APPENDICES

APPENDIX A

Culture Media

1.) Brain Heart Infusion (BHI) Broth

BHI	37.00	g
Distilled water	1000.00	ml

Sterilize by autoclaving at 121ºC 15 lbs for 15 min.

2.) BHI agar

BHI agar (dehydrate)	52.00	g
Distilled water	1000.00	ml

Sterilize by autoclaving at 121ºC 15 lbs for 15 min. Allow sterilized medium to cool and pour in Petri dishes.

3.) Potato Dextrose Agar (PDA)

PDA agar (dehydrate)	39.00	g
Distilled water	1000.00	ml

Add distilled water, mix thoroughly, heat with frequent agitation and boil for 1 min to completely dissolve the powder. Sterilize by autoclaving at 121ºC 15 lbs for 15 min. Allow sterilized medium to cool and pour in Petri dishes.

4.) Malt Extract Agar (MEA)

MEA agar (dehydrate)	50.00	g
Distilled water	1000.00	ml

Add distilled water, mix thoroughly, heat with frequent agitation and boil for 1 min to completely dissolve the powder. Sterilize by autoclaving at 115ºC 15 lbs for 10 min. Allow sterilized medium to cool and pour in Petri dishes.

5.) Sabouraud Dextrose Broth (SDB)

SDB (dehydrate)	30.00	g
Distilled water	1000.00	ml

Sterilize by autoclaving at 121ºC 15 lbs for 15 min.

6.) Luria-Bertani (LB) medium

LB	20.00	g
Deionized water up to	1000.00	ml

Shake until the solutes are dissolved. Sterilize by autoclaving at 121ºC 15 lbs for 15 min.

7.) Minimal Medium (MM) Agar

NH_4Cl	2.00	g
$(NH_4)_2SO_4$	1.00	g
KCl	0.5	g
NaCl	0.5	g
KH_2PO_4	1.00	g
$MgSO_4.7H_2O$	0.5	g
$FeSO_4.7H_2O$	0.02	g
Glucose	10.00	g
Agar	15.00	g

Dissolve in distilled water and adjust pH to 5.0 with 1 N NaOH and adjust the volume to 1000 ml final volume with distilled water. Sterilize by autoclaving at 121ºC 15 lbs for 15 min.

8.) Aspergillus minimal medium (AMM)

KCl	0.52	g
$MgSO_4.7H_2O$	0.52	g
KH_2PO_4	1.52	g
Trace element solution	1	ml

1% D-glucose, 10 mM glutamine

1% Agar

Dissolve in distilled water and adjust pH to 6.5 with 1 N NaOH and adjust the volume to 1000 ml final volume with distilled water. Sterilize by autoclaving at 121ºC 15 lbs for 15 min.

9.) Trace element solution

$Na_2B_4O_7.10H_2O$	40.00	mg
$CuSO_4.5H_2O$	400.00	mg
$FePO_4.4H_2O$	1.00	g
$MnSO_4.1H_2O$	600.00	mg
$Na_2MoO_4.2H_2O$	800.00	mg
$ZnSO_4.7H_2O$	8.00	g

Dissolve $FePO_4.4H_2O$ in 100 ml distilled water and 20 ml 4 N HCl, then add the rest elements one by one and adjust the volume to 1000 ml final volume with distilled water. Sterilize by autoclaving at 121ºC 15 lbs for 15 min.

APPENDIX B

Alkaline Lysis Buffer for Preparing Plasmid DNA

1.) Solution I

50 mM glucose

25 mM Tris-Cl (pH 8.0)

10 mM EDTA (pH 8.0)

Sterilize by autoclaving for 20 min 15 lb/sq.in. on liquid cycle at store at 4 ºC.

2.) Solution II

0.2 N NaOH (freshly dilute from a 10 N stock)

1% SDS

3.) Solution III

5 M Potassium acetate	60.00	ml
Glacial acetic acid	11.50	ml
H_2O	28.50	ml

The resulting solution is 3 M with the respect to potassium and 5 M with respect to acetate.

APPENDIX C

Solution for Working in Protoplast-mediated Genomic DNA Extraction

1.) Osmotic buffer (pH 8.0)

$MgSO_4.7H_2O$	73.94	g
1 M sodium phosphate buffer pH 7.0	2.50	ml
Distilled water to	250.00	ml

Adjust pH to 5.8. Sterilize by autoclaving at 121ºC for 15 min.

2.) ST buffer (pH 5.8)

0.6 M Sorbitol

100 mM Tri-HCl

Sterilize by autoclaving for 20 min 15 lb/sq.in. on liquid cycle at store at 4 ºC.

3.) Lysis buffer

10 mM Tris

1 mM EDTA

1% sodium dodecyl sulphate (SDS)

Sterilize by autoclaving for 20 min 15 lb/sq.in. on liquid cycle at store at 4 ºC.

APPENDIX D

Solution for Protoplast-mediated Transformation

1.) Osmotic buffer (pH 5.8)

$MgSO_4.7H_2O$	73.94	g
1 M sodium phosphate buffer pH 7.0	2.50	ml
Distilled water to	250.00	ml

Adjust pH to 5.8 with 0.2 M Na_2HPO_4. Sterilize by autoclaving at 121ºC for 15 min.

2.) Trapping Buffer

D-Sorbitol	54.65	g
1 M Tris-HCl pH 7.0	50.00	ml
Distilled water to	500.00	ml

Sterilize by autoclaving at 121ºC for 15 min.

3.) STC (Sorbitol, Tris, $CaCl_2$) Buffer

D-Sorbitol	109.30	g
1 M Tris-HCl pH 7.5	5.00	ml
$CaCl_2$	0.73	g
Distilled water to	500.00	ml

Sterilize by autoclaving at 121ºC for 15 min.

4.) 60% PEG

Polyethylene glycol 4000	150.00	g
1 M $CaCl_2$	2.5	ml
1 M Tris-HCl pH 7.5	2.50	ml

Dissolve in distilled water and adjust to 250 ml in volumetric flask. Sterilize by autoclaving at 121ºC for 15 min.

APPENDIX E

Reagents for General Molecular Biology

1.) 20X SSC

NaCl	175.33	g
Sodium citrate	88.20	g

Dissolve in distilled water and adjust pH to 7.0 with 1 N NaOH and adjust the volume to 1000 ml final volume with distilled water. Sterilize by autoclaving at 121ºC for 15 min.

2.) 1 M Tris-HCl pH 7.5

Tris base	121.11	g

Dissolve in distilled water and adjust pH to 7.5 with concentrated HCl and adjust the volume to 1000 ml final volume with distilled water. Sterilize by autoclaving at 121ºC for 15 min.

3.) 0.5 M EDTA pH 8.0

EDTA ($C_{10}H_{14}N_2O_8Na_2.2H_2O$)	18.6	g

Dissolve in distilled water and adjust pH to 8.0 with NaOH and adjust the volume to 100 ml final volume with distilled water. Sterilize by autoclaving at 121ºC for 15 min.

4.) TE Buffer

10 mM Tris-HCl pH 7.5

1 mM EDTA pH 8.0

Sterilize by autoclaving at 121ºC for 15 min.

5.) 50X Tris-acetate (TAE) buffer

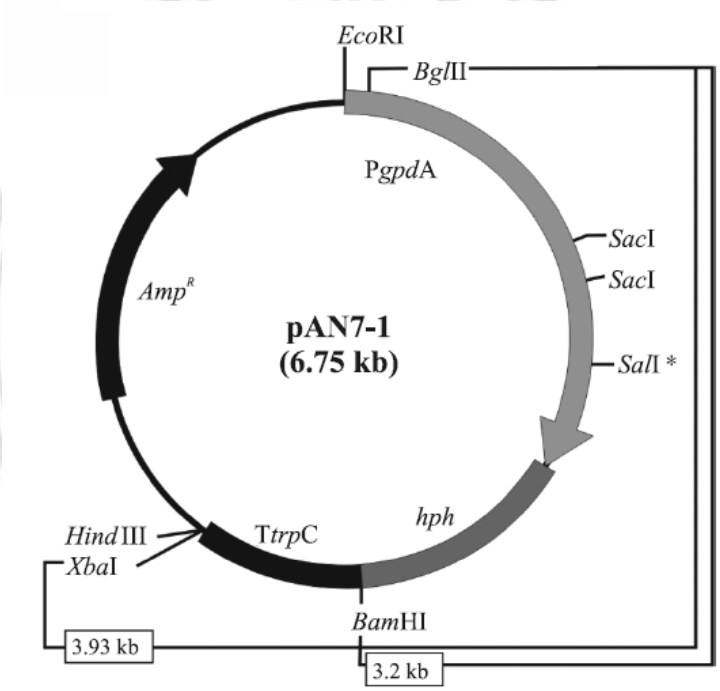
Tris base	242.00	g
Glacial acetic acid	57.10	ml
0.5 M EDTA	100.00	ml

The working solution (1X) contains 0.04 M Tris acetate and 0.0001 M EDTA

Appendix F

Plasmid Used in This Study

1.) **pAN7-1** (Cardoza *et al*., 2006)



Expression vector (PAN7-1) DNA, 6756bp

GenBank: Z32698.1 (http://www.ncbi.nlm.nih.gov/nuccore/Z32698.1)

```
LOCUS       Z32698                  6756 bp    DNA     linear   SYN 14-NOV-
2006
DEFINITION  Expression vector (PAN7-1) DNA, 6756bp.
ACCESSION   Z32698
VERSION     Z32698.1  GI:475166
KEYWORDS    hygromycin b phosphotransferase.
SOURCE      synthetic construct
ORGANISM    synthetic construct
            other sequences; artificial sequences.
REFERENCE   1
  AUTHORS   Mullaney,E.J., Hamer,J.E., Roberti,K.A., Yelton,M.M. and
            Timberlake,W.E.
  TITLE     Primary structure of the trpC gene from Aspergillus nidulans
```

```
  JOURNAL   Mol. Gen. Genet. 199 (1), 37-45 (1985)
   PUBMED   3158796
  REMARK    sites .
REFERENCE   2
AUTHORS   Punt,P.J., Oliver,R.P., Dingemanse,M.A., Pouwels,P.H. and van den
            Hondel,C.A.
  TITLE     Transformation of Aspergillus based on the hygromycin B
            resistance marker from Escherichia coli
  JOURNAL   Gene 56 (1), 117-124 (1987)
   PUBMED   2824287
  REMARK    (sites)
REFERENCE   3  (bases 999 to 2572)
  AUTHORS   Punt,P.J., Dingemanse,M.A., Kuyvenhoven,A., Soede,R.D.,
            Pouwels,P.H. and van den Hondel,C.A.
  TITLE     Functional elements in the promoter region of the Aspergillus
            nidulans gpdA gene encoding glyceraldehyde-3-phosphate
            dehydrogenase
  JOURNAL   Gene 93 (1), 101-109 (1990)
   PUBMED   2121607
REFERENCE   4  (bases 1 to 6756)
  AUTHORS   Punt,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (22-APR-1994) Punt P., TNO Nutrition and Food Research
            Institute, Lange Kleiweg 139, Rijkswik, The Netherlands
FEATURES             Location/Qualifiers
     source          1..6756
                     /organism="synthetic construct"
                     /mol_type="other DNA"
                     /db_xref="taxon:32630"
     misc_feature    1..6756
                     /product="fungal transformation vector pAN7-1"
                     /standard_name="HPH"
                     /experiment="experimental evidence, no additional
details
                     recorded"
                     /citation=[2]
                     /phenotype="gene product confers resistance to hygromycin
                     B"
     promoter        1..2129
                     /experiment="experimental evidence, no additional details
                     recorded"
                     /citation=[2]
                     /citation=[3]
                     /function="promoter region of the A.nidulans gpdA gene"
     gene            2130
                     /gene="gpdA"
     misc_feature    2130
                     /gene="gpdA"
                     /experiment="experimental evidence, no additional details
                     recorded"
                     /citation=[3]
                     /function="major transcription start site"
     intron          2178..2293
```

```
                     /experiment="experimental evidence, no additional details
                     recorded"
                     /citation=[3]
                     /number=1
     gene            2302..3321
                     /gene="HPH"
     CDS             2302..3321
                     /gene="HPH"
                     /experiment="experimental evidence, no additional details
                     recorded"
                     /note="gene-product confers resistance to hygromycin B"
                     /citation=[2]
                     /citation=[3]
                     /codon_start=1
                     /transl_table=11
                     /product="hygromycin B phosphotransferase"
                     /protein_id="CAA83647.1"
                     /db_xref="GI:475167"

/translation="MPELTATSVEKFLIEKFDSVSDLMQLSEGEESRAFSFDVGGRGY

VLRVNSCADGFYKDRYVYRHFASAALPIPEVLDIGEFSESLTYCISRRAQGVTLQDLP

ETELPAVLQPVAEAMDAIAAADLSQTSGFGPFGPQGIGQYTTWRDFICAIADPHVYHW

QTVMDDTVSASVAQALDELMLWAEDCPEVRHLVHADFGSNNVLTDNGRITAVIDWSEA

MFGDSQYEVANIFFWRPWLACMEQQTRYFERRHPELAGSPRLRAYMLRIGLDQLYQSL

VDGNFDDAAWAQGRCDAIVRSGAGTVGRTQIARRSAAVWTDGCVEVLADSGNRRPSTR
                     PRAKE"
     terminator      3339..4108
                     /standard_name="terminator region from A.nidulans trpC
                     gene (containing CDS)"
                     /experiment="experimental evidence, no additional details
                     recorded"
                     /note="this region contains the 3'end of the trpC
                     transcript including 50 codons. two major transcription
                     ends have been identified"
                     /citation=[1]
                     /citation=[2]
     polyA_site      3590..3600
     misc_difference 3600..3610
                     /note="introduction of one base at this position
generates
                     an BLGI site with was shown to be present by restriction
                     digestion"
                     /citation=[1]
                     /replace="gccttncaggc"
     misc_difference 3809..3817
                     /note="introduction of one base at this position
generates
```

```
                     an ALWNI site which was shown to be present by
restriction
                     digestion"
                     /citation=[1]
                     /replace="cagcncctg"
     polyA_site      3863..3873
     misc_difference 3897..3907
                     /note="introduction (actual sequence could be deletion as
                     well) of a base at this position results in the
                     elimination of an XMNI site which was shown to be absent
                     by restriction digestion"
                     /citation=[1]
                     /replace="gaaatcanttc"
     variation       3934..3941
                     /note="A NCOI site present at this position was removed
by
                     digestion, treatment with klenow polymerase and
                     religation"
                     /citation=[2]
                     /replace="catgcatg"
     misc_feature    4105..6756
                     /product="PUC18"
                     /note="vector sequences derived from PUC18 from SALI TO
                     ECORI"
                     /citation=[2]
```

>gi|475166|emb|Z32698.1| Expression vector (PAN7-1) DNA, 6756bp

```
   1 gaattccctt gtatctctac acacaggctc aaatcaataa gaagaacggt tcgtcttttt
  61 cgtttatatc ttgcatcgtc ccaaagctat tggcgggata ttctgtttgc agttggctga
 121 cttgaagtaa tctctgcaga tctttcgaca ctgaaatacg tcgagcctgc tccgcttgga
 181 agcggcgagg agcctcgtcc tgtcacaact accaacatgg agtacgataa gggccagttc
 241 cgccagctca ttaagagcca gttcatgggc gttggcatga tggccgtcat gcatctgtac
 301 ttcaagtaca ccaacgctct tctgatccag tcgatcatcc gctgaaggcg ctttcgaatc
 361 tggttaagat ccacgtcttc gggaagccag cgactggtga cctccagcgt ccctttaagg
 421 ctgccaacag ctttctcagc cagggccagc ccaagaccga caaggcctcc ctccagaacg
 481 ccgagaagaa ctggaggggt ggtgtcaagg aggagtaagc tccttattga agtcggagga
 541 cggagcggtg tcaagaggat attcttcgac tctgtattat agataagatg atgaggaatt
 601 ggaggtagca tagcttcatt tggatttgct ttccaggctg agactctagc ttggagcata
 661 gagggtcctt tggctttcaa tattctcaag tatctcgagt ttgaacttat tccctgtgaa
 721 ccttttattc accaatgagc attggaatga acatgaatct gaggactgca atcgccatga
 781 ggttttcgaa atacatccgg atgtcgaagg cttggggcac ctgcgttggt tgaatttaga
```

```
 841 acgtggcact attgatcatc cgatagctct gcaaagggcg ttgcacaatg caagtcaaac
 901 gttgctagca gttccaggtg gaatgttatg atgagcattg tattaaatca ggagatatag
 961 catgatctct agttagctca ccacaaaagt cagacggcgt aaccaaaagt cacacaacac
1021 aagctgtaag gatttcggca cggctacgga agacggagaa gccaccttca gtggactcga
1081 gtaccattta attctatttg tgtttgatcg agacctaata cagcccctac aacgaccatc
1141 aaagtcgtat agctaccagt gaggaagtgg actcaaatcg acttcagcaa catctcctgg
1201 ataaacttta agcctaaact atacagaata agataggtgg agagcttata ccgagctccc
1261 aaatctgtcc agatcatggt tgaccggtgc ctggatcttc ctatagaatc atccttattc
1321 gttgacctag ctgattctgg agtgacccag agggtcatga cttgagccta aaatccgccg
1381 cctccaccat ttgtagaaaa atgtgacgaa ctcgtgagct ctgtacagtg accggtgact
1441 ctttctggca tgcggagaga cggacggacg cagagagaag ggctgagtaa taagccactg
1501 gccagacagc tctggcggct ctgaggtgca gtggatgatt attaatccgg gaccggccgc
1561 ccctccgccc cgaagtggaa aggctggtgt gcccctcgtt gaccaagaat ctattgcatc
1621 atcggagaat atggagcttc atcgaatcac cggcagtaag cgaaggagaa tgtgaagcca
1681 ggggtgtata gccgtcggcg aaatagcatg ccattaacct aggtacagaa gtccaattgc
1741 ttccgatctg gtaaaagatt cacgagatag taccttctcc gaagtaggta gagcgagtac
1801 ccggcgcgta agctccctaa ttggcccatc cggcatctgt agggcgtcca aatatcgtgc
1861 ctctcctgct ttgcccggtg tatgaaaccg gaaaggccgc tcaggagctg gccagcggcg
1921 cagaccggga acacaagctg gcagtcgacc catccggtgc tctgcactcg acctgctgag
1981 gtccctcagt ccctggtagg cagctttgcc ccgtctgtcc gcccggtgtg tcggcggggt
2041 tgacaaggtc gttgcgtcag tccaacattt gttgccatat tttcctgctc tccccaccag
2101 ctgctctttt cttttctctt tcttttccca tcttcagtat attcatcttc ccatccaaga
2161 acctttattt cccctaagta agtactttgc tacatccata ctccatcctt cccatccctt
2221 attcctttga acctttcagt tcgagctttc ccacttcatc gcagcttgac taacagctac
                              hph
2281 cccgcttgag cagacatcac catgcctgaa ctcaccgcga cgtctgtcga gaagtttctg
2341 atcgaaaagt tcgacagcgt ctccgacctg atgcagctct cggagggcga agaatctcgt
2401 gctttcagct tcgatgtagg agggcgtgga tatgtcctgc gggtaaatag ctgcgccgat
2461 ggtttctaca aagatcgtta tgtttatcgg cactttgcat cggccgcgct cccgattccg
2521 gaagtgcttg acattgggga attcagcgag agcctgacct attgcatctc ccgccgtgca
2581 cagggtgtca cgttgcaaga cctgcctgaa accgaactgc ccgctgttct gcagccggtc

          YG    >>>>>>>>>>>>>>>>>>>>
```

2641 gcggaggcca tggatgcgat cgctgcggcc gatcttagcc agacgagcgg gttcggccca

2701 ttcggaccgc aaggaatcgg tcaatacact acatggcgtg atttcatatg cgcgattgct

2761 gatccccatg tgtatcactg gcaaactgtg atggacgaca ccgtcagtgc gtccgtcgcg

2821 caggctctcg atgagctgat gctttgggcc gaggactgcc ccgaagtccg gcacctcgtg

2881 cacgcggatt tcggctccaa caatgtcctg acggacaatg gccgcataac agcggtcatt

2941 gactggagcg aggcgatgtt cggggattcc caatacgagg tcgccaacat cttcttctgg

3001 aggccgtggt tggcttgtat ggagcagcag acgcgctact tcgagcggag gcatccggag

<<<<<<<<<<<<<<<<<<< HY

3061 cttgcaggat cgccgcggct ccgggcgtat atgctccgca ttggtcttga ccaactctat

3121 cagagcttgg ttgacggcaa tttcgatgat gcagcttggg cgcagggtcg atgcgacgca

3181 atcgtccgat ccggagccgg gactgtcggg cgtacacaaa tcgcccgcag aagcgcggcc

3241 gtctggaccg atggctgtgt agaagtactc gccgatagtg gaaaccgacg ccccagcact

3301 cgtccgaggg caaaggaata gagtagatgc cgaccgcggg atccacttaa cgttactgaa

3361 atcatcaaac agcttgacga atctggatat aagatcgttg gtgtcgatgt cagctccgga

3421 gttgagacaa atggtgttca ggatctcgat aagatacgtt catttgtcca agcagcaaag

3481 agtgccttct agtgatttaa tagctccatg tcaacaagaa taaaacgcgt tttcgggttt

3541 acctcttcca gatacagctc atctgcaatg cattaatgca ttgactgcaa cctagtaacg

3601 ccttncaggc tccggcgaag agaagaatag cttagcagag ctattttcat tttcgggaga

3661 cgagatcaag cagatcaacg gtcgtcaaga gacctacgag actgaggaat ccgctcttgg

3721 ctccacgcga ctatatattt gtctctaatt gtactttgac atgctcctct tctttactct

3781 gatagcttga ctatgaaaat tccgtcacca gcncctgggt tcgcaaagat aattgcatgt

3841 ttcttccttg aactctcaag cctacaggac acacattcat cgtaggtata aacctcgaaa

3901 tcanttccta ctaagatggt atacaatagt aaccatgcat ggttgcctag tgaatgctcc

3961 gtaacaccca atacgccggc cgaaactttt ttacaactct cctatgagtc gtttacccag

4021 aatgcacagg tacacttgtt tagaggtaat ccttctttct agaagtcctc gtgtactgtg

4081 taagcgccca ctccacatct ccactcgacc tgcaggcatg caagcttggc actggccgtc

4141 gttttacaac gtcgtgactg ggaaaaccct ggcgttaccc aacttaatcg ccttgcagca

4201 catccccctt tcgccagctg gcgtaatagc gaagaggccc gcaccgatcg cccttcccaa

4261 cagttgcgca gcctgaatgg cgaatggcgc ctgatgcggt attttctcct tacgcatctg

4321 tgcggtattt cacaccgcat atggtgcact ctcagtacaa tctgctctga tgccgcatag

4381 ttaagccagc cccgacaccc gccaacaccc gctgacgcgc cctgacgggc ttgtctgctc

4441 ccggcatccg cttacagaca agctgtgacc gtctccggga gctgcatgtg tcagaggttt

4501 tcaccgtcat caccgaaacg cgcgagacga aagggcctcg tgatacgcct atttttatag

4561 gttaatgtca tgataataat ggtttcttag acgtcaggtg gcacttttcg gggaaatgtg

4621 cgcggaaccc ctatttgttt atttttctaa atacattcaa atatgtatcc gctcatgaga

4681 caataaccct gataaatgct tcaataatat tgaaaaagga agagtatgag tattcaacat

4741 ttccgtgtcg cccttattcc ctttttgcg gcattttgcc ttcctgtttt tgctcaccca

4801 gaaacgctgg tgaaagtaaa agatgctgaa gatcagttgg gtgcacgagt gggttacatc

4861 gaactggatc tcaacagcgg taagatcctt gagagttttc gccccgaaga acgttttcca

4921 atgatgagca cttttaaagt tctgctatgt ggcgcggtat tatcccgtat tgacgccggg

4981 caagagcaac tcggtcgccg catacactat tctcagaatg acttggttga gtactcacca

5041 gtcacagaaa agcatcttac ggatggcatg acagtaagag aattatgcag tgctgccata

5101 accatgagtg ataacactgc ggccaactta cttctgacaa cgatcggagg accgaaggag

5161 ctaaccgctt ttttgcacaa catgggggat catgtaactc gccttgatcg ttgggaaccg

5221 gagctgaatg aagccatacc aaacgacgag cgtgacacca cgatgcctgt agcaatggca

5281 acaacgttgc gcaaactatt aactggcgaa ctacttactc tagcttcccg gcaacaatta

5341 atagactgga tggaggcgga taaagttgca ggaccacttc tgcgctcggc ccttccggct

5401 ggctggttta ttgctgataa atctggagcc ggtgagcgtg ggtctcgcgg tatcattgca

5461 gcactggggc cagatggtaa gccctcccgt atcgtagtta tctacacgac ggggagtcag

5521 gcaactatgg atgaacgaaa tagacagatc gctgagatag gtgcctcact gattaagcat

5581 tggtaactgt cagaccaagt ttactcatat atactttaga ttgatttaaa acttcatttt

5641 taatttaaaa ggatctaggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa

5701 cgtgagtttt cgttccactg agcgtcagac cccgtagaaa agatcaaagg atcttcttga

5761 gatccttttt ttctgcgcgt aatctgctgc ttgcaaacaa aaaaaccacc gctaccagcg

5821 gtggtttgtt tgccggatca agagctacca actcttttte cgaaggtaac tggcttcagc

5881 agagcgcaga taccaaatac tgtccttcta gtgtagccgt agttaggcca ccacttcaag

5941 aactctgtag caccgcctac atacctcgct ctgctaatcc tgttaccagt ggctgctgcc

6001 agtggcgata agtcgtgtct taccgggttg gactcaagac gatagttacc ggataaggcg

6061 cagcggtcgg gctgaacggg gggttcgtgc acacagccca gcttggagcg aacgacctac

6121 accgaactga gatacctaca gcgtgagcta tgagaaagcg ccacgcttcc cgaagggaga

6181 aaggcggaca ggtatccggt aagcggcagg gtcggaacag gagagcgcac gagggagctt

6241 ccagggggaa acgcctggta tctttatagt cctgtcgggt ttcgccacct ctgacttgag

6301 cgtcgatttt tgtgatgctc gtcaggggg cggagcctat ggaaaaacgc cagcaacgcg

6361 gccttttac ggttcctggc cttttgctgg ccttttgctc acatgttctt tcctgcgtta

6421 tcccctgatt ctgtggataa ccgtattacc gcctttgagt gagctgatac cgctcgccgc

6481 agccgaacga ccgagcgcag cgagtcagtg agcgaggaag cggaagagcg cccaatacgc

6541 aaaccgcctc tccccgcgcg ttggccgatt cattaatgca gctggcacga caggtttccc

6601 gactggaaag cgggcagtga gcgcaacgca attaatgtga gttagctcac tcattaggca

6661 ccccaggctt tacactttat gcttccggct cgtatgttgt gtggaattgt gagcggataa

6721 caatttcaca caggaaacag ctatgaccat gattac 6756

1-2129 = promoter
2130-2177 = gpdA major transcription start site
2178-2293 = intron
2302-3321 = hph
3339-4108 = terminator
3590-3600 = polyA
3863-3873 = polyA
4105-6756 = pUC18
4173-4195 = M3
6711-6735 = M2

List of single cutter restriction enzymes in pAN7-1

0 cutter

#	Enzyme	Specificity
1	Acc65I	G▼GTAC▲C
2	AfeI	AGC▼▲GCT
3	AflII	C▼TTAA▲G
4	AleI	CACNN▼▲NNGTG
5	ApaI	G▲GGCC▼C
6	AscI	GG▼CGCG▲CC
7	BsaAI	YAC▼▲GTR
8	BsaBI	GATNN▼▲NNATC
9	BsiWI	C▼GTAC▲G
10	BspDI	AT▼CG▲AT
11	BssHII	G▼CGCG▲C
12	BstXI	CCAN▲NNNN▼NTGG
13	Bsu36I	CC▼TNA▲GG
14	ClaI	AT▼CG▲AT
15	CspCI	▲NN▼(N)$_{11}$CAA(N)$_5$GTGG(N)$_{10}$▲NN▼
16	EcoNI	CCTNN▼N▲NNAGG
17	EcoRV	GAT▼▲ATC
18	FseI	GG▲CCGG▼CC
19	HpaI	GTT▼▲AAC
20	KpnI	G▲GTAC▼C
21	NotI	GC▼GGCC▲GC
22	NruI	TCG▼▲CGA
23	PacI	TTA▲AT▼TAA
24	PmeI	GTTT▼▲AAAC
25	PmlI	CAC▼▲GTG
26	PsiI	TTA▼▲TAA
27	PspOMI	G▼GGCC▲C
28	SexAI	A▼CCWGG▲T
29	SfiI	GGCCN▲NNN▼NGGCC
30	SgrAI	CR▼CCGG▲YG
31	SmaI	CCC▼▲GGG
32	SnaBI	TAC▼▲GTA
33	SpeI	A▼CTAG▲T
34	SwaI	ATTT▼▲AAAT
35	TspMI	C▼CCGG▲G
36	XmaI	C▼CCGG▲G

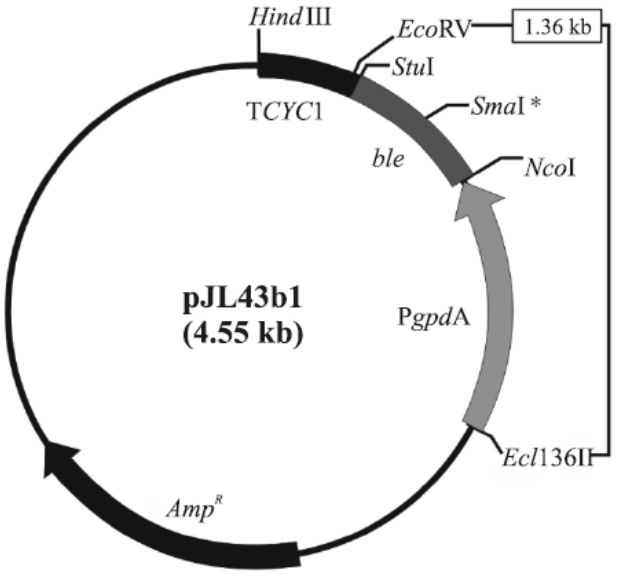
1 cutter

#	Enzyme	Specificity	Sites & flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	AhdI	GACNN▲N▼NNGTC	list	5513/5512
2	AsiSI	GCG▲AT▼CGC	list	*2660/2658
3	AvrII	C▼CTAG▲G	list	1718/1722
4	BamHI	G▼GATC▲C	list	3339/3343
5	BbvCI	CC▼TCA▲GC	list	1976/1979
6	BclI	T▼GATC▲A	list	#853/857
7	BglII	A▼GATC▲T	list	138/142
8	BlpI	GC▼TNA▲GC	list	3631/3634
9	BmgBI	CAC▲GTC	list	*374
10	BmtI	G▲CTAG▼C	list	908/904
11	BsrGI	T▼GTAC▲A	list	1422/1426
12	BstEII	G▼GTNAC▲C	list	396/401
13	BstZ17I	GTA▲TAC	list	3921
14	HindIII	A▼AGCT▲T	list	4122/4126
15	KasI	G▼GCGC▲C	list	*4286/4290
16	MfeI	C▼AATT▲G	list	1734/1738
17	MluI	A▼CGCG▲T	list	*3525/3529
18	NaeI	GCC▲GGC	list	*3977
19	NarI	GG▼CG▲CC	list	*4287/4289
20	NcoI	C▼CATG▲G	list	2648/2652
21	NgoMIV	G▼CCGG▲C	list	*3975/3979
22	NheI	G▼CTAG▲C	list	904/908
23	PciI	A▼CATG▲T	list	6401/6405
24	PluTI	G▲GCGC▼C	list	*4290/4286
25	PshAI	GACNN▲NNGTC	list	*2323
26	PspXI	VC▼TCGA▲GB	list	*1076/1080
27	RsrII	CG▼GWC▲CG	list	*2704/2707
28	SalI	G▼TCGA▲C	list	*1944/1948
29	SbfI	CC▲TGCA▼GG	list	4114/4110
30	SfoI	GGC▲GCC	list	*4288
31	StuI	AGG▲CCT	list	465
32	XbaI	T▼CTAG▲A	list	4058/4062
33	XcmI	CCANNNN▲N▼NNNNTGG	list	1163/1162

2 cutters

#	Enzyme	Specificity	Sites & flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	AatII	G▲ACGT C	list	*2323/2319, *4594/4590
2	AccI	GT MK▲AC	list	*1945/1947, 3920/3922
3	AflIII	A CRYG▲T	list	3525/3529, 6401/6405
4	AgeI	A CCGG▲T	list	*1283/1287, *1431/1435
5	AvaI	C YCGR▲G	list	*694/698, *1076/1080
6	BanII	G▲RGCY C	list	1257/1253, 1420/1416
7	BbsI	GAAGACNN NNNN▲	list	368/372, 1056/1060
8	BciVI	GTATCC(N)$_5$▲N	list	4676/4675, 6203/6202
9	BmrI	ACTGGGNNNN▲N	list	4167/4166, 5473/5472
10	Bpu10I	CC TNA▲GC	list	1900/1903, 1976/1979
11	BseYI	C CCAG▲C	list	3292/3296, 6097/6101
12	BsgI	GTGCAG(N)$_{14}$▲NN	list	1547/1545, 1947/1945
13	BsmI	GAATG▲CN	list	3958/3956, 4026/4024
14	BsoBI	C YCGR▲G	list	694/698, 1076/1080
15	BspQI	GCTCTTCN NNN▲	list	323/326, 6518/6521
16	BstBI	TT CG▲AA	list	*354/356, *786/788
17	BtgZI	GCGATG(N)$_{10}$ NNNN▲	list	*2242/2246, *2968/2972
18	DraIII	CAC▲NNN GTG	list	2584/2581, *2877/2874
19	Eco53kI	GAG▲CTC	list	1255, 1418
20	EcoRI	G AATT▲C	list	1/5, 2539/2543
21	FspI	TGC▲GCA	list	*4267, *5290
22	MscI	TGG▲CCA	list	1501, 1911
23	NdeI	CA TA▲TG	list	2746/2748, 4339/4341
24	PaeR7I	C TCGA▲G	list	*694/698, *1076/1080
25	PpuMI	RG GWC▲CY	list	664/667, 1980/1983
26	SacI	G▲AGCT C	list	1257/1253, 1420/1416
27	SacII	CC▲GC GG	list	*3076/3074, *3337/3335
28	SapI	GCTCTTCN NNN▲	list	323/326, 6518/6521
29	SspI	AAT▲ATT	list	681, 4708
30	StyI	C CWWG▲G	list	1718/1722, 2648/2652
31	XhoI	C TCGA▲G	list	*694/698, *1076/1080
32	XmnI	GAANN▲NNTTC	list	707, 4913
33	ZraI	GAC▲GTC	list	*2321, *4592

2.) pJL43b1



CTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAAC
CAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTC
CAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCA
GGGCGATGGCCCACTACGTGAACCATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTA
AATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGG
AAGGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCAC
CACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCATTCAGGCTGCGCAACTGTTGG
GAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGAT
TAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAGCGCGCGTAATA
CGACTCACTATAGGGCGAATTGGAGCTCTGTACAGTGACCGGTGACTCTTTCTGGCATGCGGAGAGACGG
ACGGACGCAGAGAGAAGGGCTGAGTAATAAGCCACTGGCCAGACAGCTCTGGCGGCTCTGAGGTGCAGTG
GATGATTATTAATCCGGGACCGGCCGCCCCTCCGCCCCGAAGTGGAAAGGCTGGTGTGCCCCTCGTTGAC
CAAGAATCTATTGCATCATCGGAGAATATGGAGCTTCATCGAATCACCGGCAGTAAGCGAAGGAGAATGT
GAAGCCAGGGGTGTATAGCCGTCGGCGAAATAGCATGCCATTAACCTAGGTACAGAAGTCCAATTGCTTC
CGATCTGGTAAAAGATTCACGAGATAGTACCTTCTCCGAAGTAGGTAGAGCGAGTACCCGGCGCGTAAGC
TCCCTAATTGGCCCATCCGGCATCTGTAGGGCGTCCAAATATCGTGCCTCTCCTGCTTTGCCCGGTGTAT
GAAACCGGAAAGGCCGCTCAGGAGCTGGCCAGCGGCGCAGACCGGGAACACAAGCTGGCAGTCGACCCAT
CCGGTGCTCTGCACTCGACCTGCTGAGGTCCCTCAGTCCCTGGTAGGCAGCTTTGCCCCGTCTGTCCGCC
CGGTGTGTCGGCGGGGTTGACAAGGTCGTTGCGTCAGTCCAACATTTGTTGCCATATTTTCCTGCTCTCC
CCACCAGCTGCTCTTTTCTTTTCTCTTTCTTTTCCCATCTTCAGTATATTCATCTTCCCATCCAAGAACC
TTTATTTCCCCTAAGTAAGTACTTTGCTACATCCATACTCCATCCTTCCCATCCCTTATTCCTTTGAACC
TTTCAGTTCGAGCTTTCCCACTTCATCGCAGCTTGACTAACAGCTACCCCGCTTGAGCAGACATCACCAT
GGCCAAGTTGACCAGTGCCGTTCCGGTGCTCACCGCGCGCGACGTCGCCGGAGCGGTCGAGTTCTGGACC
GACCGGCTCGGGTTCTCCCGGGACTTCGTGGAGGACGACTTCGCCGGTGTGGTCCGGGACGACGTGACCC
TGTTCATCAGCGCGGTCCAGGACCAGGTGGTGCCGGACAACACCCTGGCCTGGGTGTGGGTGCGCGGCCT
GGACGAGCTGTACGCCGAGTGGTCGGAGGTCGTGTCCACGAACTTCCGGGACGCCTCCGGGCCGGCCATG
ACCGAGATCGGCGAGCAGCCGTGGGGGCGGGAGTTCGCCCTGCGCGACCCGGCCGGCAACTGCGTGCACT
TCGTGGCCGAGGAGCAGGACTGACCGACGCCGACCAACACCGCCGGTCCGACGCGGCCCGACGGGTCCGA
GGCCTCGGAGATCCGTCCCCCTTTTCCTTTGTCGATATCATGTAATTAGTTATGTCACGCTTACATTCAC
GCCCTCCCCCCACATCCGCTCTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTTA
TTTTTTTATAGTTATGTTAGTATTAAGAACGTTATTTATATTTCAAATTTTTCTTTTTTTTCTGTACAGA
CGCGTGTACGCATGTAACATTATACTGAAAACCTTGCTTGAGAAGGTTTTGGGACGCTCGAAGGCTTTAA
TTTGCAAGCTTATCGATACCGTCGACCTCGAGGGGGGGCCCGGTACCCAGCTTTTGTTCCCTTTAGTGAG
GGTTAATTGCGCGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAAT

TCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACA
TTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCG
GCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCG
CTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCA
GGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGT
TGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTG
GCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTT
CCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCT
CACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGT
TCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG
CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGA
AGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTAC
CTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTT
TGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTG
ACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTA
GATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGT
TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGAC
TCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG
AGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGT
GGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGC
CAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTAT
GGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCG
GTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGG
CAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAAC
CAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACC
GCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGA
TCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTAC
TTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACA
CGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCA
TGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAA
AGTGCCAC

653-1538 (in red) gpdA promoter (886 bp)
1539-1965 (in blue) phleomycin gene (427 bp)
1966-2251 (in green) terminator region of CYC1 gene

List of single cutter restriction enzymes in pJL43b1

#	Enzyme	Specificity	Sites & flanks	Cut positions (blunt - 5' ext. - 3' ext.)
1	AatII	G▲ACGT▼C	list	*1585/1581
2	Acc65I	G▼GTAC▲C	list	*2282/2286
3	AgeI	A▼CCGG▲T	list	*668/672
4	AhdI	GACNN▲N▼NNGTC	list	3573/3572
5	ApaI	G▲GGCC▼C	list	*2280/2276
6	AvrII	C▼CTAG▲G	list	955/959
7	BaeI	▲(N)5▼(N)10ACNNNNGTAYC(N)7▲(N)5▼	list	951/946+984/979
8	BbvCI	CC▼TCA▲GC	list	1213/1216

9	BfuAI	ACCTGCNNNN▼NNNN▲	list	1217/1221
10	BmgBI	CAC▲GTC	list	*1673
11	BpmI	CTGGAG(N)$_{14}$▲NN▼	list	3643/3641
12	BsaAI	YAC▲GTR	list	*227
13	BsaI	GGTCTCN▼NNNN▲	list	3634/3638
14	BseRI	GAGGAG(N)$_{8}$▲NN▼	list	1914/1912
15	BsmBI	CGTCTCN▼NNNN▲	list	*688/692
16	BspDI	AT▼CG▲AT	list	*2253/2255
17	BspMI	ACCTGCNNNN▼NNNN▲	list	1217/1221
18	BspQI	GCTCTTCN▼NNN▲	list	2564/2567
19	ClaI	AT▼CG▲AT	list	*2253/2255
20	Eco53kI	GAG▲CTC	list	655
21	EcoRV	GAT▲ATC	list	1996
22	FseI	GG▲CCGG▼CC	list	*1815/1811
23	HindIII	A▼AGCT▲T	list	2246/2250
24	KpnI	G▲GTAC▼C	list	2286/2282
25	MfeI	C▼AATT▲G	list	971/975
26	MluI	A▼CGCG▲T	list	*2170/2174
27	NcoI	C▼CATG▲G	list	1537/1541
28	PaeR7I	C▼TCGA▲G	list	*2267/2271
29	PciI	A▼CATG▲T	list	2680/2684
30	PflFI	GACN▼N▲NGTC	list	1282/1283
31	PsiI	TTA▲TAA	list	99
32	PspOMI	G▼GGCC▲C	list	*2276/2280
33	PspXI	VC▼TCGA▲GB	list	*2267/2271
34	RsrII	CG▼GWC▲CG	list	*1935/1938
35	SacI	G▲AGCT▼C	list	657/653
36	SapI	GCTCTTCN▼NNN▲	list	2564/2567
37	SexAI	A▼CCWGG▲T	list	#1702/1707
38	SgrAI	CR▼CCGG▲YG	list	*1653/1657
39	SmaI	CCC▲GGG	list	*1629
40	StuI	AGG▲CCT	list	1962
41	TliI	C▼TCGA▲G	list	*2267/2271
42	TspMI	C▼CCGG▲G	list	*1627/1631
43	Tth111I	GACN▼N▲NGTC	list	1282/1283
44	XhoI	C▼TCGA▲G	list	*2267/2271
45	XmaI	C▼CCGG▲G	list	*1627/1631
46	XmnI	GAANN▲NNTTC	list	4172
47	ZraI	GAC▲GTC	list	*1583

CURRICULUM VITAE

Name Ms. Panjaphorn Nimmanee

Date of Birth June 24, 1980

Education

1997	Certificate of Mathayomsuksa VI, Streesmutprakan School, Samut Prakarn
2001	Bachelor Degree of Science (Medical Technology), Faculty of Allied Health Science, Thammasart University, Thailand
2007	Master Degree of Science (Microbiology), Faculty of Science, Mahidol University, Thailand

Scholarship 2010-2014: The Royal Golden Jubilee Ph.D. program, The Thailand Research Fund

Publication Rangsipanurat W, Paungmoung P, Chuwongwattana S, Phumeesat P, **Nimmanee P**, Janwithayanuchit I. Microbiological quality of vegetable and juice beverages in sealed containers in Samut Prakarn Province. *Journal of Health Science*. 2009; 18: 703-709.