Appendices

APPENDIX A

List of Amino Acids and Their Abbreviations

Nonpolar Amino Acids (hydrophobic)

amino acid	three letter code	single letter code
glycine	Gly	G
alanine	Ala	A
valine	Val	V
leucine	Leu	L
isoleucine	Ile	I
methionine	Met	M
phenylalanine	Phe	F
tryptophan	Trp	W
proline	Pro	P

Polar (hydrophilic)

serine	Ser	S
threonine	Thr	T
cysteine	Cys	C
tyrosine	Tyr	Y
asparagine	Asn	N
glutamine	Gln	Q

Electrically Charged (negative and hydrophilic)

aspartic acid	Asp	D
glutamic acid	Glu	E

Electrically Charged (positive and hydrophilic)

lysine	Lys	K
arginine	Arg	R
histidine	His	H

The Standard Genetic Code

First Position (5' end)	Second Position				Third Position (3' end)
	U	C	A	G	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met Start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Start Codon
Stop Codon
Nonpolar Side Chain
Uncharged Polar Side Chain
Charged Polar Side Chain

From: http://www.cbs.umn.edu/~amundsen/chlamy/code.html

Nucleotide Base Codes
IUPAC

Symbol	Nucleotide
A	adenine
C	cytosine
G	guanine
T	Thymine - DNA Uracil - RNA

Single letter amino acids and wobble predictions for designing degenerate oligonucleotide primers

AA	Predicted 3' codon	AA	Predicted 3' codon
B	C, G, T	M	A, C
D	A, G, T	N	A, C, G, T
H	A, C, T	R	A, G
K	G, T	S	C, G
V	A, C, G	W	A, T
Y	C, T		

APPENDIX B

Composition of buffers and solutions

- **Agarose Diffussion Assay medium**

  100mM Citrate

  200mM Na_2HPO_4

  0.5% (w/v) Ammonium oxalate

  0.01% (w/v) Polygalcturonic acid (PGA)

  1% (w/v) TypeII agarose (Sigma)

  pH 5.3

- **Antibiotic Stock Solutions**

  Ampicillin

  Cefotaxime

  Kanamycin

  Rifampicin

- **Blocking Solution**

  1 x working solution : 1% (w/v) dissolved in Maleic acid buffer

- **Citrate/Phosphate Buffer**

  0.1M Citric acid

  0.2M Na_2HPO_4

  pH 5.3

- **Co-cultivation Medium**

  Composition per liter:

  4.42g MS salts (Highveld Biological)

  1ml 1000 X vitamin stock

  0.1mg NAA

  1mg BAP (6-Benzylaminopurine)

30g Sucrose

8g Agar

pH 5.8

- **Denaturation Solution**

  0.5M NaOH

  1.5M NaCl

- **Detection Buffer**

  0.1M Tris-HCl

  0.1M NaCl

  pH 9.5

- **0.5M EDTA**

  Dissolve 186.1g disodium-EDTA in 1 liter ddH_2O

  Adjust pH to 8.0

- **GUS Assay Solution**

  0.1% (w/v) X-Gluc

  0.01% (v/v) Tween 20

  10mM EDTA (pH8.0)

  50mM NaH_2PO_4

- **0.1M IPTG Stock**

  Dissolve 1.2 g isopropyl-β-D-thiogalactopyranoside in 50ml dH_2O. Filter sterilize and store at 4°C.

- **Luria Bertani (LB) Medium**

  Composition per litre:

  10g Bacto®-tryptone

  5g Bacto®-yeast extract

  5g NaCl

  Adjust pH to 7.0

- **LB Agar**

  Add 15g Bacto®agar to 1 liter of LB medium.

- **2M Mg^{2+} Stock**

  20.33g $MgCl_2.6H_2O$

  24.65g $MgSO_4.7H_2O$

  Add distilled water to 100ml.

  Filter-sterilize.

- **Maleic Acid Buffer**

  0.1M Maleic acid

  0.15M NaCl

  pH 7.5

- **2% Malt Extract Agar (MEA)**

  Composition per liter:

  20g Malt extract

  15g Bacto®Agar

- **Minimal Salts Medium**

  Composition per liter:

  2g NH_4NO_3

  1g KH_2PO_4

  0.1g $MgSO_4$

  0.5g yeast extract

  1g NaOH

  3g $_{DL}$-Malic acid

  Supplement with 0.5% sodium polygalacturonic acid (PGA)

- **MS (Murashige and Skoog) Medium for Tobacco**

  Composition per liter:

  4.42g MS Salts (Highveld Biological)

1ml 1000 x Vitamin stock
30g Sucrose
0.1g Myo-inositol
7g Agar
pH 5.8

- **Neutralising Solution**

  1M Tris-HCl pH 8.0
  1.5M NaCl
  10mM EDTA

- **PAHBAH**

  1% ρ-4-amino-2-hydroxybenzoic acid hydrazide
  0.4M NaOH
  0.1M HCl

- **RNase A (10mg/ml)**

  Dissolve 10mg RNase A in 1ml ddH_2O.
  Heat to 100°C for 10min. Allow cooling to room temperature.
  Store at -20°C.

- **Regeneration Medium**

  Composition per liter:
  4.42g MS salts (Highveld Biological)
  1ml 1000 x Vitamin stock
  1mg BAP (6-Benzylaminopurine)
  0.5mg IAA (Indole-3-Acetic Acid)
  30g Sucrose
  7g Agar
  pH 5.8

- **Ruthenium Red Solution**

  0.05% (w/v) in ddH_2O

- **10% SDS**

  Dissolve 100g electrophoresis grade SDS in 1 liter water.

  Heat to 68°C. Adjust pH to 7.2.

- **Solution 1**

  25mM Tris-HCl p H 8.0

  10mM EDTA

  50mM Glucose

- **Solution 2**

  0.2N NaOH

  1% SDS

- **Solution 3**

  3M Potassium Acetate p H4.8

- **SOC Medium**

  Composition per 100ml:

  2g Bacto®-tryptone

  0.5g Bacto®-yeast extract

  1ml 1M NaCl

  0.25ml 1M KCl

  1ml 2M Mg^{2+} stock, filter-sterilized

  1ml 2M Glucose, filter-sterilized

- **20 x SSC**

  0.3M NaCitrate

  1.5M NaCl

  pH 7

- **50 x TAE**

  Composition per liter:

  242g Tris

  57.1ml Glacial acetic acid

  100ml 0.5M EDTA pH 8.0

- **TE Buffer**

  10mM Tris-HCl pH 8.0

  1mM EDTA pH 8.0

- **1 x TNE Buffer**

  10mM Tris-HCl p H 8.0

  1mM EDTA pH 8.0

  0.2M NaCl

  pH 7.4

- **Washing Buffer**

  0.1M Maleic acid

  0.15M NaCl

  pH 7.5

  0.3% (v/v) Tween 20

- **2 x Washing Solution**

  2 x SSC

  0.1% SDS

- **0.5 x Washing Solution**

  0.5 x SSC

  0.1% SDS

- **X-gal (50mg/ml)**

  Dissolve 100mg 5-Bromo-4-chloro-3-indolyl-β-D-galactoside in 2ml N,N'-dimethylformamide. Cover with aluminum foil and store at -20°C.

- **YEP Medium**

  10g Bacto®-peptone

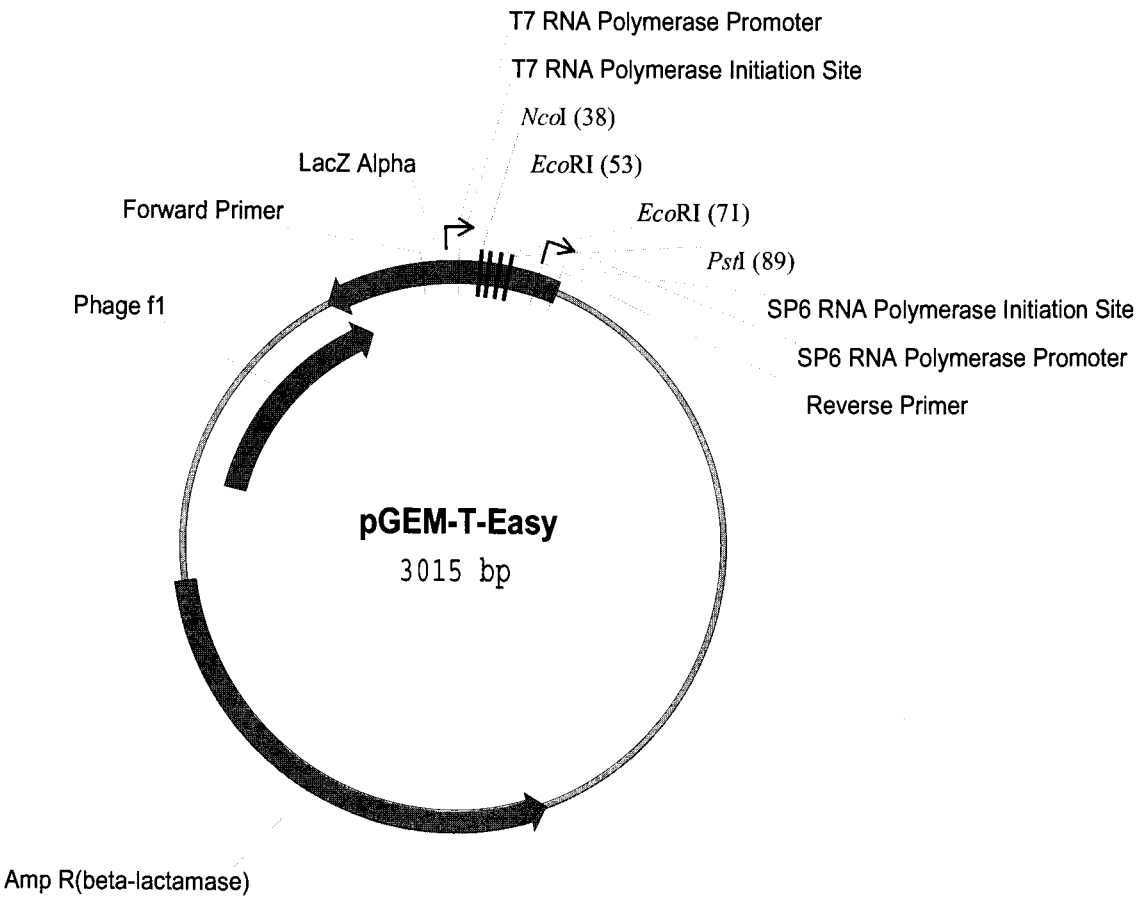
  10g Bacto®-yeast extract

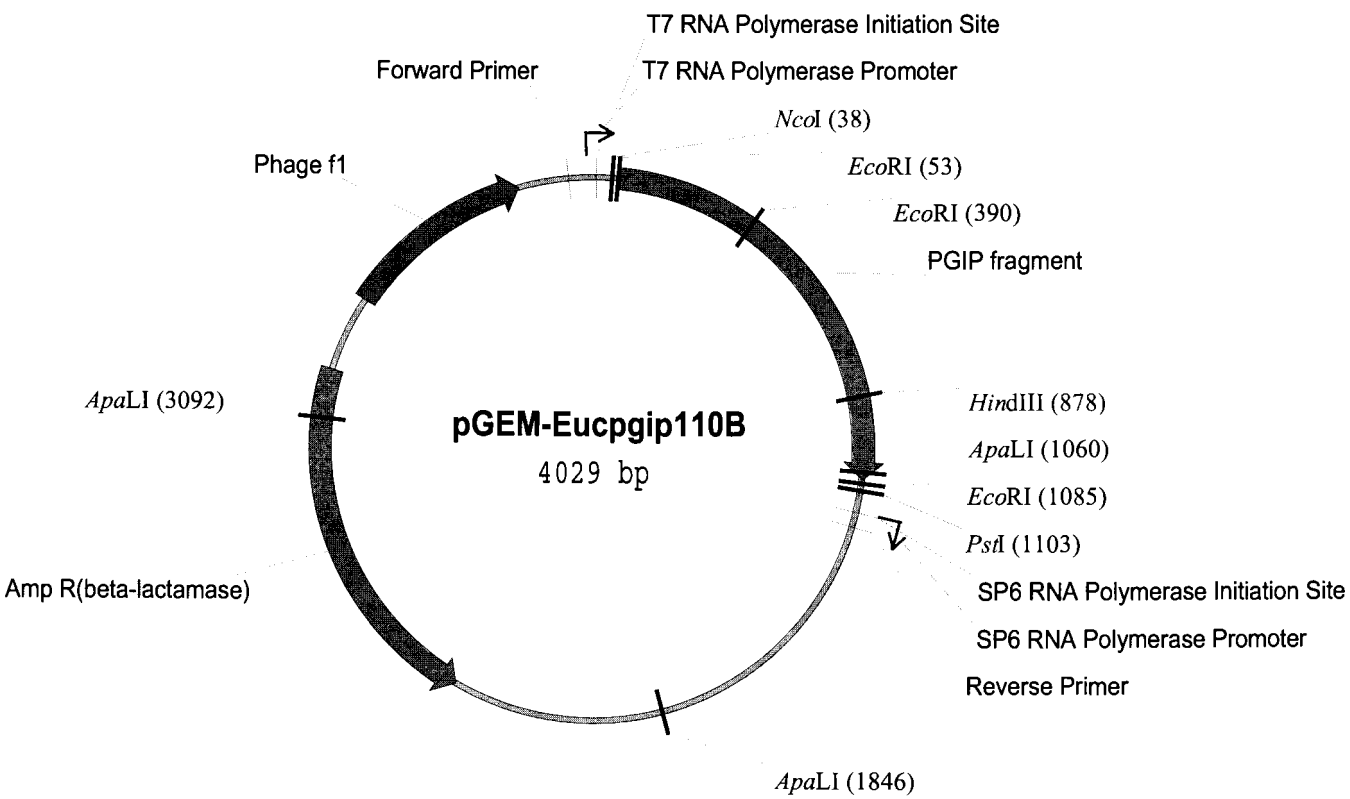
  5g NaCl

  Add 15g Bacto®-Agar for YEP-Agar

APPENDIX C

Plasmid Maps

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EcoRI (390)
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pGEM-Eucpgip110B
4029 bp
HindIII (878)
ApaLI (1060)
EcoRI (1085)
PstI (1103)
Amp R(beta-lactamase)
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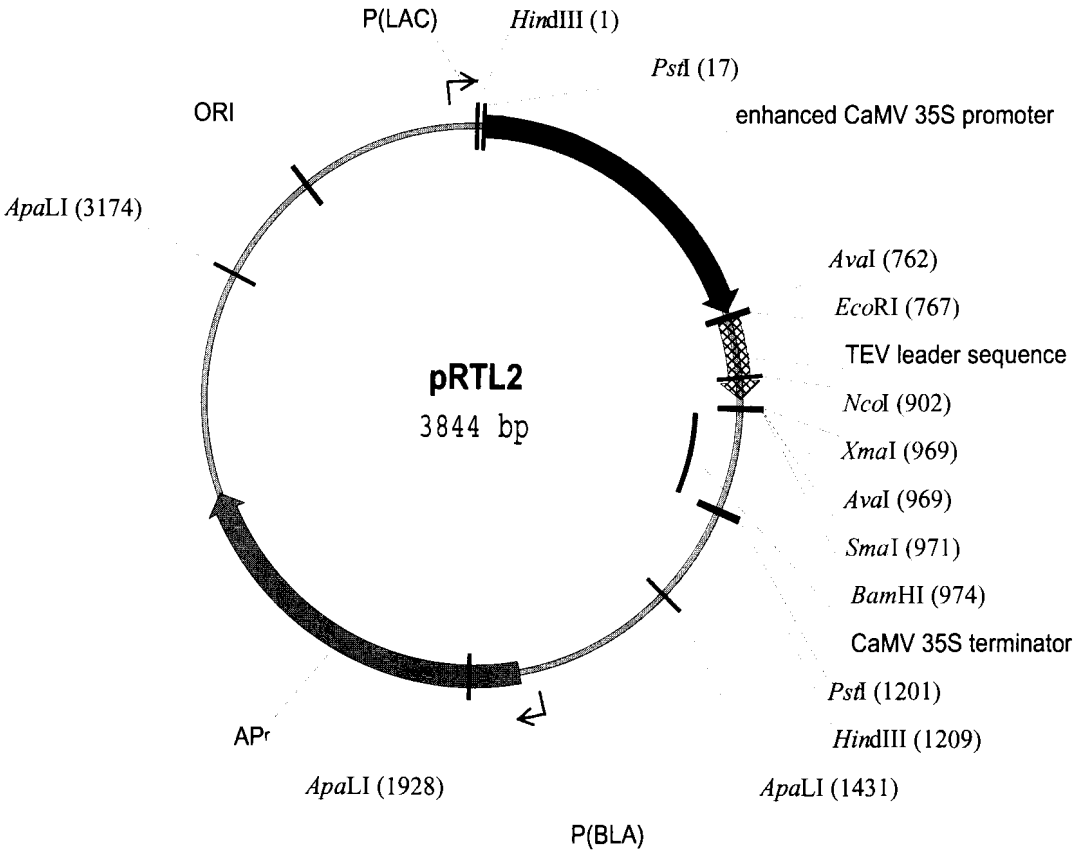
SP6 RNA Polymerase Promoter
Reverse Primer
ApaLI (1846)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AvaI (762)
EcoRI (767)
TEV leader sequence
NcoI (902)
XmaI (969)
AvaI (969)
SmaI (971)
BamHI (974)
CaMV 35S terminator
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HindIII (1209)
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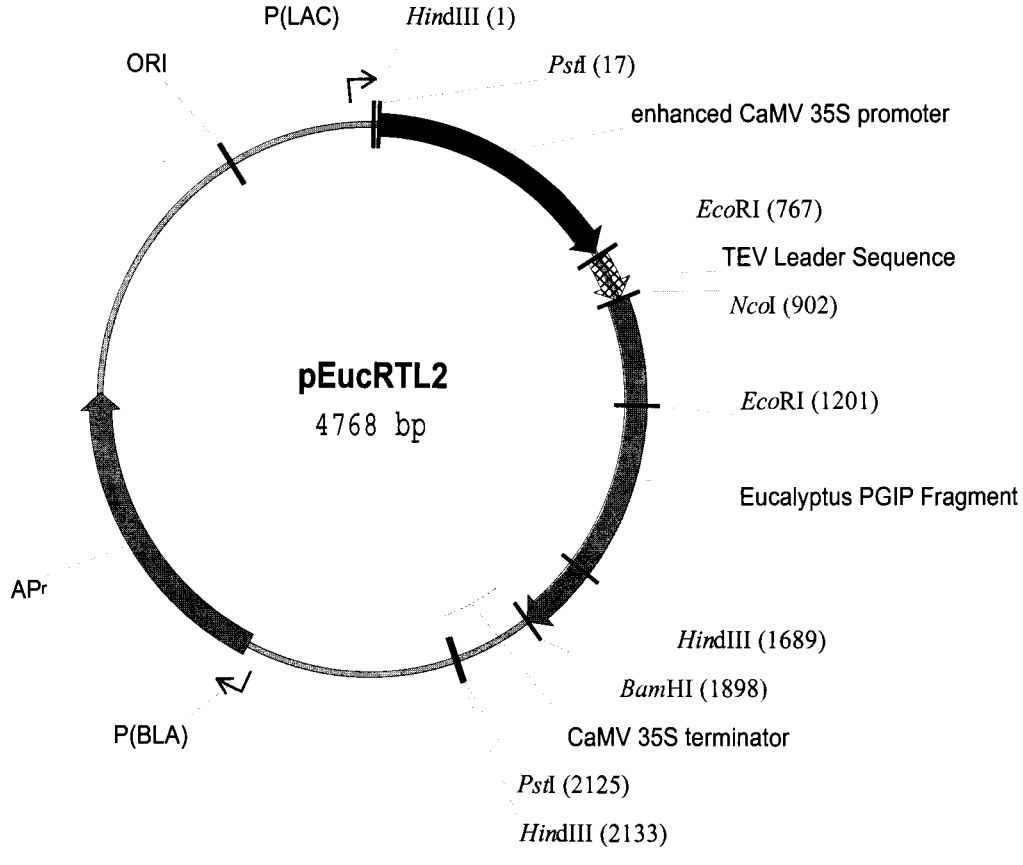
APr
ApaLI (1928)
ApaLI (1431)
P(BLA)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ORI
PstI (17)
enhanced CaMV 35S promoter
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TEV Leader Sequence
NcoI (902)
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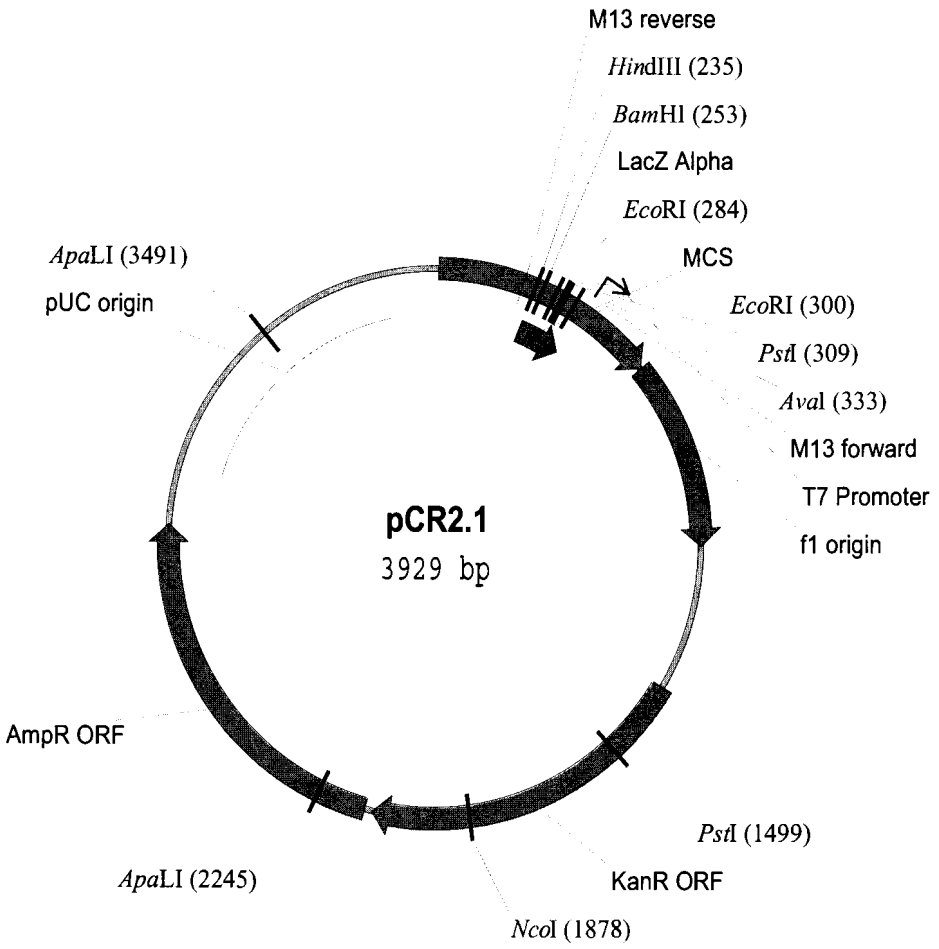
4768 bp
EcoRI (1201)
Eucalyptus PGIP Fragment
APr
HindIII (1689)
BamHI (1898)
P(BLA)
CaMV 35S terminator
PstI (2125)
HindIII (2133)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BamHI (253)
LacZ Alpha
EcoRI (284)
MCS
EcoRI (300)
PstI (309)
AvaI (333)
M13 forward
T7 Promoter
f1 origin
pCR2.1
3929 bp
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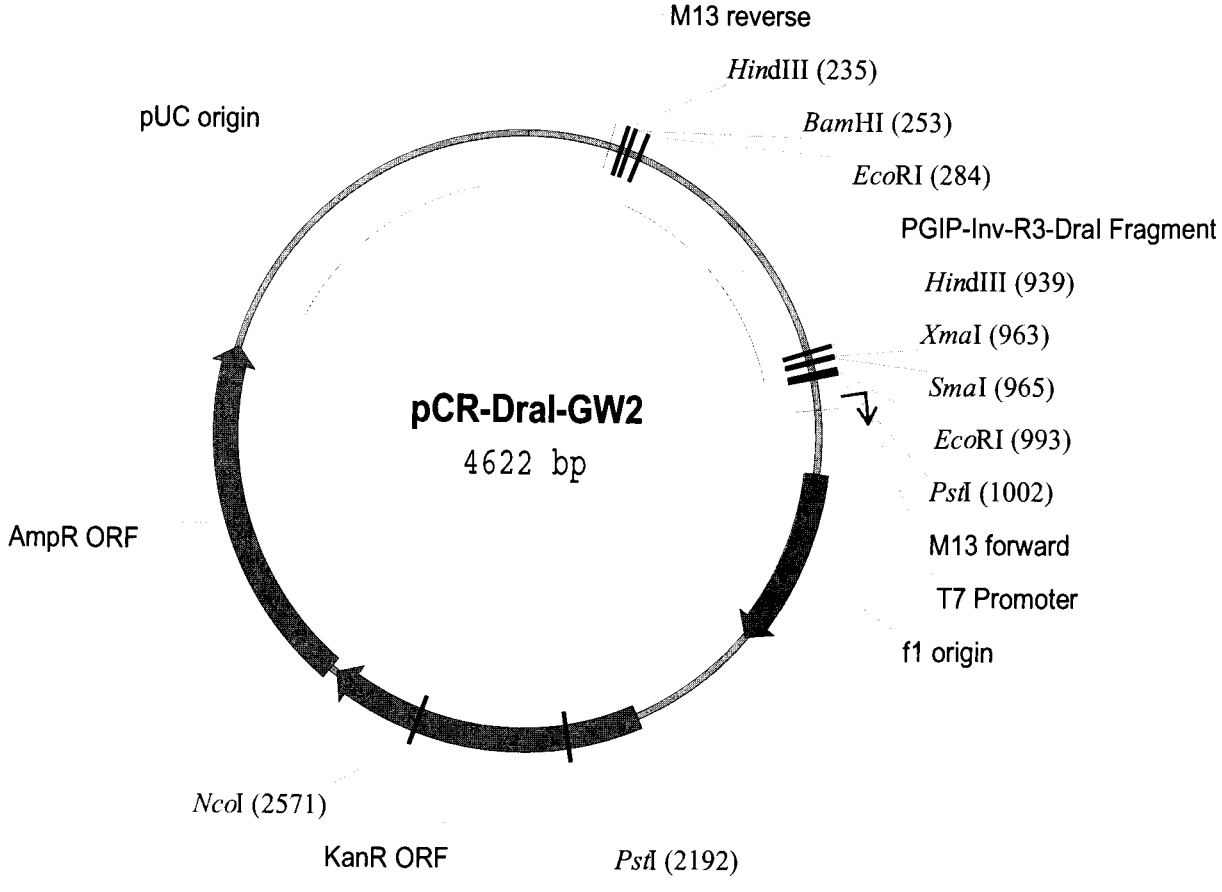
pUC origin
AmpR ORF
ApaLI (2245)
NcoI (1878)
KanR ORF
PstI (1499)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M13 forward
T7 Promoter
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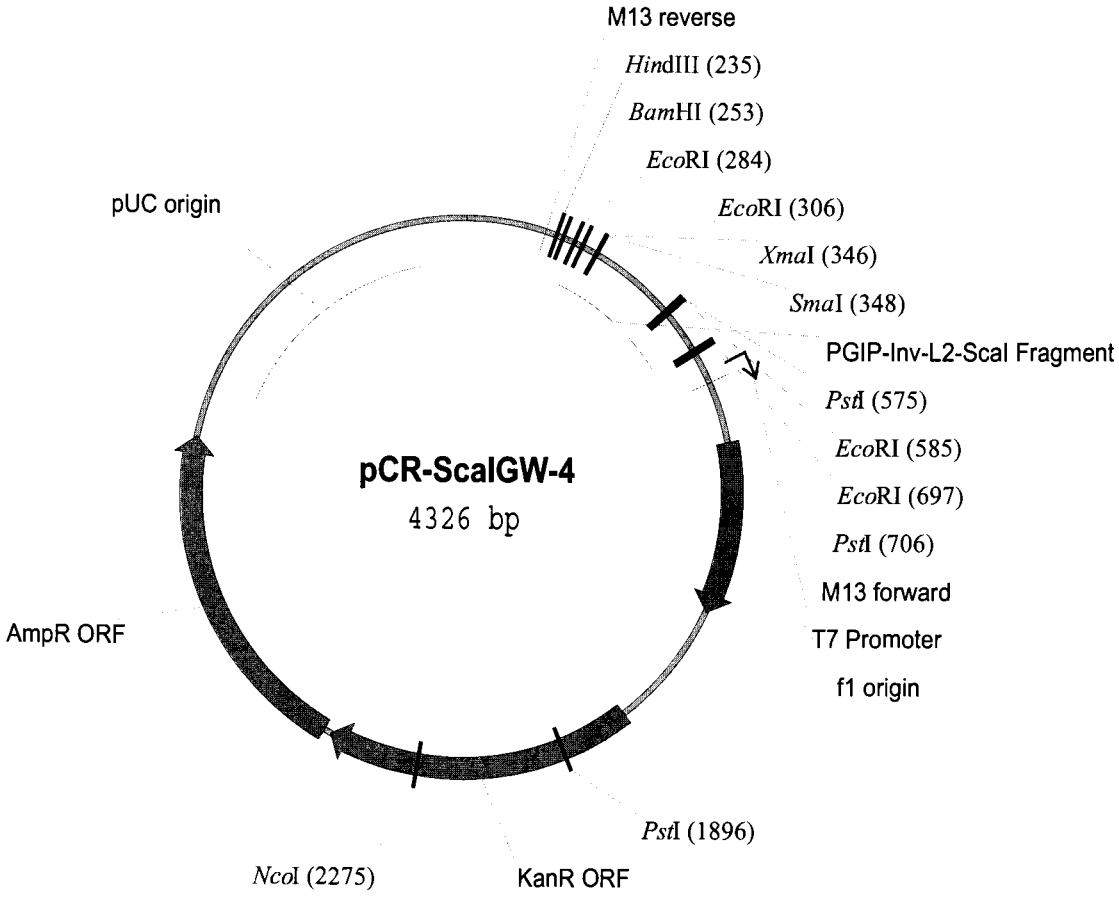
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PstI (706)
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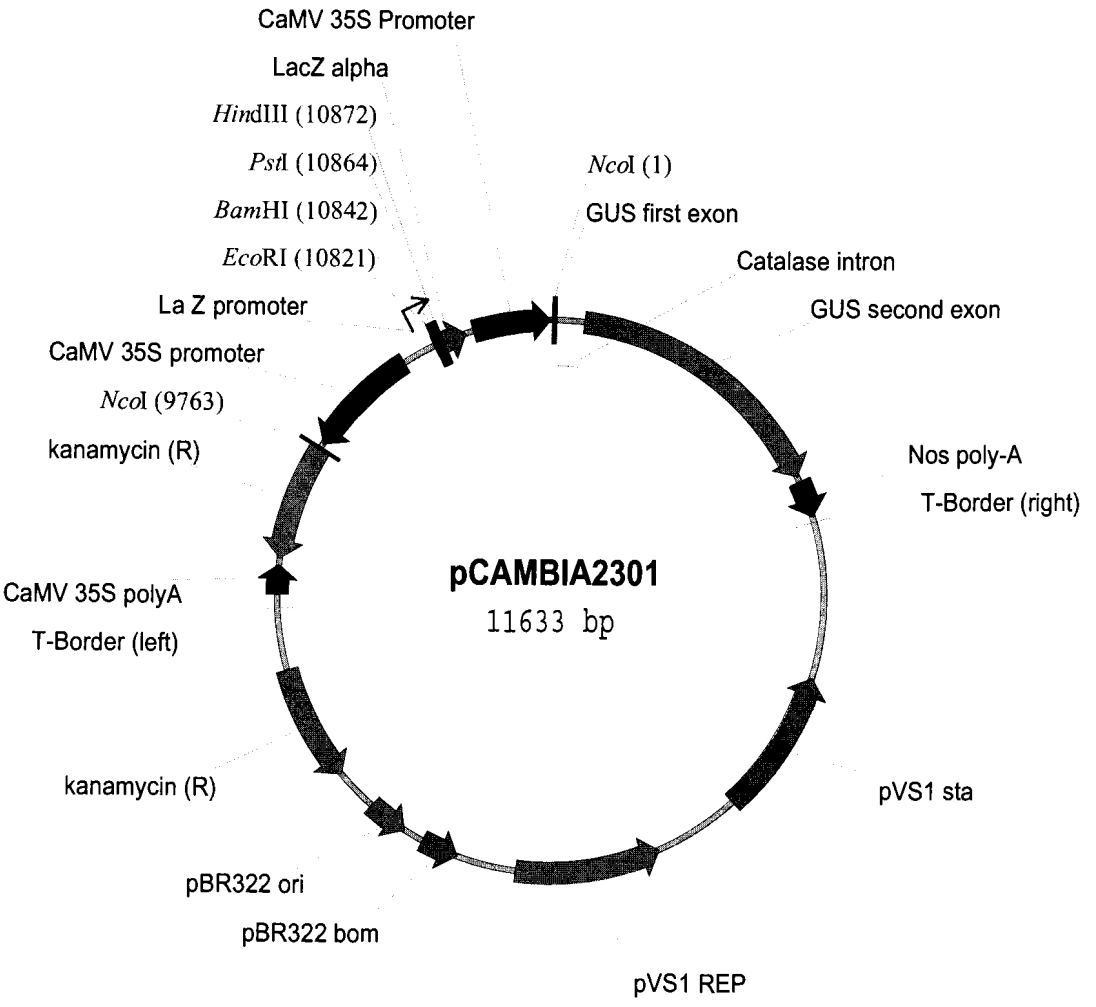
NcoI (2275)
KanR ORF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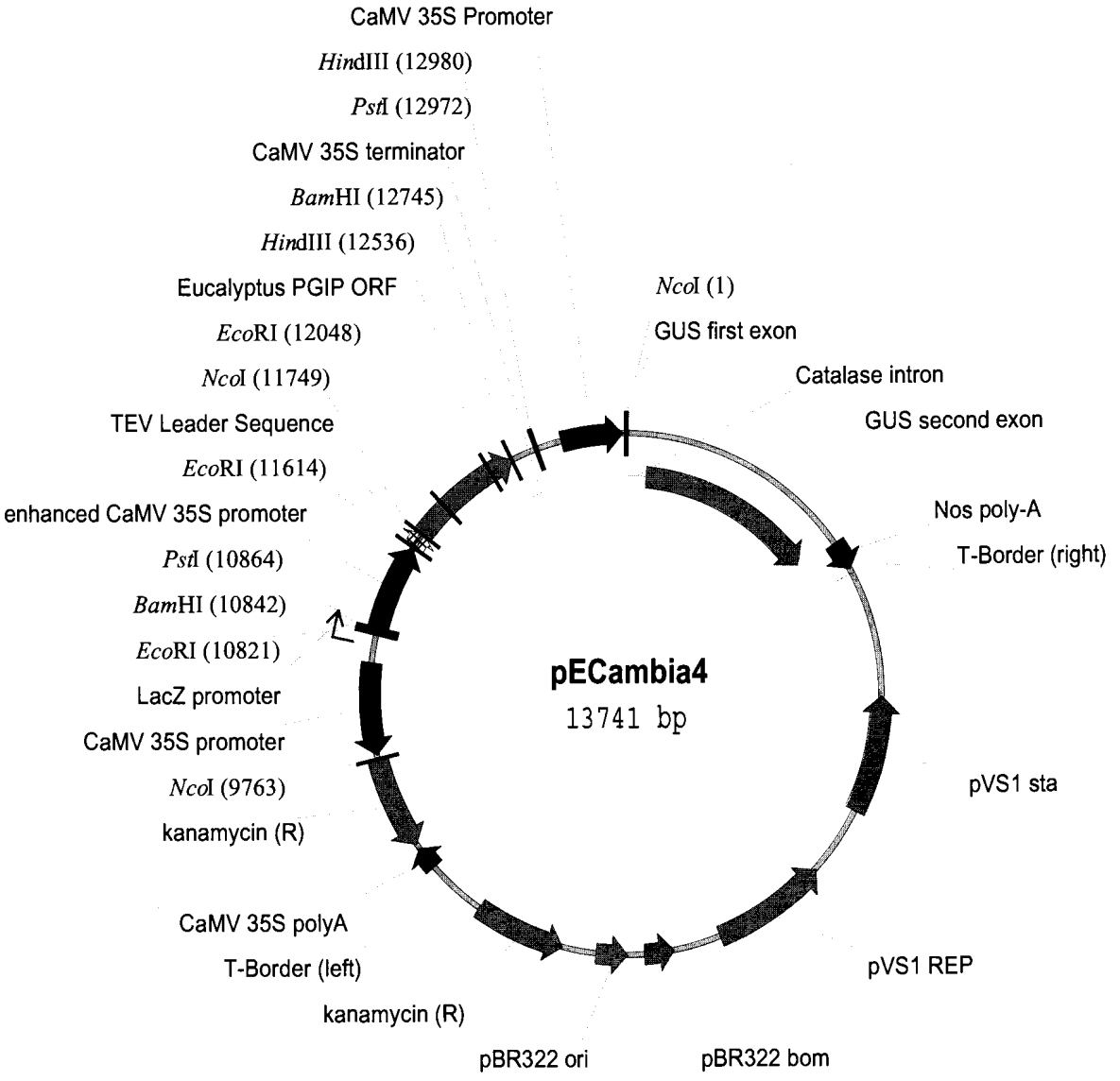
LacZ alpha
HindIII (10872)
PstI (10864)
BamHI (10842)
EcoRI (10821)
La Z promoter
CaMV 35S promoter
NcoI (9763)
kanamycin (R)
CaMV 35S polyA
T-Border (left)
kanamycin (R)
pBR322 ori
pBR322 bom
pVS1 REP
pVS1 sta
NcoI (1)
GUS first exon
Catalase intron
GUS second exon
Nos poly-A
T-Border (right)
pCAMBIA2301
11633 bp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HindIII (12536)
Eucalyptus PGIP ORF
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NcoI (11749)
TEV Leader Sequence
EcoRI (11614)
enhanced CaMV 35S promoter
PstI (10864)
BamHI (10842)
EcoRI (10821)
LacZ promoter
CaMV 35S promoter
NcoI (9763)
kanamycin (R)
CaMV 35S polyA
T-Border (left)
kanamycin (R)
pBR322 ori
pBR322 bom
pVS1 REP
pVS1 sta
Nos poly-A
T-Border (right)
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GUS first exon
Catalase intron
GUS second exon
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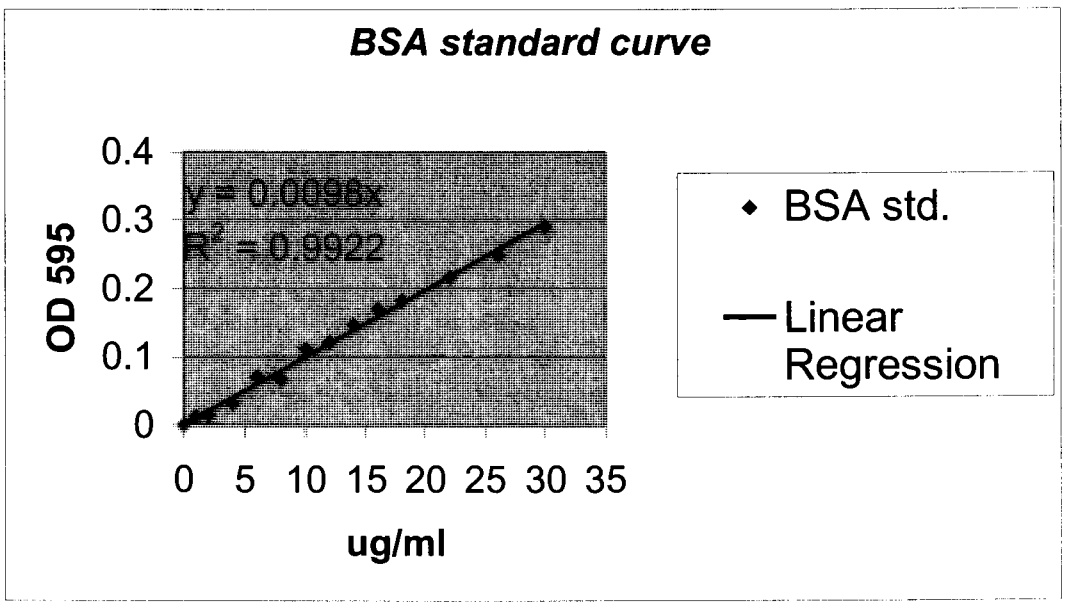
APPENDIX D

BSA Standard Curve



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