gene.

FIG. 6: Nucleotide sequence of the intergenic region of the 2.2 kb fragment. 6(a): oligodeoxynucleotides used in the PCR reactions [SEQ ID NOS: 5-8]. 6(b): *Sa*II-*Hind*III fragment corresponding to the nucleotides 1343 to 2246 on the sequence in FIG. 1 [SEQ ID NO: 2].

FIG. 7: Strategy for the construction of the plasmid pYG45.

FIG. 8: Strategy for the construction of human serum albumin expression cassettes.

FIG. 9: Strategy for the construction of the plasmid pYG65.

FIG. 10: Strategy for the construction of the plasmid pYG70.

FIG. 11: Strategy for the construction of the plasmid pYG72.

FIG. 12: Strategy for the construction of the vector pYG621.

FIG. 12: Visualization, by Northern blotting, of the expression of the human albumin gene under the control of the *K. lactis* PGK promoter. The samples correspond to 10  $\mu$ g of the total RNA. 18S and 28S are the positions of the 18S and 28S ribosomal RNAs. ALB=fragments recognized by the probe corresponding to the albumin gene; URA=fragments recognized by the probe corresponding to the *K. lactis* URA A gene serving as loading reference.

FIG. 14: Visualization of albumin production in strains transformed by the expression vector pYG621 containing the *K. lactis* PGK promoter. The samples correspond to 30  $\mu$ l of the culture supernatant; the bands at the position of the 66 kd marker correspond to albumins. M=molecular weight markers: bovine carbonic anhydrase (31 kd), ovalbumin (45 kd), BSA (66 kd), rabbit phosphorylase b (92 kd).

#### EXAMPLES

The following examples describe the isolation and use of the promoter of the present invention and are not intended to be limiting unless so expressly stated.

##### Example 1

##### Isolation of the Promoter Region of the *K. lactis* PGK Gene

The sequence presented in FIG. 1 was obtained by screening a total genomic DNA library from *Kluyveromyces lactis* CBS2359 using a heterologous probe derived from the *S. Cerevisiae* PGK gene (Dobson et al., *Nucleic Acids Res.* 10:2625, 1982). More specifically, the probe used corresponds to the 1.3 kb N-terminal *Pvu*I-*Eco*RI fragment of the *S. Cerevisiae* PGK gene.

In Southern blotting (Southern et al., *J. Biol. Chem.* 98:503, 1975), the probe used hybridizes to two different fragments when the genomic DNA is digested with *Xba*I. One fragment, of about 2.5 kb, was isolated by screening a small genomic library from *K. lactis* CBS2359 consisting of

XbaI-cut DNA fragments of between 2 and 3 kb in size, which were introduced inside the plasmid pUC18 at the XbaI site. A library with 500 clones was thus produced and then screened with the heterologous probe described above.

A clone was identified by colony hybridization and its plasmid DNA was prepared. This plasmid (pYG610) contains a 2.5 kb genomic DNA fragment whose restriction map is presented in FIG. 3. The plasmid pYG611 contains the same insert in the opposite direction (see FIG. 8).

In a second stage, the 2.5 kb fragment thus isolated was sequenced using the Sanger method (Sanger et al., Proc. Nat. Acad. Sci USA 74:5463, 1977). For that, the fragment derived from pYG611 was first subcloned in the bacteriophages M13tg130 and M13tg131. The strategy for the sequencing of the fragment is schematically represented in FIG. 4.

Analysis of the sequence obtained showed that the fragment isolated contains a part encoding the N-terminal region of the protein PGK from *Kluyveromyces lactis* (0.3 kb), and 2.2 kb corresponding to the promoter region situated upstream of the site of initiation of translation. It shows furthermore that a second reading frame, situated about 0.9 kb upstream of the ATG of the PGK gene, is situated in the opposite direction relative to the PGK gene (FIG. 2).

Comparison of this sequence with that of the promoter of the *S. Cerevisiae* PGK gene reveals the absence of specific homology, especially with its regulatory element. This sequence therefore corresponds to a completely novel promoter region, which is very distinct from those previously described, from the point of view of its structure and consequently from the point of view of its regulation.

#### Example 2

##### Construction of Expression Vectors for the Production of Heterologous Proteins

This example illustrates the use of the promoter capabilities of the 2.2 kb sequence of the sequence in FIG. 1 [SEQ ID NO: 1] and of desired sequences.

a) Insertion of a restriction site at position -6 relative to the ATG

The insertion of this size subsequently enables any gene which is to be expressed to be introduced downstream of the promoter. For reasons of compatibility with existing expression vectors (EP 361,991), "portable" promoters were constructed in the form of SaII—HindIII fragments.

A HindIII site was introduced at position -6 relative to the site of initiation of translation (ATG) of the PGK gene using the PCR amplification technique (Mullis et al., Meth. Enzymol. 155:335, 1987). Two oligodeoxynucleotides, which are presented in FIG. 5 [SEQ ID NOS: 3 and 4], were used for this purpose.

The oligodeoxynucleotide A [SEQ ID NO:3] corresponds to the sequence simulated at 467 bp upstream of the ATG codon, at a HindIII site which is replaced by a SaII site during the amplification. The oligodeoxynucleotide B [SEQ ID NO:4] corresponds to the sequence upstream of the initiation site, and enables a HindIII site to be introduced at position -6.

The fragment obtained by PCT was inserted between the SaII and HindIII sites of the bacteriophage M13tg130 in order to verify, by sequencing, that mutation did not occur during the amplification.

b) Construction of human serum albumin expression cassettes: FIG. 8

The 474 bp recombinant DNA obtained above was introduced into the SaII and HindIII sites of the plasmid pYG45

(FIG. 7), in order to obtain the vector pYG614 (FIG. 8). The plasmid pYG45 contains an expression cassette consisting of the promoter and the terminator of the *S. cerevisiae* PGK gene between which the prepro-human serum albumin encoding gene (prepro-HSA sequence) is inserted into a HindIII site. pYG45 is derived from pYG18 (see Patent EP 361,999) by subcloning the SaII—BamHI fragment containing the HSA expression cassette into the corresponding sites of the vector pIC-20RDH (FIG. 7). pIC-20RDH is obtained by digesting the plasmid pIC-20R (March et al., Gene 32:481, 1984) with the enzyme HindIII, filling the ends using the Klenow fragment of *E. Coli* polymerase I and recircularization with T4 DNA ligase.

The SaII—SacI fragment may be isolated from the plasmid pYG614 by digestion. It contains: a promoter region derived from the sequence in FIG. 1, the albumin gene and the terminator of the *S. cerevisiae* PGK gene. It constitutes an expression cassette which may be inserted inside a plasmid so as to constitute an expression vector.

Another expression cassette may be obtained from the plasmid pYG614 by cloning the AflIII—SacI fragment containing part of the PGK promoter of the invention, the albumin gene (prepro-HSA) and the *S. cerevisiae* PGK terminator inside the plasmid pYG611 described above. This generates the plasmid pYG615. The SaII—SacI fragment (containing the complete promoter region of FIG. 1, the prepro-serum albumin-encoding gene, and the terminator of the *S. cerevisiae* PGK gene) may then be isolated by digestion. This fragment constitutes a second albumin expression cassette using the promoter sequence of the invention.

c) Construction of albumin expression vectors

Albumin expression vectors may be constructed by inserting the expression cassettes obtained above inside *K. lactis*/*E. coli* shuttle plasmids such as pYG72 (FIG. 10). In particular, an expression vector was obtained (vector pYG621) by inserting the SaII—SacI fragment from pYG615, containing the albumin-expression cassettes, inside the vector pYG72 (see FIG. 10). This vector corresponds to the plasmid pKan 707 (see EP 361,999) from which the SacI fragment, containing the URA3 gene, has been removed, as well as the unique HindIII site present in the *aph* gene, so as to facilitate subsequent constructions. The *aph* gene encodes aminoglycoside 3'-phosphotransferase I (Oka et al., J. Mol. Biol. 147:217, 1981) and is used in yeast as a marker for resistance to G418. The PstI fragment of the plasmid pKan 707, containing the *aph* gene, was subcloned into the bacteriophage M13mp7 to give the vector pYG64 (FIG. 9). The HindIII site present in this gene was destroyed by site-directed mutagenesis according to the method described by Taylor et al., (Nucleic Acid Res. 13:8749, 1985). The resulting plasmid was called pYG65 (FIG. 9). The oligodeoxynucleotide used for the mutagenesis had the sequence 5'-GAA ATG CAT AAG CTC TTG CCA TTC TCA CCG-3 [SEQ ID NO: 9] and transformed the triplet CTT encoding the amino acid 185 (Leu) to CTC. This change does not modify the resulting protein sequence. To construct the plasmid pYG272, the part containing the bacterial replicon of the vector pKan 707 was isolated by digestion with the enzyme EcoRI and recircularization with T4 DNA ligase so as to obtain pYG69. The PstI fragment present in this latter vector, containing the *aph* gene, was replaced by the equivalent mutant fragment derived from pYG65. This construct was called pYG70. The 4.7 kb pKDI sequence between the EcoRI and SacI sites was introduced inside this latter vector so as to obtain pYG72. The vector pYG621 (FIG. 11) was obtained by insertion of the SaII—SacI fragment containing the albumin expression cassette derived from pYG615.

## Example 3

## Construction of a Cassette Enabling the Promoter Region to be Used in the 2 Directions

This construct was obtained by introducing a SaII site and a HindIII site on either side of the region between the two open reading frames identified in FIG. 2: ORF PGK and ORF X, that is, at nucleotides 1343 and 2246 in FIG. 1.

This construct was produced by the PCT technique using oligodeoxynucleotide A [SEQ ID NO: 5], which introduces a SaII site in the -1 position relative to the site of initiation of translation of the PGK gene, and oligodeoxynucleotide B [SEQ ID NO: 6], which introduces a HindIII site in the -1 position relative to the site of initiation of translation of the X gene (see FIG. 6(a)). Three PCR reactions were carried out using the plasmid pYG610 as the template, so as to remove a HindIII site present in the promoter region:

The first 2 PCRs amplified the regions on either side of the HindIII site using the oligodeoxynucleotides A and B [SEQ ID NOS: 5 and 6] coupled to oligodeoxynucleotides [SEQ ID NO: 7] and D [SEQ ID NO: 8] respectively (FIG. 6). Oligodeoxynucleotides C and D are complementary and enable a point mutation to be introduced at the level of the inner hindIII site.

The last PCR generated the final fragment, containing the modified promoter region, using the previous 2 amplification products as primer.

This region is then introduced into the vectors described in Example 2, and used as a bi-directional promoter.

## Example 4

## Expression of Albumin

The vector pYG621 was introduced, by transformation, into the *K. lactis* strain MW98-8C (CBS 579.88), using the ethylene glycol/dimethyl sulphoxide technique (Durrens et al. *Curr. Genet.* 18:7, 1990). This strain is derived from the wild strain CBS2359 and is of the genotype: Mat  $\alpha$ , *uraA*, *lysA*, *argA*, R+, *cir*. The transformant yeast are selected for the G418-resistant phenotype, which the plasmid pYG621 confers, using YPD media (10 g/l yeast extract, 20 g/l peptone, 20 g/l glucose) containing 0.2 g/l of geneticin. Plasmid pYG72-transformed strains not containing an expression cassette were selected to serve as controls in the production tests. Moreover, vector pYG19-transformed strains were also selected in order to compare the efficiency of the *K. lactis* PGK promoter according to the invention with that from *S. cerevisiae*. The vector pYG19 is similar to the vector pYG621 except that the albumin gene is under the control of the *S. cerevisiae* PGK promoter (EP 361,991).

## a) Analysis of the mRNAs

The cells were cultured at 28° C. in selective YPD media containing 0.2 g/l of geneticin. The total RNAs were extracted (Sherman et al., *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory, 143, 1986) and separated by electrophoresis on an agarose gel. The RNAs were hybridized to a probe corresponding to the albumin structural gene (1.9 kb HindIII—HindIII fragment) derived from the vector pYG18 (FIG. 7) following the Northern blot method (Maniatis et al., *Molecular cloning*, Cold Spring Harbor, Laboratory Press, 1982). Autoradiography showed a 2.3 kb band specific to albumin (FIG. 13). Moreover, it is clearly evident that the level of transcription of the albumin gene is substantially higher in the strains containing a promoter region of the invention (pYG621) than in those containing the intact *S. cerevisiae* PGK promoter (pYG 19).

## b) Analysis of the proteins

The cells were cultured in Erlenmeyer flasks in selective YPD media containing 0.2 g/l geneticin at 28° C. with shaking. After 96 hours in culture, 30  $\mu$ l of supernatant were collected and mixed with an equivalent volume of 2xLaemmli buffer (Laemmli, *Nature* 227:680, 1970). After heating at 96° C. for 10 minutes, the sample proteins were separated on an 8.5% SDS polyacrylamide gel. The production of albumin was then visualized by staining the gel with coomassie blue, and production from the different vectors was compared. FIG. 1d shows that the 4 clones which were obtained separately by transforming the strain MW98-8C using the vector pYG621, secreted substantially more albumin than those obtained by transformation using the vector pYG19.

It is evident that the promoter region of the invention permits excellent albumin production by yeast, greater than that obtained with the *S. cerevisiae* PGK promoter. This region, or reduced forms or derivatives thereof, constitute(s) an important industrial tool for microbiological, and more particularly eukaryotic, production systems.

One skilled in the art will readily appreciate the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The DNA sequences, recombinant DNAs, vectors, cells, methods, procedures, and techniques described herein are presented as representative of the preferred embodiments, or intended to be exemplary and not intended as limitations on the scope of the present invention. Changes therein and other users will occur to those of skill in the art which are encompassed within the spirit of the invention or defined by the scope of the appended claims.

## Deposit of Strains Useful in Practicing the Invention

Deposits of biologically pure cultures of the following strains were made pursuant to, and in secretion of, the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. The Accession Numbers indicated were assigned after successful viability testing, and the requisite fees were paid. Access to said cultures will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122. All restriction on availability of said culture to the public will be irrevocably removed upon the granting of a patent based upon the application and said cultures will remain permanently available for a term of at least five years after the most recent request for the furnishing of a sample and in any case for a period of at least 30 years after the date of the deposit. Should the cultures become nonviable or be inadvertently destroyed, they will be replaced with viable culture (s) of the same taxonomic description.

Strain/Plasmid	ATCC No.	Deposit Date
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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 9

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2250 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM:
- Kluyveromyces lactis*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TCTAGATTTA GCGGGTCATC GAAATTTAGT AGCGAGTCTA TTAGGGACCA GAGTTGCAAC 60  
 CTGAGGTTTA ATGCGTCATC CTGTCGTTGC TTCAAGTTCC CCACTTGAAT CACTTGGACA 120  
 AACCGTTTCA TTGGTTTGAG GAAGGTGACG GATCTGGGTA GAAACTGGAC TACTGCATCT 180  
 GTTGGTAGTC TTGATGCCAT GGTGATGAGC CATTGCCATT GGAAAAGAGT GAATTCAGAT 240  
 TCCAAGATTT GGTC AATGAT TGATTTTGTA AGATTGAGAT CGTAATCCTG ATACTCTTTG 300  
 AGCCATGTTT CCAACAGTTC TTCGGAATCT GCCGTTGTTG AACGAGTAT TTCGGAGTAC 360  
 AATCTCGGTG GTTTCGTTAT CTGAGAGGAT GGTGTAGTGG TTTGATGTTG CTGTGTGAAA 420  
 GATGATGCAG AGCTGATCAA CGATTGGAAC TGGGAGATCA CTTGTTTCC TTTCTCTGG 480  
 TTCCCGTTAC CTGTTTGCCT TTCCTCATA CATTGTTGCG TATCCTCATC TTCAGATAAC 540  
 GAAATATCAA ACTCATCGGA ATCGGACGCG TCGTTCAAAT CGCCCTCATC CTGGTAAATG 600  
 TTCTTGAACC GGTCGAGAAG GTTGAGAATC TCTGTCGGA CACCACCTG CGGCGTATAC 660  
 CAGAACCAGA ATAAATTGTA GCACATCTTA ACTTCTCTA AGGAAACATC TGAACCTGTA 720  
 TCAACGCATT CCGTAAGTAT ACTGTTTGCC TTGTCTCTG TGAATTTATG AGGGTAAGAC 780  
 TCTGAGATCA TAAGTAAC TGAGCATCG AAGTTGTTGT AGTTTGAAT TAGGGATCTG 840  
 GAAAGATGCG GTACCACTGC TTTGATGACA TTATCTGGCG GGTTCACCG TACCAATTCC 900  
 TGCAAGAATA GCGAATCCAA CGGTTTAAAC TCAGAGTAAT GGTGATCAA CTCGATGAAA 960  
 ACGTCCCAAT GGATGGATTG CATCAAGTGT TGATGTTCCA CCAAATTAAG ACAATATTTT 1020  
 GTAACGTTTT CGAGTGAAAC TGACACGGGC CTGCCCTCAG CACTCGTAGA CACGAGTAAC 1080  
 GTCTTGAGAC CTCTCGTACA GGAAGCGAC ATATCGTTCA ATAGACTATG GAACAAAGTG 1140  
 TACACCCGAG CGATATCCTT GCATTGCAA AACGATTGAA TAAGTGACGT CGATGCTAAA 1200  
 TCCTGGATAA GTACGCTGGT ATCGTGTAAG CCCATGAGAA CGACACGTTT CTCATCACTA 1260  
 GAAGCCGAAC TGTGTTCTT AGTGGGGATT GGTTCGACAT TTTGCCAATT GCTGTCGATG 1320  
 TACCCTTTCA AAGCCATGTA CCTTAAATCT TCATCCTTGG CCAAGTAGAT TCATCGGGTG 1380  
 TGTTTGAAGT AAGAATATTT GCTTGTTTTT ATGGTATCAA AGGTATATGT TGTAAGAGAC 1440  
 AATTTCCGGT AATCCAATTG TCTGTCTGCT CAGTTTAGCA CATGTATAGT ACGTTGCACA 1500  
 TAGTCTACAA TATTCAGCAT TCAGATTCA GTATACAGCA TATGGCTAAA TGATCACAAA 1560

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TGTGATTGAT GATTTGACAC GACTAGAAAA GAGAACGAAA AAGGGAAATT CATGTCACGT	1620
GCGTTGGCAC GTGACATGGA ATATCGAAGA AAGAAAAAAA AAAACGATCT CGTCCTAGTG	1680
GAAGCCCAGA GTCTGGTCCC CCCGGAGTCT TCCCAAAACA AGAAGCTGAC ACATGTTGAC	1740
ACAGAACACC CCACAGCAA TGCACCACGC TACGTAGATC AGGAAGCTTA ACTCTAGCGA	1800
CCTGTCGCTC GCCCCACAGA ACCTCACCCG AGAACACAC ATTACACGCC GCCAGCTCCC	1860
ACTATACTCA TCTTGCTTCC CTTAAGCGTT CTCACGATTC GTTCGCTGCC CTTCTTCAAG	1920
AGTCTTCTGA TTCTAATTCT CATTGCGAAT CCTCTACAGT TAATGAATTG CTTGACATGA	1980
CATTCATTGT CTCATGGTTT TGGCTTTTGG GCTTTTGTCT TTTAAAGCTA TATCAACTTT	2040
ACATATAAAT ATACGTCAAA AGGGGATTCA TTAATTAGAA AATTCTCTTT TTCAATAGTT	2100
GCTATTCTAT ATCAATCTAT TCAACTCAAT TGGTTATTAT TTTTCATCTT TTGTCATCCT	2160
AAACCATCAA CAATATTTAA ATATATCTGT TGCTACATTA AGAGTTACTT CAGAAATAAC	2220
AAAAAATCG ATCAAGAATT AATAAAAAATG	2250

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 917 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Kluyveromyces lactis*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AAGCTTTTAA ATCTTCATCC TTGGCAAGTA GATTCATCGG GTGTGTTTGA AGTAAGAATA	60
TTTGCTTGTT TTTATGGTAT CAAAGGTATA TGTTGTAGAA GACAATTTC GGTAAATCCAA	120
TTGTCTGTCT GCTCAGTTTA GCACATGTAT AGTACGTTGC ACATAGTCTA CAATATTCAG	180
CATTCAGCAT TCAGTATACA GCATATGGCT AAATGATCAC AAATGTGATT GATGATTGA	240
CACGACTAGA AAAGAGAACG AAAAAGGGAA ATTCATGTCA CGTGCGTTGG CACGTGACAT	300
GGAATATCGA AGAAAGAAAA AAAAAACGA TCTCGTCCTA GTGGAAGCCC AGAGTCTGGT	360
CCCCCCGGAG TCTTCCAAA ACAAGAAGCT GACACATGTT GACACAGAAC ACCCCACAGC	420
AAATGCACCA CGCTACGTAG ATCAGGAAGC TTAACCTTAG CGACCTGTCG CTCGCCCCAC	480
AGAACCTCAC CCGAGAACCA CACATTACAC GCCGCCAGCT CCCACTATAC TCATCTTGCT	540
TCCCTTAAGC GTTCTCACGA TTCGTTTCGCT GCCCTTCTTC AAGAGTCTTC TGATTCTAAT	600
TCTCATTCGA AATCCTCTAC AGTTAATGAA TTGCTTGACA TGACATTCAT TGTCTCATGG	660
TTTTGGCTTT TTGGCTTTTG TCTTTTAAAG CTATATCAAC TTTACATATA AATATACGTC	720
AAAAGGGGAT TCATTAATTA GAAAATTCTC TTTTCAATA GTTGCTATTC ATTATCAATC	780
TATTCAACTC AATTGGTTAT TATTTTCATC TTTTGTGTCAT CCTAAACCAT CAACAATATT	840
TAATATATAT TGTTGTCTACA TTAAGAGTTA CTTCAGAAAT AACAAAAAAA TCGATCAAGA	900
ATTAATAAAA AGTCGAC	917

(2) INFORMATION FOR SEQ ID NO: 3:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 33 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:  
 GGGGAATTCGT CGACTTAACT CTAGCGACCT GTC 33
- (2) INFORMATION FOR SEQ ID NO: 4:
- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 36 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:  
 GGGGAATCAA GCTTTAATTC TTGATCGATT TTTTIG 36
- (2) INFORMATION FOR SEQ ID NO: 5:
- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 31 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:  
 CATGTCGACT TTTTATTAAT TCTTGATCGA T 31
- (2) INFORMATION FOR SEQ ID NO: 6:
- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 26 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:  
 ATGAAGCTTA AATCTTCATC CTTGGC 26
- (2) INFORMATION FOR SEQ ID NO: 7:
- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 53 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:  
 GGGTGAGGTT CTGTGGGGCG AGCGACAGGT CGCTAGAGTT AAGCATCCTG ATC 53
- (2) INFORMATION FOR SEQ ID NO: 8:
- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 53 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GATCAGGATG CTTAACTCTA GCGACCTGTC GCTCGCCCCA CAGAACCTCA CCC

53

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 30 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAAATGCATA AGCTCTTGCC ATTCTCACCG

30

We claim:

1. An isolated and purified DNA [sequence] selected from the group consisting of:

(a) the [sequence] DNA presented in FIG. 1 (SEQ ID NO: 1);

(b) a derivative of the [sequence] DNA of (a), wherein the derivative comprises at least nucleotides 1787 to 2242 of SEQ ID NO: 1; and

(c) a fragment of the [sequence] DNA of (a), wherein the fragment comprises at least nucleotides 1787 to 2242 of SEQ ID NO: 1;

wherein said DNA [sequence] possesses transcriptional promoter activity.

2. A recombinantly prepared DNA comprising a DNA [sequence] according to claim 1.

3. A recombinantly prepared DNA according to claim 2, further comprising one or more structural genes.

4. A recombinantly prepared DNA according to claim 3, wherein said one or more structural genes is human serum albumin.

5. A recombinantly prepared DNA according to claim 3, further comprising signals permitting the secretion of the expression product of said structural gene(s).

6. A recombinantly prepared DNA according to claim 3, wherein said [sequence] DNA is a bidirectional promoter and [said genes are] a first structural gene is inserted on [each] one side of the promoter and a second structural gene is inserted on the other side of the promoter, in the 2 opposite orientations, provided that said first structural gene and said second structural gene are different.

7. An autonomous or integrative expression plasmid comprising the recombinantly prepared DNA according to claim 3.

8. A recombinantly prepared cell containing a DNA [sequence] according to claim 1.

9. The recombinantly prepared cell according to claim 8, characterized in that it is a yeast cell.

10. The recombinantly prepared cell according to claim 9, characterized in that said yeast cell is the Kluyveromyces genus.

11. A process for the preparation of a recombinant protein by expression of [the] a first gene encoding said protein in a cellular host comprising expression of the said gene under the transcriptional control of a [sequence] DNA according to claim 1.

12. A process according to claim 11, characterized in that the protein is human serum albumin.

13. The process of claim 11, further comprising simultaneous expression of [isolated and purified genes] a second gene, wherein said [sequence] DNA is a bidirectional promoter and [said genes are] said first gene is inserted on [each] one side of the promoter and said second gene is inserted on the other side of the promoter, in the 2 opposite orientations, provided that said first and second genes are different.

14. An isolated and purified DNA [sequence] according to claim 1, comprising [all or part of] the [sequence] DNA presented in FIG. 6(b) (SEQ ID NO: 2).

15. A recombinantly prepared DNA comprising a DNA [sequence] according to claim 14.

16. A recombinantly prepared DNA according to claim 15, further comprising, one or more structural genes.

17. A recombinantly prepared DNA according to claim 16, further comprising signals permitting the secretion of the expression product of the said structural gene(s).

18. An autonomous or integrative expression plasmid comprising the recombinantly prepared DNA according to claim 16.

19. A recombinantly prepared cell containing a DNA [sequence] according to claim 14.

20. The recombinantly prepared cell according to claim 19, characterized in that it is a yeast cell.

21. The recombinantly prepared cell according to claim 20, wherein said yeast cell is of the Kluyveromyces genus.

22. A process for the preparation of a recombinant protein comprising expression of [the] a first gene encoding said protein in a cellular host wherein the expression of said gene is under the transcriptional control of a [sequence] DNA according to claim 14.

23. The process of claim 22, further comprising simultaneous expression of [isolated and purified genes] a second gene, wherein said [sequence] DNA is a bidirectional promoter and [said genes are] said first gene is inserted on [each] one side of the promoter and said second gene is inserted on the other side of the promoter, in the 2 opposite orientations, provided that said first and second genes are different.

\* \* \* \* \*

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : RE 40,940 E  
APPLICATION NO. : 09/790512  
DATED : October 20, 2009  
INVENTOR(S) : Fleer et al.

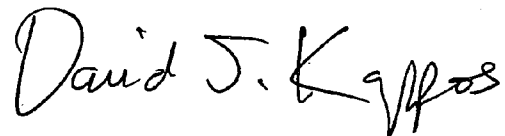
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It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, item (57), line 2, "activity." should read --activity,--.

Signed and Sealed this

Twenty-second Day of December, 2009

A handwritten signature in black ink that reads "David J. Kappos". The signature is written in a cursive, slightly slanted style.

David J. Kappos  
*Director of the United States Patent and Trademark Office*