Fig.1

Pre	Pro	pre-albumin																		pro region				
HSA	HSA	Met	Lys	Trp	Val	Thr	Phe	Ile	Ser	Leu	Leu	Phe	Leu	Phe	Ser	Ser	Ala	Tyr	Ser	Arg	Gly	Val	Phe	Arg	Arg
HSA	MFα-1	Met	Lys	Trp	Val	Thr	Phe	Ile	Ser	Leu	Leu	Phe	Leu	Phe	Ser	Ser	Ala	Tyr	Ser	Arg	Ser	Leu	Asp	Lys	Arg
		-24	-23	-22	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1
Preferred mutations of the invention:							Phe	Ile	Val		Ile														

Fig.2

Standard genetic code

	T	C	A	G
T	TTT Phe (F) TTC Phe (F) TTA Leu (L) TTG Leu (L)	TCT Ser (S) TCC Ser (S) TCA Ser (S) TCG Ser (S)	TAT Tyr (Y) TAC Tyr (Y) TAA **Ter** TAG **Ter**	TGT Cys (C) TGC Cys (C) TGA **Ter** TGG Trp (W)
C	CTT Leu (L) CTC Leu (L) CTA Leu (L) CTG Leu (L)	CCT Pro (P) CCC Pro (P) CCA Pro (P) CCG Pro (P)	CAT His (H) CAC His (H) CAA Gln (Q) CAG Gln (Q)	CGT Arg (R) CGC Arg (R) CGA Arg (R) CGG Arg (R)
A	ATT Ile (I) ATC Ile (I) ATA Ile (I) **ATG** Met (M)	ACT Thr (T) ACC Thr (T) ACA Thr (T) ACG Thr (T)	AAT Asn (N) AAC Asn (N) AAA Lys (K) AAG Lys (K)	AGT Ser (S) AGC Ser (S) AGA Arg (R) AGG Arg (R)
G	GTT Val (V) GTC Val (V) GTA Val (V) GTG Val (V)	GCT Ala (A) GCC Ala (A) GCA Ala (A) GCG Ala (A)	GAT Asp (D) GAC Asp (D) GAA Glu (E) GAG Glu (E)	GGT Gly (G) GGC Gly (G) GGA Gly (G) GGG Gly (G)

Single letter code:

A = adenosine

C = cytidine

G = guanosine

T = thymidine

B = C or G or T

D = A or G or T

H = A or C or T

K = G or T

M = A or C

N = A or C or G or T

R = A or G

S = C or G

V = A or C or G

W = A or T

Y = C or T

Fig.3

Modified list of preferred yeast codons

	T	C	A	G
T	TTC Phe (F) TTG Leu (L)	TCT Ser (S) TCC Ser (S)	TAC Tyr (Y) TAA **Ter**	TGT Cys (C) TGG Trp (W)
C		CCA Pro (P)	CAT His (H) CAA Gln (Q)	
A	ATT Ile (I) ATC Ile (I) **ATG** Met (M)	ACT Thr (T) ACC Thr (T)	AAC Asn (N) AAG Lys (K)	AGA Arg (R)
G	GTT Val (V) GTC Val (V)	GCT Ala (A)	GAT Asp (D) GAC Asp (D) GAA Glu (E)	GGT Gly (G)

Fig.4

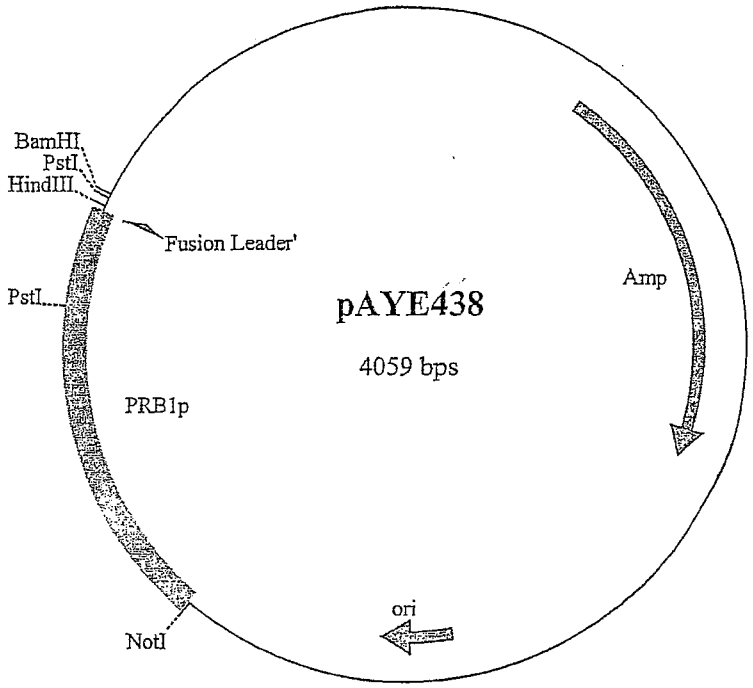

BamHI
PstI
HindIII
Fusion Leader'
PstI
pAYE438
4059 bps
Amp
PRB1p
ori
NotI

Fig. 5

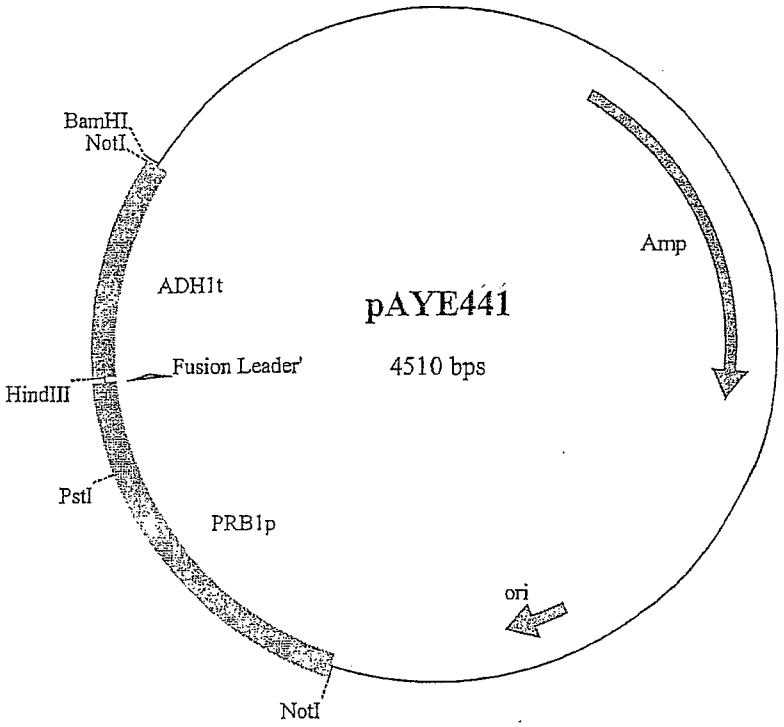

BamHI
NotI
ADH1t
Fusion Leader'
HindIII
PstI
PRB1p
NotI
pAYE441
4510 bps
Amp
ori

Fig.6

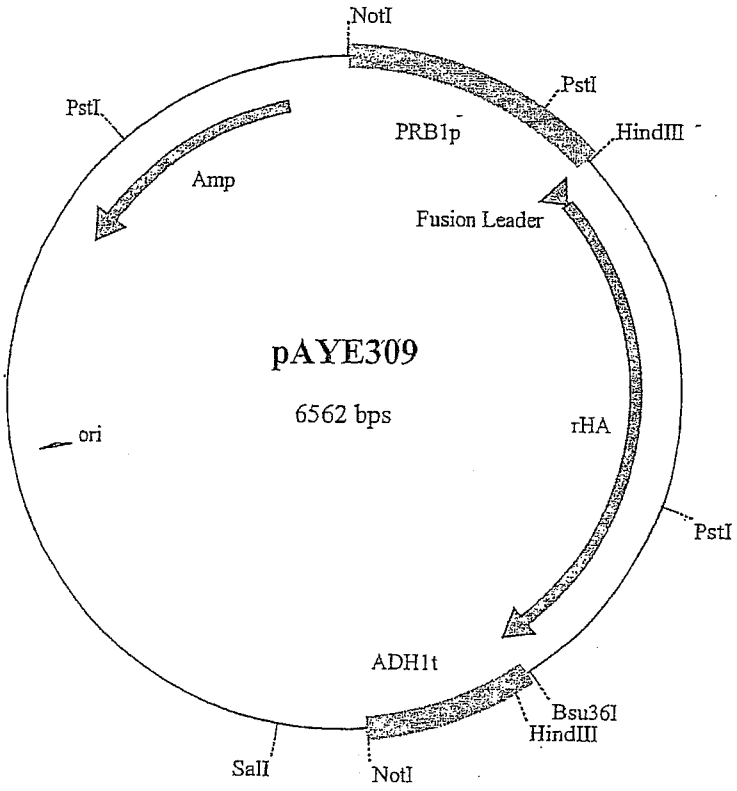
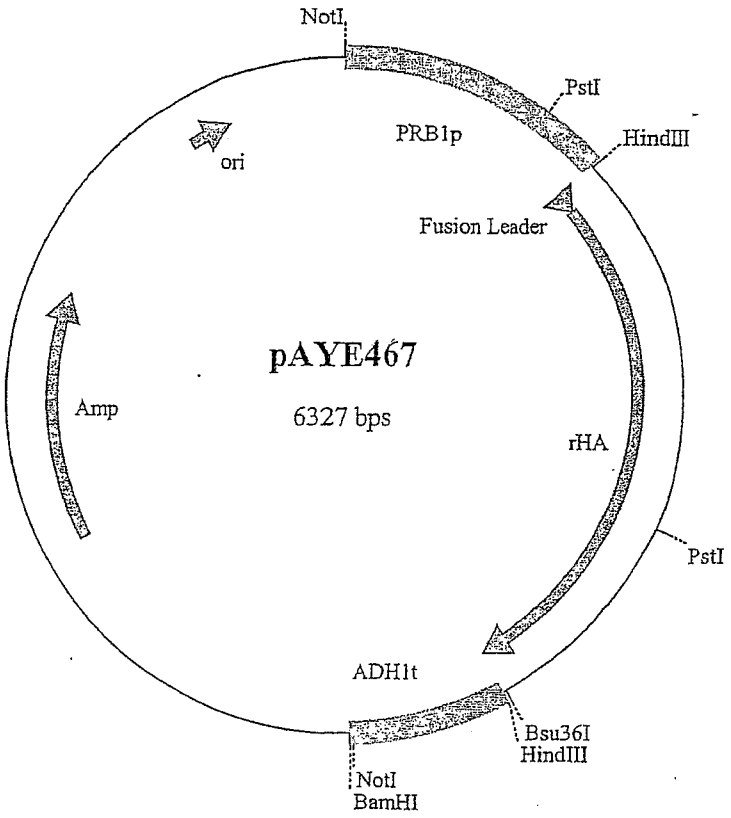

NotI
PstI
PstI
PRB1p
HindIII
Amp
Fusion Leader
pAYE309
6562 bps
rHA
ori
PstI
ADH1t
Bsu36I
HindIII
SalI
NotI

Fig. 7


NotI
PstI
PRB1p
HindIII
ori
Fusion Leader
pAYE467
6327 bps
Amp
rHA
PstI
ADH1t
Bsu36I
HindIII
NotI
BamHI

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Fig. 8

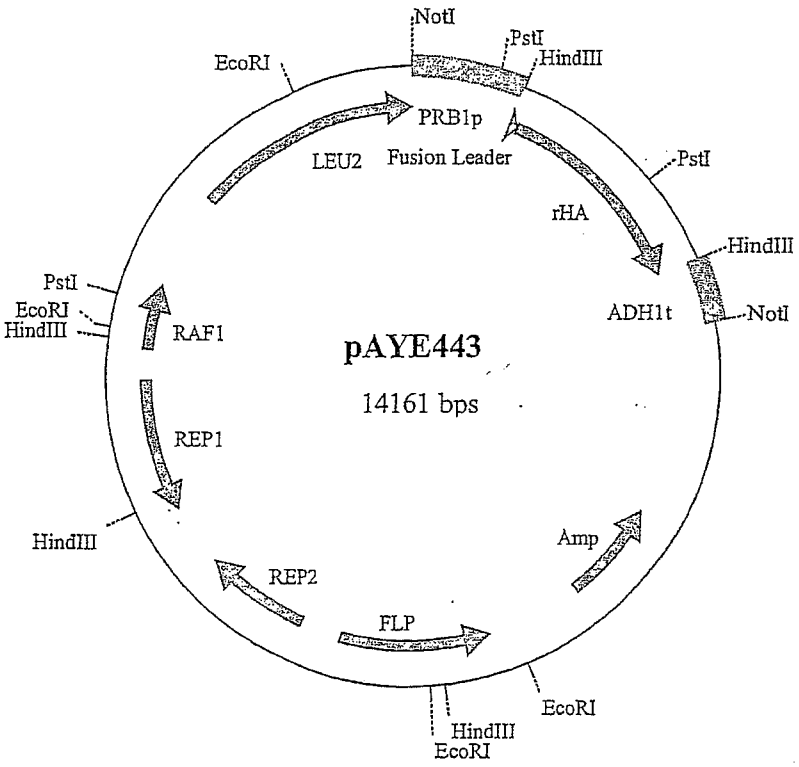


Fig.9

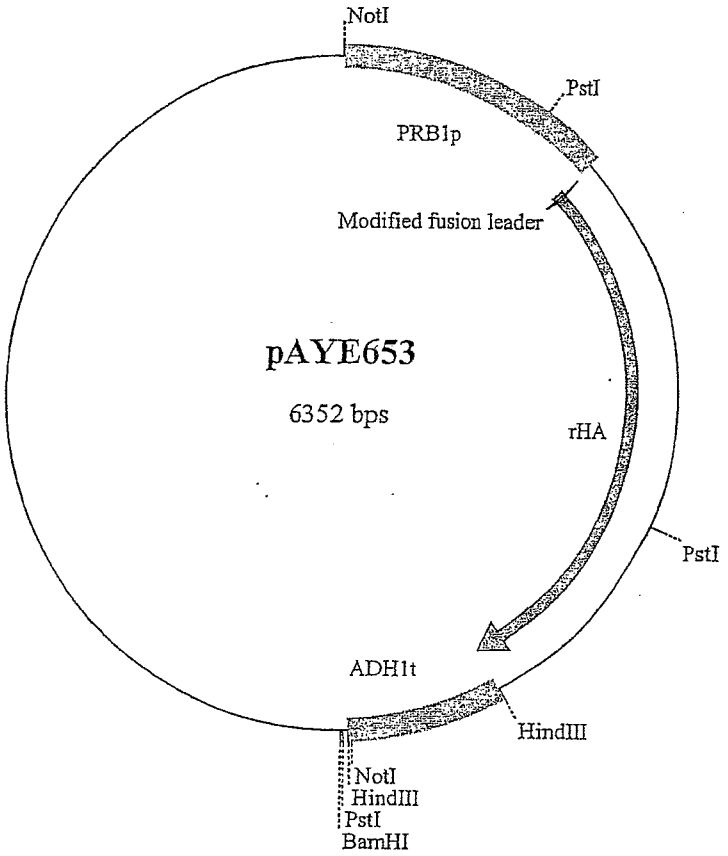

NotI
PstI
PRB1p
Modified fusion leader
pAYE653
6352 bps
rHA
PstI
ADH1t
HindIII
NotI
HindIII
PstI
BamHI

Fig.10

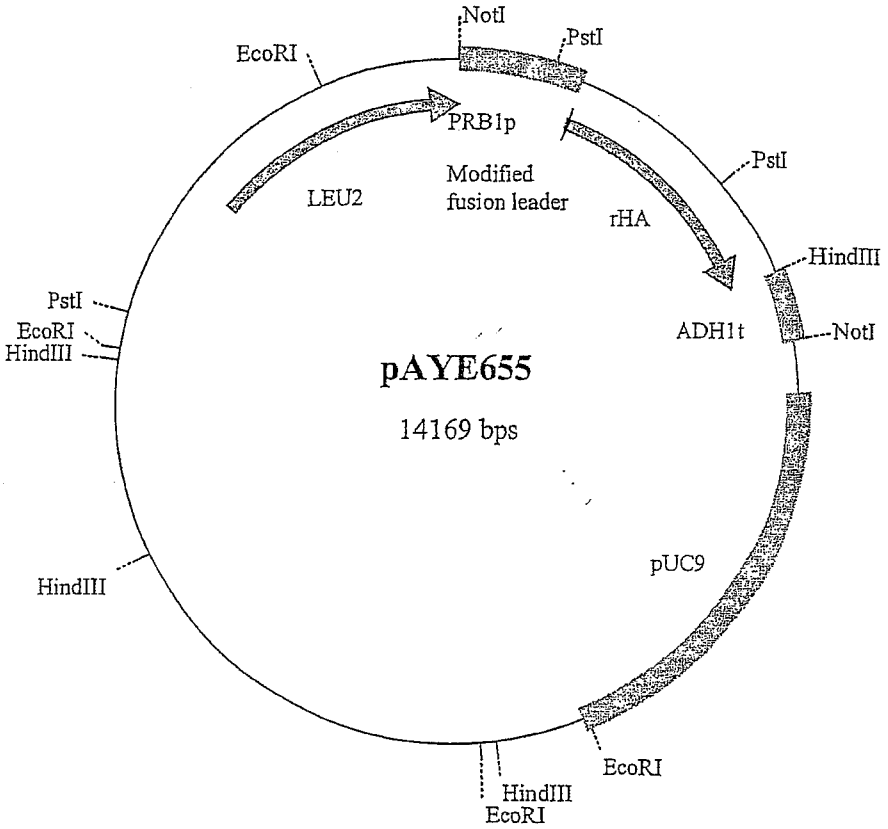

NotI
PstI
EcoRI
PRB1p
LEU2
Modified
fusion leader
rHA
PstI
HindIII
NotI
ADH1t
PstI
EcoRI
HindIII
pAYE655
14169 bps
pUC9
HindIII
EcoRI
HindIII
EcoRI

Fig.11

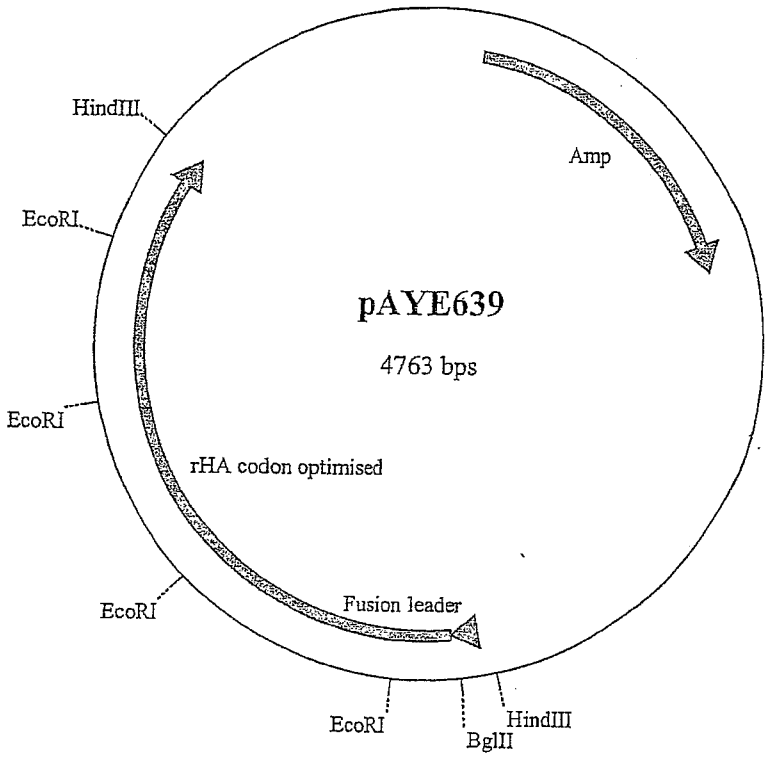

HindIII
EcoRI
EcoRI
EcoRI
EcoRI
BglII
HindIII
Amp
pAYE639
4763 bps
rHA codon optimised
Fusion leader

Fig.12

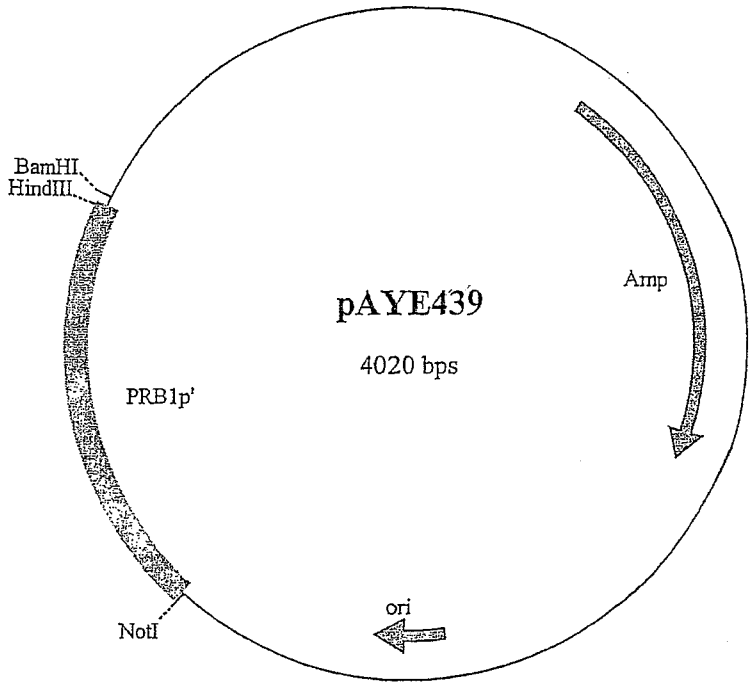


Fig.13

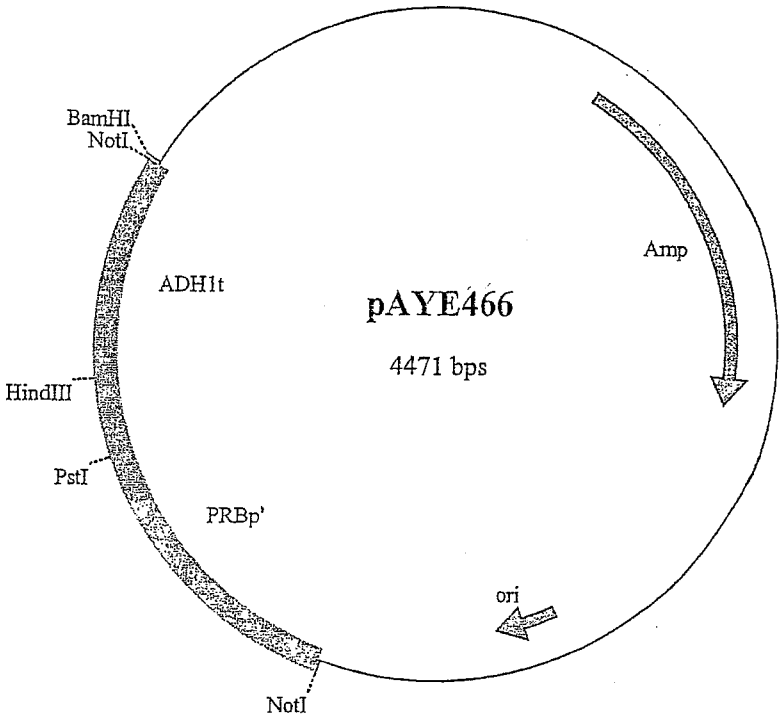

BamHI
NotI
ADH1t
pAYE466
4471 bps
Amp
HindIII
PstI
PRBp'
ori
NotI

Fig.14

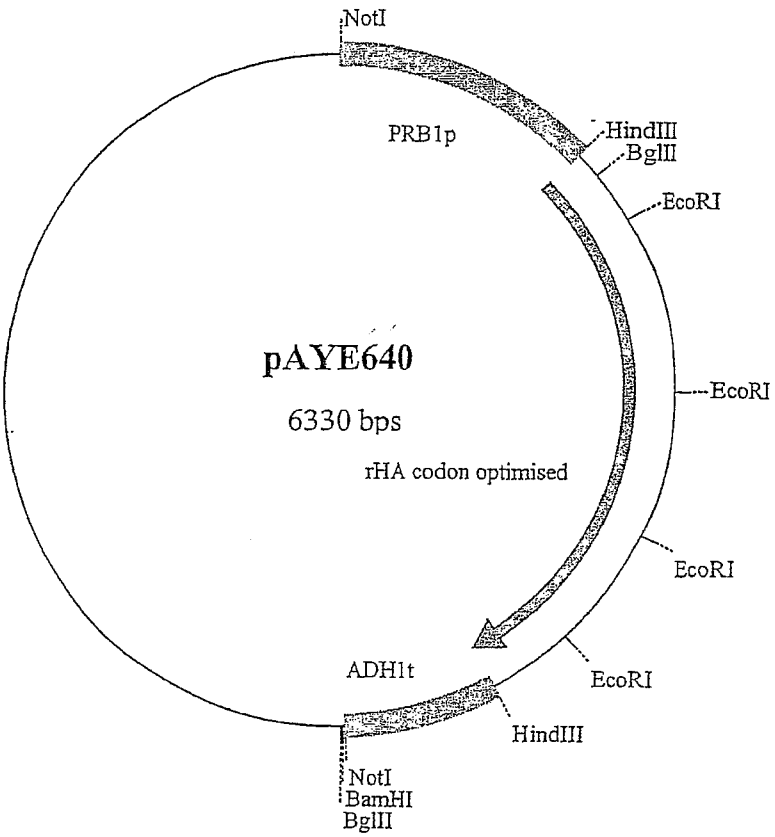
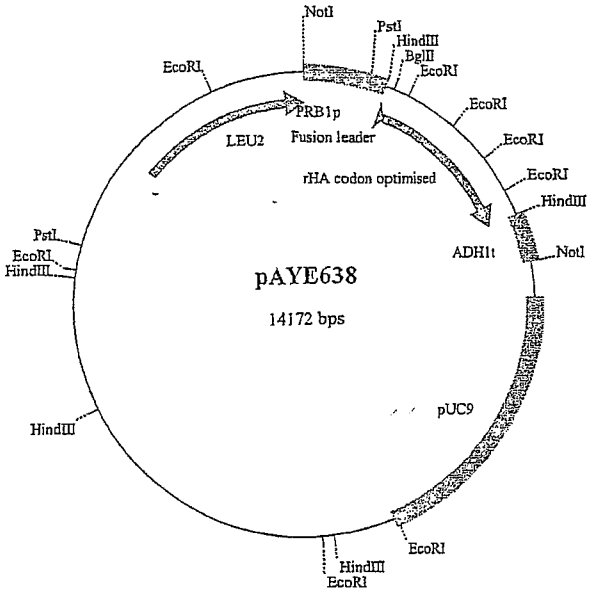

NotI
PRB1p
HindIII
BglII
EcoRI
pAYE640
6330 bps
rHA codon optimised
EcoRI
EcoRI
ADH1t
EcoRI
HindIII
NotI
BamHI
BglII

Fig.15


NotI
PstI
HindIII
BglII
EcoRI
EcoRI
EcoRI
EcoRI
EcoRI
HindIII
NotI
ADH1t
rHA codon optimised
Fusion leader
PRB1p
LEU2
pAYE638
14172 bps
pUC9
EcoRI
HindIII
EcoRI
HindIII
PstI
EcoRI
HindIII

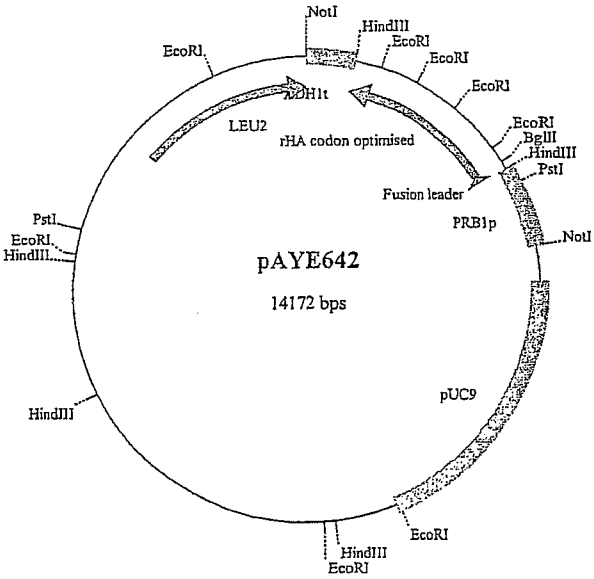

NotI
HindIII
EcoRI
EcoRI
EcoRI
EcoRI
EcoRI
BglII
HindIII
PstI
NotI
PRB1p
Fusion leader
rHA codon optimised
ADH1t
LEU2
pAYE642
14172 bps
pUC9
EcoRI
HindIII
EcoRI
HindIII
PstI
EcoRI
HindIII

Fig.16

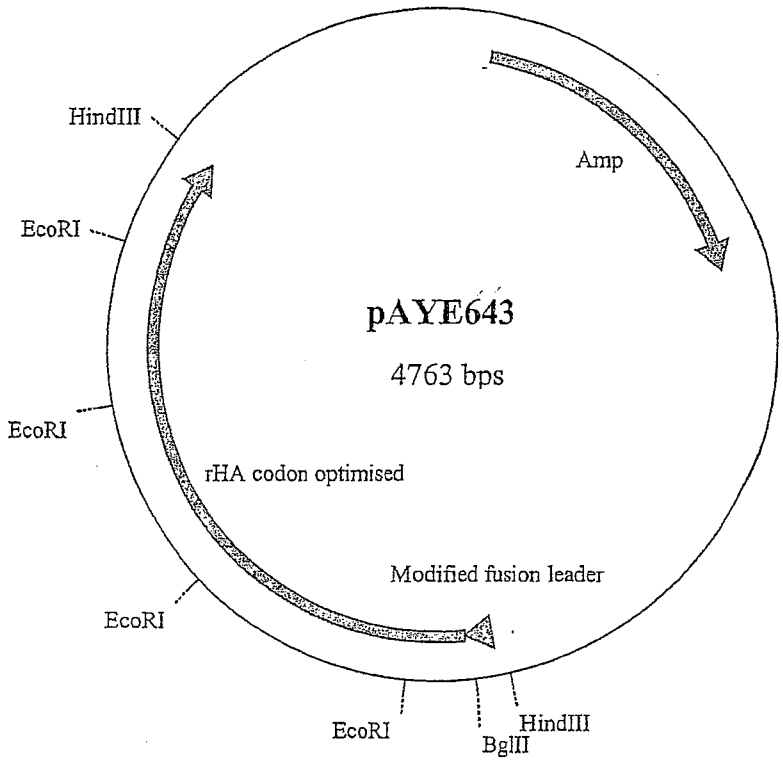

HindIII
EcoRI
EcoRI
EcoRI
EcoRI
HindIII
BglII
Amp
pAYE643
4763 bps
rHA codon optimised
Modified fusion leader

Fig.17

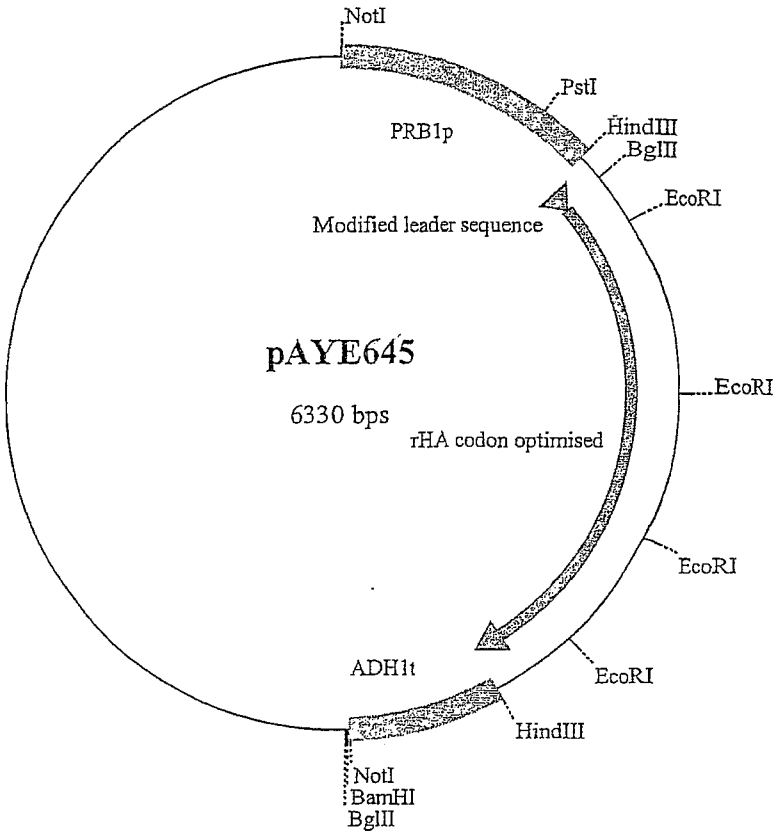
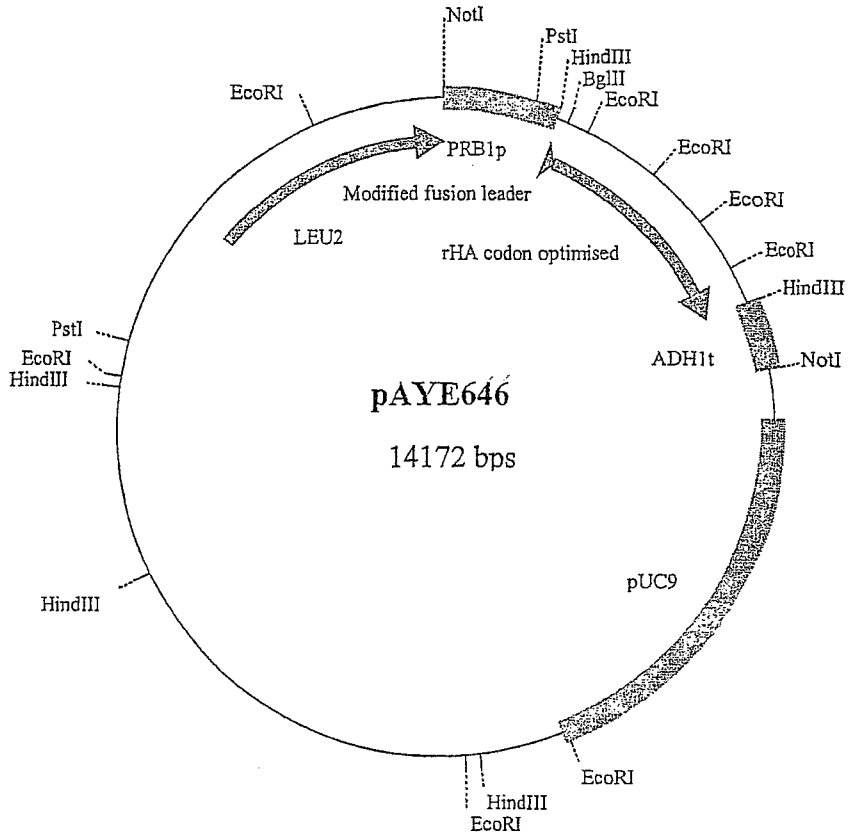
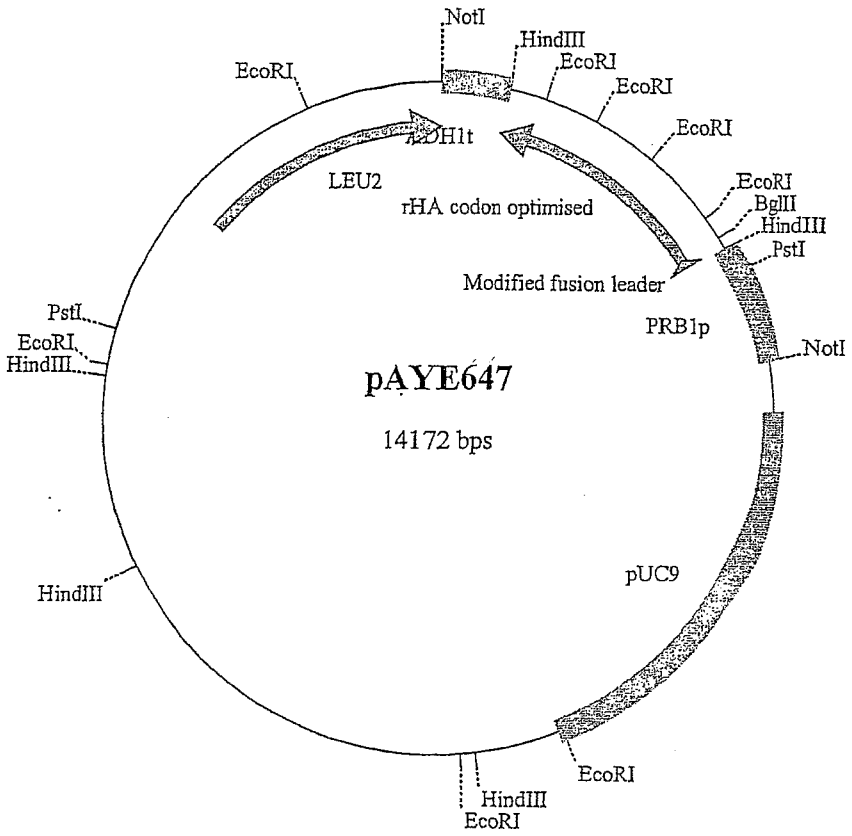

NotI
PstI
HindIII
BglII
PRB1p
EcoRI
Modified leader sequence
pAYE645
6330 bps
rHA codon optimised
EcoRI
EcoRI
ADH1t
EcoRI
HindIII
NotI
BamHI
BglII

Fig.18


NotI
PstI
HindIII
BglII
EcoRI
EcoRI
EcoRI
EcoRI
EcoRI
HindIII
NotI
ADH1t
pUC9
EcoRI
HindIII
EcoRI
HindIII
EcoRI
PstI
PstI
EcoRI
HindIII
LEU2
PRB1p
Modified fusion leader
rHA codon optimised
pAYE646
14172 bps

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Fig.19



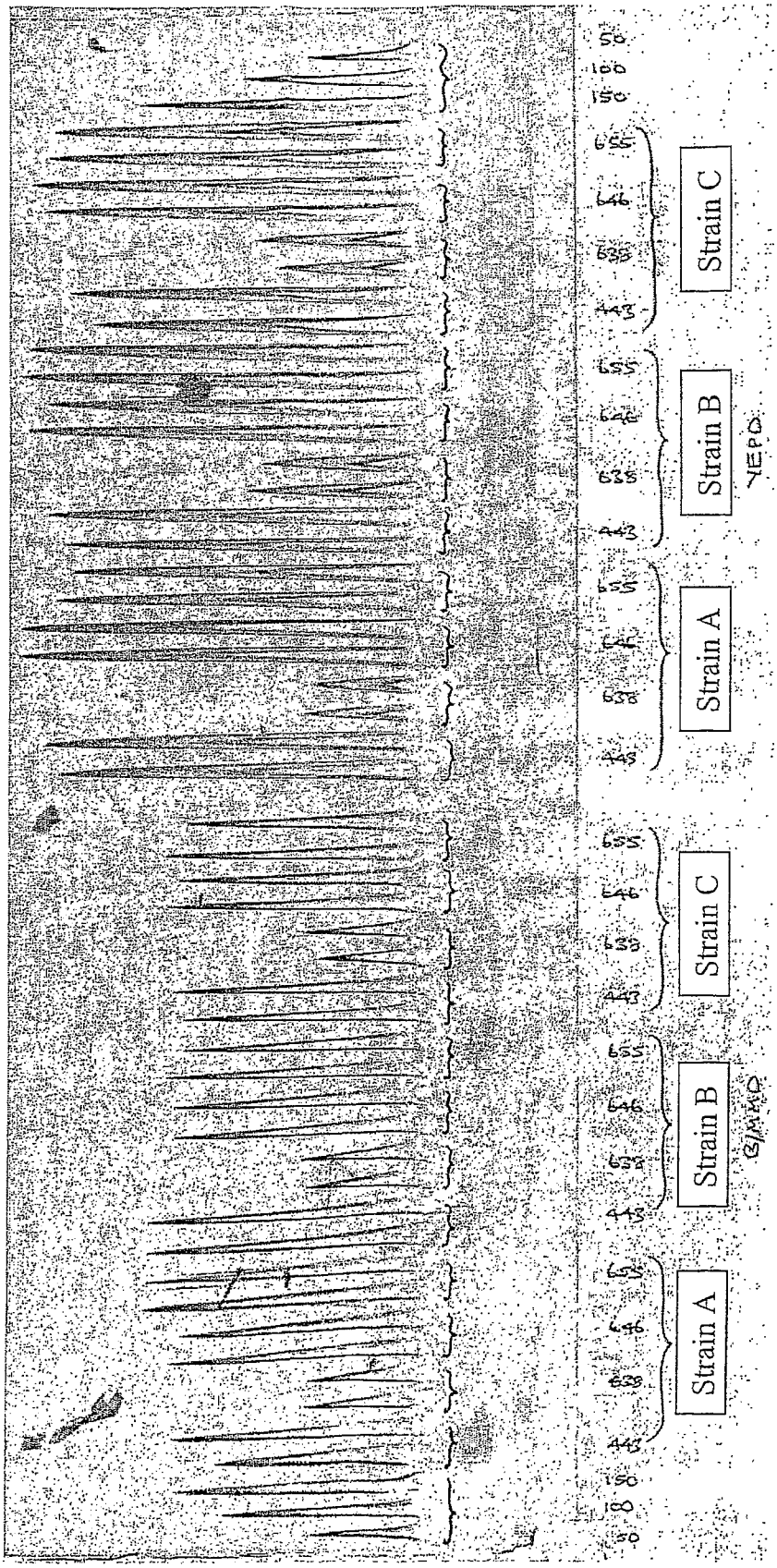


Fig.20

Fig.21

Strain	Plasmid	Yx/s (g/g)	Yp/s (mg/g)	rHA (g/L)
C	pAYE443 (1st feeds)	0.33	10.4	2.9
	(fill and draw)	0.34	11.5	3.2
	pAYE638 (1st feeds)	0.36	*	*
	(fill and draw)	0.36	2.4	0.7
	pAYE646 (1st feeds)	0.33	11.6	3.2
	(fill and draw)	0.35	12.2	3.5
	pAYE655 (1st feeds)	0.37	12.1	3.4
	(fill and draw)	0.35	13.0	3.7
B	pAYE443 (1st feeds)	0.35	10.5	2.8
	pAYE646 (1st feeds)	0.35	13.0	3.5
	(fill and draw)	0.33	12.8	3.6

Fig.22

Plasmid designation	Leader	Desired Protein
pAYE443 pAYE467	HSA/MFα-1 natural codon bias	HSA natural codon bias
pAYE655 pAYE643	modified HSA/MFα-1 FIVSI fully codon biased the rest has natural codon bias	HSA natural codon bias
pAYE638 PAYE639 pAYE640 pAYE642	HSA/MFα-1 all fully codon biased	HSA fully codon biased
pAYE645 pAYE646 pAYE647	modified HSA/MFα-1 all fully codon biased	HSA fully codon biased