

APPENDICES

Appendix A

Chemicals and reagents

Chemicals and reagents

1. Somogyi-Nelson method

Somogyi I : NaSO₄ 288 g

C₄H₄KNaO₆.4H₂O 24 g

Na₂CO₃ 48 g

Dissolve in boiled distill water 1,000 ml

Somogyi II: Na₂SO₄ 72 g, dissolve in boiled distill water 300 ml

add CuSO₄.5H₂O 8 g, adjust volume to 400 ml

Mix Somogyi I : Somogyi II ; 4:1

Nelson : (NH₄)₆Mo₇O₂₄.4H₂O 100 g, dissolve in boiled distill water 1,800 ml

add H₂SO₄ 84 ml

Na₂HASO₄.7H₂O 12 g, dissolve in boiled distill water 100 ml

Adjust total volume to 2 liters

2. Southern Blotting

HCl 250 mM HCl

Denaturation solution 1 0.5 N NaOH, 1.5 m NaCl

Neutralisation solution 1 0.5 M Tris-HCl, pH 7.5; 3 M NaCl

20X SSC buffer 3 M NaCl, 300 mM Sodium citrate, pH 7.0

5X SSC buffer 750 mM NaCl, 75 mM Sodium citrate, pH 7.0

2X Wash solution 2X SSC, containing 0.1% SDS

0.5X Wash solution 0.5X SSC, containing 0.1% SDS

3. Colony Hybridisation

Denaturation solution 1 0.5 N NaOH, 1.5 m NaCl

Neutralisation solution 2 1.0 M Tris-HCl, pH 7.5; 1.5 M NaCl

20X SSC buffer 3 M NaCl, 300 mM Sodium citrate, pH 7.0

2X SSC buffer 300 mM NaCl, 75 mM Sodium citrate, pH 7.0

2X Wash solution 2X SSC, containing 0.1% SDS

0.5X Wash solution 0.5X SSC, containing 0.1% SDS

4. Northern Blotting

2X Wash solution	2X SSC, containing 0.1% SDS
0.5X Wash solution	0.5X SSC, containing 0.1% SDS
20X SSC buffer	3 M NaCl, 300 mM Sodium citrate, pH 7.0
10X SSC buffer	1.5 M NaCl, 150 mM Sodium citrate, pH 7.0

5. Colorimetric Detection with NBT and BCIP

Anti-Digoxigenin-AP*	750 units/ml Anti-Digoxigenin, Fab fragments conjugated to alkaline phosphatase
NBT solution	75 mg/ml nitroblue tetrazolium salt in 70% (v/v) dimethylformamide
BCIP solution	50 mg/ml 5-bromo-4-chloro-3-indolyl phosphate (BCIP), toluidinium salt in 100% dimethylformamide
Washing buffer	100 mM maleic acid, 150 mM NaCl; pH 7.5 (+20°C)
(dilute 1:10 with H ₂ O)	0.3% (v/v) Tween [®] 20
Maleic acid buffer	100 mM maleic acid, 150 mM NaCl; pH 7.5 (+20°C)
(dilute 1:10 with H ₂ O)	
Blocking solution	1% (w/v) Blocking reagent for nucleic acid hybridisation dissolved in Maleic acid buffer. Blocking solution is cloudy and should not be filtered. It is stable for at least two weeks when stored at +4°C, but must then be brought to room temperature before use.
(dilute 1:10 with 1X Maleic acid buffer)	
Detection buffer	100 mM Tris-HCl, 100 mM NaCl; pH 9.5 (+20°C)
(dilute 1:10 with H ₂ O)	
TE buffer	10 mM Tris, 1 mM EDTA; pH8.0 (dilute 1:10 with H ₂ O)

Medias

1 LB (Luria-Bertani) Medium

1% Tryptone

0.5% Yeast Extract

1% NaCl

pH 7.0

1. For 1 liter, dissolve
10 g tryptone
5 g yeast extract
10 g NaCl

In 950 ml distill water

2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave for 20 minutes at 15 lb./sq.in. Let cool to ~55°C and add desired antibiotics at this point.
4. Store at room temperature or at +4°C.

2 LB agar plates

1. Make LB Medium above and add 15 g/liter agar before autoclaving.
2. Autoclave for 20 minutes at 15 lb./sq.in.
3. Let cool to ~55°C and add desired antibiotics at this point. Pour into 10 cm petri plates. Let the plates harden, then invert, and store at +4°C.

3 *Pichia* Media

Stock Solutions

(1) **10X YNB: (13.4 % Yeast Nitrogen Base with Ammonium Sulfate without amino acids)**

Dissolve 134 g of yeast nitrogen base (YNB) with ammonium sulfate and without amino acids in 1000 ml of water sterilise. Heat the solution to dissolve YNB completely. Store at +4°C. Alternatively, use 34 g of YNB without ammonium sulfate and amino acids and 100 g of ammonium sulfate. The shelf life of this solution is one

year. If you are using the YNB pouch included in the kit, follow the directions on the pouch.

Note: *Pichia* cells exhibit optimal growth with higher YNB concentration, therefore, the amount of YNB used in this kit is twice as concentrated as YNB formulation for *Saccharomyces*.

(2) 500X B: (0.02 % Biotin)

Dissolve 20 g biotin in 100 ml of water and filter sterilize. Store at +4°C. The shelf life of this solution is approximately one year.

(3) 100X H: (0.4% Histidine)

Dissolve 400 mg of L-histidine in 100 ml of water. Heat the solution, if necessary, to no greater than 50°C in order to dissolve. Filter sterilize and store at +4°C. The shelf life of this solution is approximately one year.

(4) 10X D: (20% Dextrose)

Dissolve 200 g of D-glucose in 1,000 ml of water. Autoclave for 15 minutes or filter sterilize. The shelf life of this solution is approximately one year.

(5) 10X M: (5% Methanol)

Mix 5 ml of methanol with 95 ml of water. Filter sterilize and store at +4°C. The shelf life of this solution is approximately two months.

(6) 10X GY: (10% Glycerol)

Mix 100 ml of glycerol with 900 ml of water. Sterilize either by filtering or autoclaving. Store at room temperature. The shelf life of this solution is greater than one year.

(7) 100X AA: (0.5% of each Amino Acid)

Dissolve 500 g each of L-glutamic acid, L-methionine, L-lysine and L-isoleucine in 100 ml of water. Filter sterilize and store at +4°C. The shelf life of this solution is approximately one year.

(8) Potassium phosphate buffer (1 M), pH 6.0

Combine 132 ml of 1 M K_2HPO_4 , 868 ml of 1 M KH_2PO_4 and confirm that the pH=6.0 ± 0.1 (If the pH need to be adjusted, use phosphoric acid or KOH). Autoclave and store at room temperature. The shelf life of this solution is greater than one year.

3.1 YPD: Yeast Extract Peptone Dextrose Medium (1liter)

1% yeast extract

2 % peptone

2 % dextrose (glucose)

Note: If you are using the YPD Base Medium or the YPD Base Agar medium pouches included with the kit, follow the directions on the pouch.

1. Dissolve 10 g yeast extract and 20 g peptone in 900 ml of water.

Note: Add 20 g of agar if making YPD slants or plates.

2. Autoclave for 20 minutes on liquid cycle.

3. Add 100 ml of 10X D.

Store the liquid medium at room temperature. Store YPD slants or plates at +4°C. The shelf life is several months.

3.2 MGY and MGYH: Minimal Glycerol Medium ± Histidine (1 liter)

1.34% YNB

1% glycerol

4×10^{-5} % biotin

± 0.004% histidine

1. Combine aseptically 800 ml autoclave water with 100 ml of 10X YNB, 2 ml of 500X B and 100 ml of 10X GY.

2. For growth of his 4 strains in this medium, a version can be made that contains histidine (called MGYH) by adding 10 ml of 100X H stock solution.

Store at +4°C. The shelf life of this solution is approximately two months.

3.3 RD and RDH Liquid Media: Regeneration Dextrose Medium ± Histidine

(1 liter)

1 M sorbitol

2% dextrose

1.34% YNB

4×10^{-5} % biotin

0.005 % amino acids

± 0.004% histidine

1. Dissolve 186 g of sorbitol in 700 ml of water and proceed to Step 2.
2. Autoclave 20 minutes on liquid cycle.
3. Cool and maintain the liquid medium in at 45°C water bath.
4. Prepare a prewarmed (45°C) mixture of the following stock solution:
 - 100 ml of 10X D
 - 100 ml of 10X YNB
 - 2 ml of 500X B
 - 10 ml of 100X AA
 - 88 ml of sterile water
 - Add to sorbitol solution
5. For growth of his 4 strains you must add histidine to the media. Add 10 ml of 100X H (histidine) to the prewarmed mixture in Step 4. Store liquid medium at +4°C.

3.4 RDB and RDHB Agar Plates

1. Dissolve 186 g of sorbitol in 700 ml of water and add 20 g of agar.
2. Autoclave 20 minutes on liquid cycle.
3. Place the autoclaved solution in at 60°C water bath prior to addition of prewarmed mixture of stock solution. This will keep the medium from becoming too thick to mix reagent.
4. Prepare the prewarmed (45°C) mixture from RDB and RDHB Liquid Media, Step 4. Add to sorbitol/agar solution. If you are selecting for His+ transformants, do not add histidine.
5. Place the solution to 45°C after adding the solutions in Step 4. During transformation, use as a molten solution at 45°C.

3.5 MD and MDH

1.34% YNB

4×10⁻⁵% biotin

2% dextrose

1. For medium, autoclave 800 ml of water for 20 minutes on liquid cycle.
2. Cool to about 60°C and then add:
 - 100 ml of 10X YNB

2 ml of 500X B

100 ml of 10X D

3. To make DE, add 10 ml of 100X H stock solution. Mix and store at +4°C.

4. For plates, add 15 g agar to the water in to Step 1 and proceed.

5. If preparing plate, pour the plates immediately. MD store well for several months at +4°C.

3.6 MM and MMH: Minimal Methanol ± Histidine (1 liter)

1.34% YNB

4×10^{-5} % biotin

0.5% methanol

1. For medium, autoclave 800 ml of water for 20 minutes on liquid cycle.

2. Cool autoclaved water to 60°C and add:

100 ml of 10X YNB

2 ml of 500X B

100 ml of 10X M

3. To make MMH, add 10 ml of 100X H stock solution. Mix and store at +4°C.

4. For plates, add 15 g agar to the water in Step 1 and proceed.

5. After mixing, pour the plates immediately. MM and MMH store well for several months +4°C.

3.7 BMG and BMM: Buffered Minimal Glycerol and Buffered Minimal Methanol (1 liter)

100 mM potassium phosphate, pH 6.0

1.34% YNB

4×10^{-5} % biotin

1% glycerol or 0.5% methanol

1. Autoclave 700 ml water for 20 minutes on liquid cycle.

2. Cool to room temperature, then add the following and mix well:

100 ml 1 M potassium phosphate buffer, pH 6.0

100 ml of 10X YNB

2 ml of 500X B

100 ml of 10X GY

4. For BMM, add 100 ml 10X M instead of glycerol.

5. Store media +4°C. The shelf life of this solution is approximately two months.

3.8 BMGY and BMMY: Buffered Glycerol-complex Medium and Buffered Methanol-complex Medium (1 liter)

1% yeast extract

2% peptone

1% glycerol or 0.5% methanol

1.34% YNB

4×10^{-5} % biotin

1. Dissolve 10 g of yeast extract, 20 g peptone in 700 ml water.

2. Autoclave 20 minutes on liquid cycle.

3. Cool to room temperature, then add the following and mix well:

100 ml 1 M potassium phosphate buffer, pH 6.0

100 ml of 10X YNB

2 ml of 500X B

100 ml of 10X GY

4. For BMMY, add 100 ml 10X instead of glycerol.

5. Store media +4°C. The shelf life of this solution is approximately two months.

Appendix B

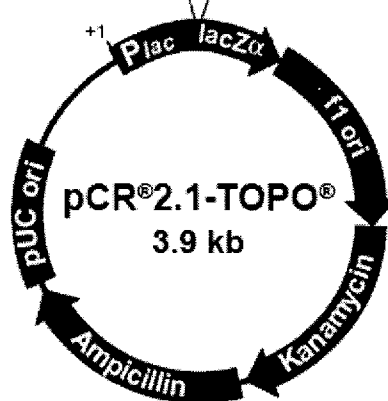
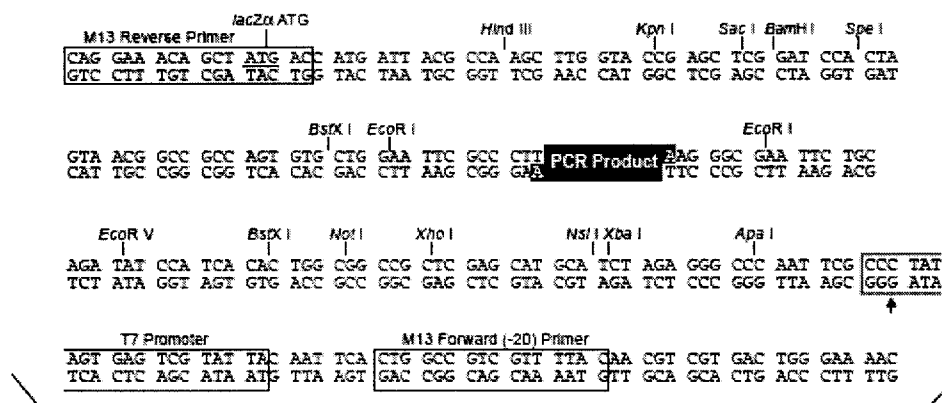
Plasmid vectors

Plasmid vectors

1. TOPO TA Cloning[®]

The plasmid vector (pCR[®]2.1-TOPO[®] (Fig 2.4)) is supplied linearized with: Single 3'-thymidine (T) overhangs for TA Cloning[®], Topoisomerase I covalently bound to the vector (referred to as “activated” vector) and *Taq* polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products. The linearized vector supplied in this kit has single, overhanging 3' deoxythymidine(T) residues. This allows PCR inserts to ligate efficiently with the vector.

Topoisomerase I from *Vaccinia* virus binds to duplex DNA at specific sites and cleaves the phosphodiester backbone after 5'-CCCTT in one strand. The energy from the broken phosphodiester backbone is conserved by formation of a covalent bond between the 3' phosphate of the cleaved strand and a tyrosyl residue (Tyr-274) of topoisomerase I. The phospho-tyrosyl bond between the DNA and enzyme can subsequently be attacked by the 5' hydroxyl of the original cleaved strand, reversing the reaction and releasing topoisomerase.



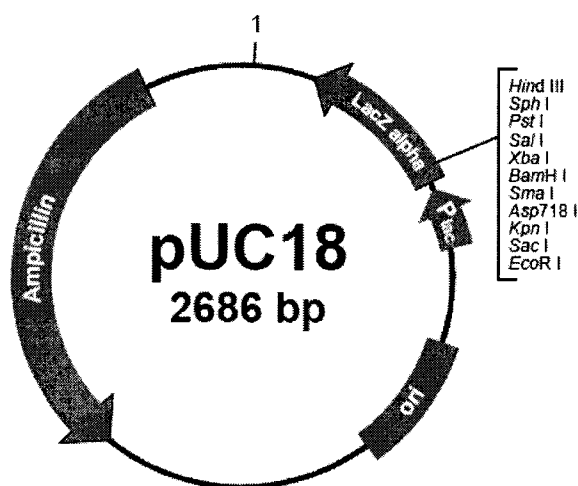
Comments for pCR[®]2.1-TOPO[®]
3931 nucleotides

LacZα fragment: bases 1-547
 M13 reverse priming site: bases 205-221
 Multiple cloning site: bases 234-357
 T7 promoter/priming site: bases 364-383
 M13 Forward (-20) priming site: bases 391-406
 f1 origin: bases 548-985
 Kanamycin resistance ORF: bases 1319-2113
 Ampicillin resistance ORF: bases 2131-2991
 pUC origin: bases 3136-3809

The map shows the features of pCR[®]2.1-TOPO[®] and sequence surrounding the TOPO[®] Cloning site. Restriction sites are labeled to indicate the actual cleavage site

2. pUC 18

pUC 18 vector is small, high copy number, *E. coli* plasmids, 2686bp in length (Fig 2.5). pUC 18 plasmid contain 1) the pMB1 replicon *rep* responsible for the replication of plasmid (source-plasmid pBR322). The high copy number of pUC plasmids is a result of the lack of the *rop* gene and a single point mutation in *rep* of pMB1; 2) *bla* gene, coding for beta-lactamase that confers resistance to ampicillin (source-plasmid pBR322). It differs from that of pBR322 by two point mutations; 3) region of *E. coli* operon *lac* containing CAP protein binding site, promoter *Plac'* *lac* repressor binding site and 5'-terminal part of the *lacZ* gene encoding the N-terminal fragment of beta-galactosidase (source-M13mp18). This fragment, whose synthesis can be induced by IPTG, is capable of intra-allelic (alpha) complementation with a defective form of beta-galactosidase encoded by host (mutation *lacZ*^{DM15}). In the presence of IPTG, bacteria synthesise both fragments of the enzyme and form blue colonies on media with X-Gal. Insertion of DNA into the MCS located within the *lacZ* gene (codons 6-7 of *lacZ* are replaced by MCS) inactivated the N-terminal fragment of beta-galactosidase and abolishes alpha-complementation. Bacteria carrying recombinant plasmids therefore give rise to white colonies.

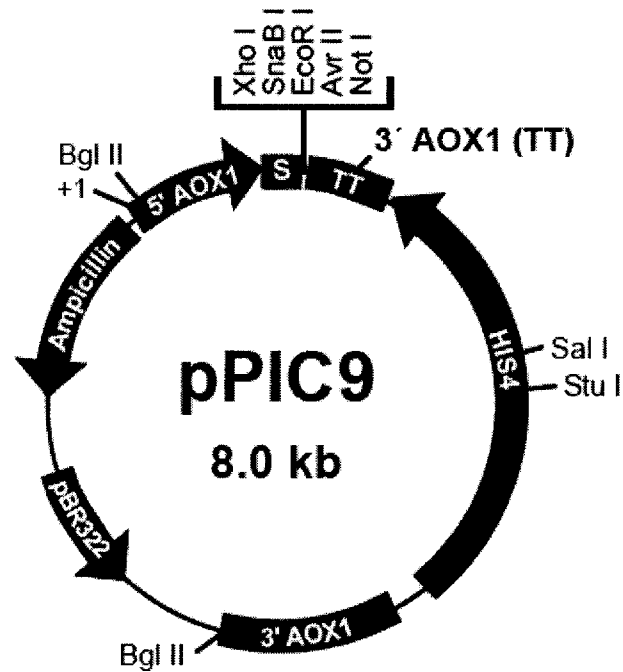


Comments for pUC18
2686 nucleotides

LacZ alpha peptide: bases 149-469 (complementary strand)
lac promoter: bases 470-543 (complementary strand)
 pUC origin: bases 808-1481 (complementary strand)
 Ampicillin (*bla*) resistance gene: bases 1629-2486 (complementary strand)
bla promoter: bases 2487-2585 (complementary strand)

The map shows enzymes that cut pUC18.

3. pPIC9



**Comments for pPIC9:
8023 nucleotides**

5' AOX1 promoter fragment: bases 1-948
 5' AOX1 primer site: bases 855-875
 α -Factor secretion signal(s): bases 949-1215
 α -Factor primer site: bases 1152-1172
 Multiple Cloning Site: bases 1192-1241
 3' AOX1 primer site: bases 1327-1347
 3' AOX1 transcription
 termination (TT): bases 1253-1586
 HIS4 ORF: bases 4514-1980
 3' AOX1 fragment: bases 4870-5626
 pBR322 origin: bases 6708-6034
 Ampicillin resistance gene: bases 7713-6853

The map shows the location and size of each feature pPIC9.

773
 ACAGCAATAT ATAAACAGAA GGAAGCTGCC CTGTCTTAAA CCTTTTTTTT TATCATCATT ATTAGCTTAC
 AOX1 mRNA 5' end (824)

5' AOX1 Primer Site (855-875)
 TTTATAATT GCGACTGGTT CCAATTGACA AGCTTTTGAT TTTAAGCACT TTTAAGACA ACTTGAGAAG

α-Factor (949-1215)
 ATCAAAAAC AACTAATTAT TCGAAGGATC CAAACG ATG AGA TTT CCT TCA ATT TTT ACT GCA
 Met Arg Phe Pro Ser Ile Phe Thr Ala

GTT TTA TTC GCA GCA TCC TCC GCA TTA GCT GCT CCA GTC AAC ACT ACA ACA GAA GAT
 Val Leu Phe Ala Ala Ser Ser Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp

GAA ACG GCA CAA ATT CCG GCT GAA GCT GTC ATC GGT TAC TCA GAT TTA GAA GGG GAT
 Glu Thr Ala Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp

TTC GAT GTT GCT GTT TFG CCA TTT TCC AAC AGC ACA AAT AAC GGG TTA TTG TTT ATA
 Phe Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu Phe Ile

α-Factor Primer Site (1152-1172) Xho I
 AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG GTA TCT CTC GAG AAA AGA
 Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val Ser Leu Glu Lys Arg

Signal cleavage (1204) SmaBI EcoRI AvrII NotI
 GAG GCT GAA GCT TAC GTA GAA TTC CCT AGG GCG GCC GCG AAT TAA TTCCCTTAG
 Glu Ala Glu Ala Tyr Val Glu Phe Pro Arg Ala Ala Ala Asn ***

ACATGACTGT TCCTCAGTTC AAGTTGGGCA CTTACGAGAA GACCGGTCTT GCTAGATTCT AATCAAGAGG

3' AOX1 Primer Site (1327-1347)
 ATGTCAGAAAT GCCATTTGCC TGAGAGATGC AGGCTTCATT TTGATACTT TTTTATTIGT AACCTAATA

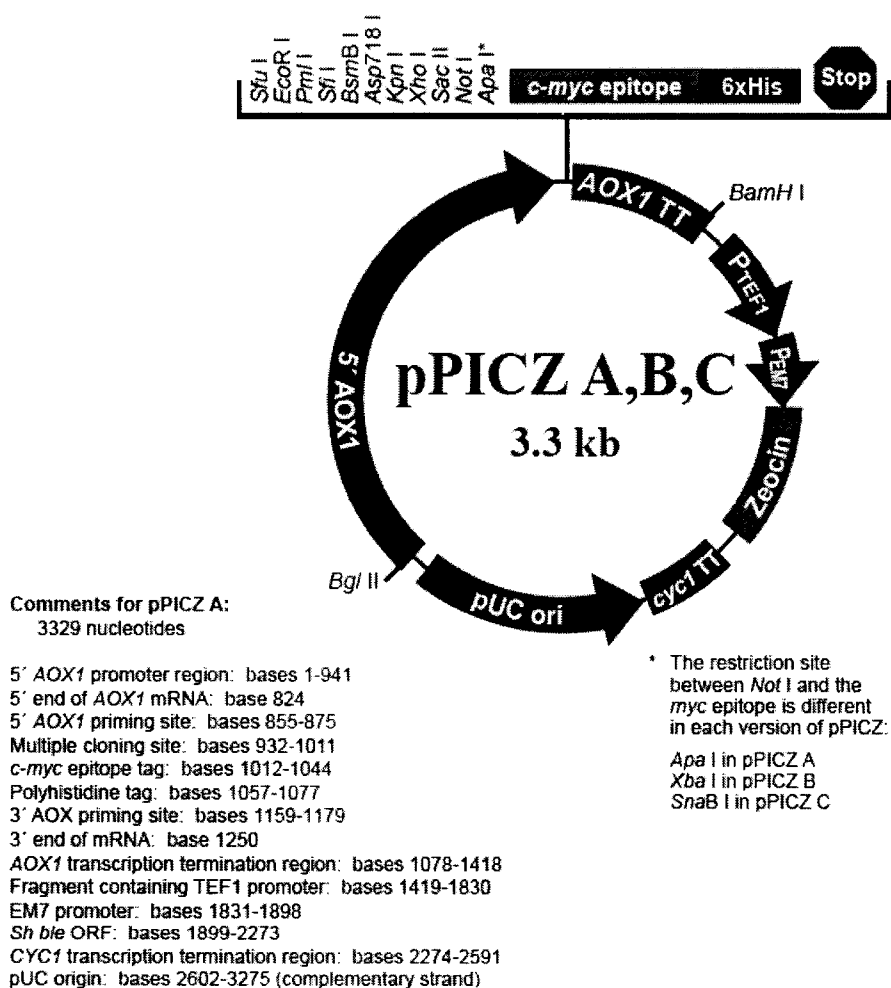
AOX1 mRNA 3' end (1418)
 GTATAGGATT TTTTTTGTC

* If cloning into the Xho I site, the sequence between the Xho I site and SmaB I site (underlined) must be recreated in order for efficient cleavage of the fusion protein to occur.

The sequence shows the details of the multiple cloning sites and surrounding sequences of the pPIC 9 vector.

4. pPICZ A

pPICZ A is 3.3 kb vectors and contains Zeocin™ resistance (*Sh ble*) gene to allow selection of the plasmid using Zeocin™. To propagate and maintain the pPICZ A plasmids. Select transformants on low salt LB plates containing 25 µg/ml Zeocin™ and on yeast's medium containing 100-1,000 µg/ml. (Fig 2.8).



The map shows the feature of the pPICZ A.

pPICZ A Multiple Cloning Site

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          5' end of AOX1 mRNA                                     5' AOX1 priming site
811  AACCTTTTTT TTTATCAICA TATTAGCTT ACTTTCATAA TTGGGACTGG TTCCAATTGA

871  CAAGCTTTTG ATTTTAACGA CTTTAAACGA CAACTTGAGA AGATCAAAAA ACAACTAATT

          Sfu I      EcoR I      Pml I      Sfi I      BsmB I Asp718 I Kpn I Xho I
931  ATTCGAAACG AGGAATTCAC GTGGCCGAGC CGGCCGCTC GGATCGGTAC CTCGAGCCGC

          Sac II Not I      Apa I      myc epitope
991  GCGGCCCGCC AGCTT GGGCCC GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG
          Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu

          Polyhistidine tag
1042 AAT AGC GCC GTC GAC CAT CAT CAT CAT CAT TGA GTTTTAGCCT TAGACATGAC
          Asn Ser Ala Val Asp His His His His His His ***

1099 TGTTCCCTCAG TTCARGTGG GCACTTACGA GAAGACCGGT CTGCTAGAT TCTAATCAAG

          3' AOX1 priming site
1159 AGGATGTCAG AATGCCATTT GCGTGAGAGA TGCAGGCTTC ATTTTGATA CTTTTTATT

          3' polyadenylation site
1218 TGTAACCTAT ATAGTATAGG ATTTTTTTTG TCATTTTGT

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The sequence shows the details of the multiple cloning sites and surrounding sequences of the pPICZ A vector.

Appendix C

Analysis of variance

Statistical analysis of variance for pretreatment of sugarcane bagasse by acid hydrolysis

1. Analysis of variance: size of bagasse

Source	DF	Sum of Squares	Mean Square	F Value	Sig
Glucose Between Groups	3	121.422	40.474	0.189	0.903
With Groups	92	19654.238	213.633		
Total	95	19775.660			
Xylose Between Groups	3	95.139	31.713	0.163	0.921
With Groups	92	17888.348	194.439		
Total	95	17983.487			

2. Analysis of variance: H₂SO₄ concentration

Source	DF	Sum of Squares	Mean Square	F Value	Sig
Glucose Between Groups	3	5175.562	2587.781	16.484	0.000
With Groups	92	14600.098	156.990		
Total	95	19775.660			
Xylose Between Groups	3	4617.347	31.713	16.063	0.000
With Groups	92	13366.140	194.439		
Total	95	17983.487			

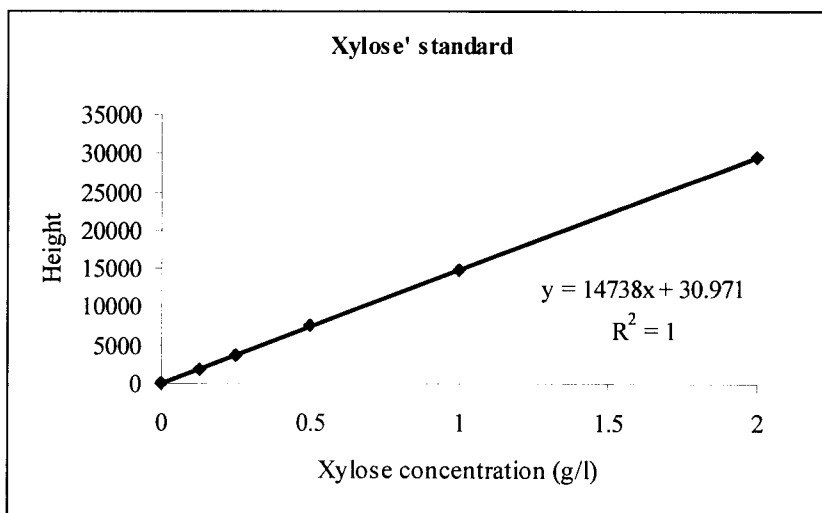
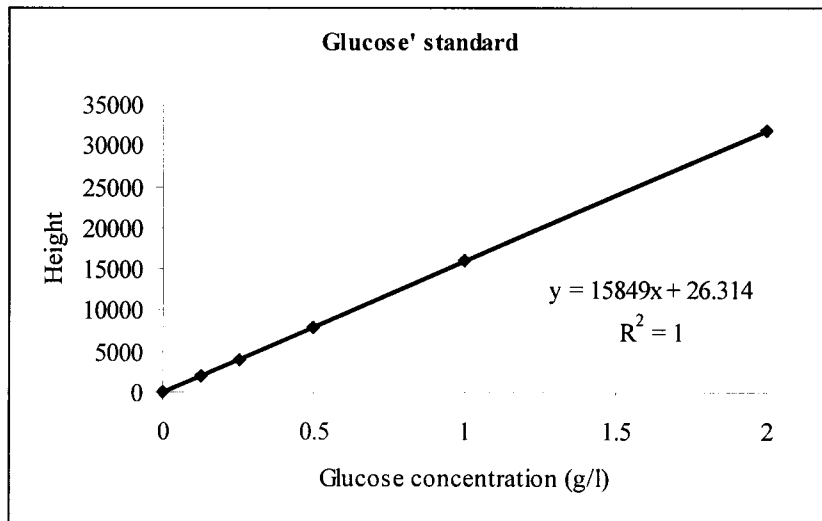
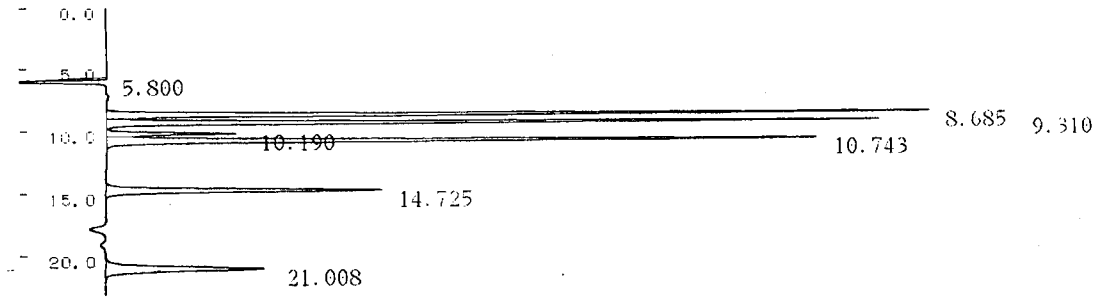
3. Analysis of variance: reaction time for acid hydrolysis

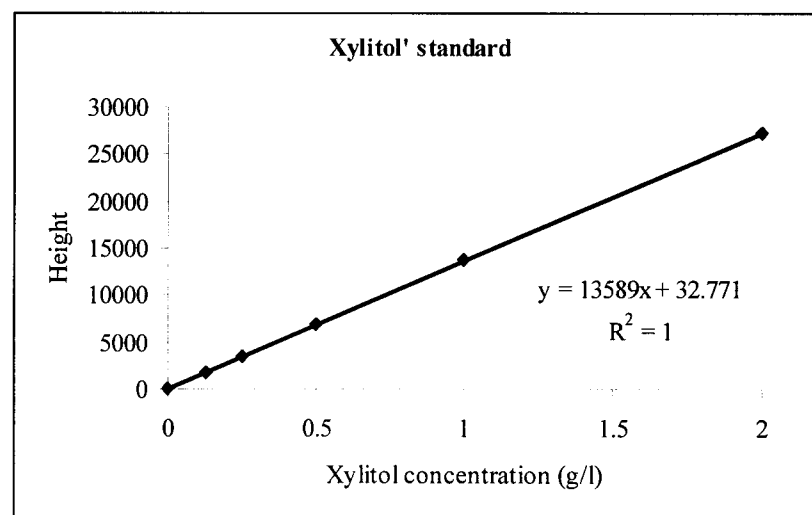
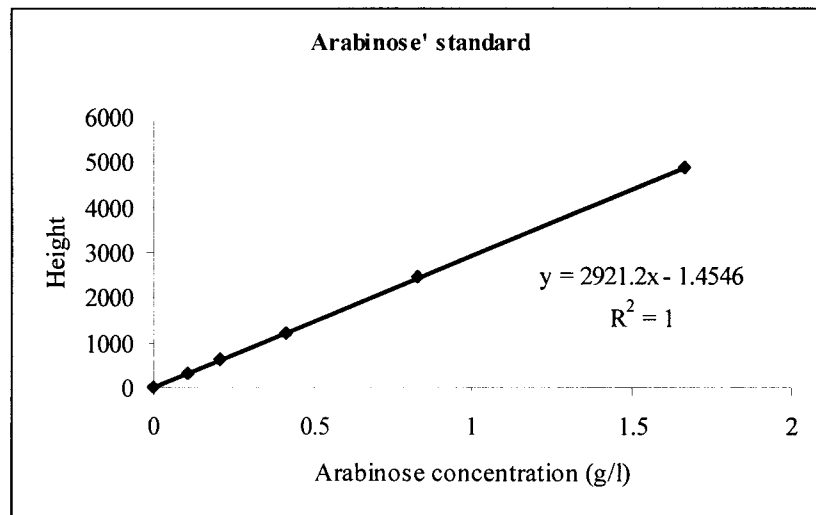
Source	DF	Sum of Squares	Mean Square	F Value	Sig
Glucose Between Groups	3	13661.141	4553.714	68.516	0.000
With Groups	92	6114.519	66.462		
Total	95	19775.660			
Xylose Between Groups	3	12484.284	4161.428	69.619	0.000
With Groups	92	5499.202	59.774		
Total	95	17983.487			

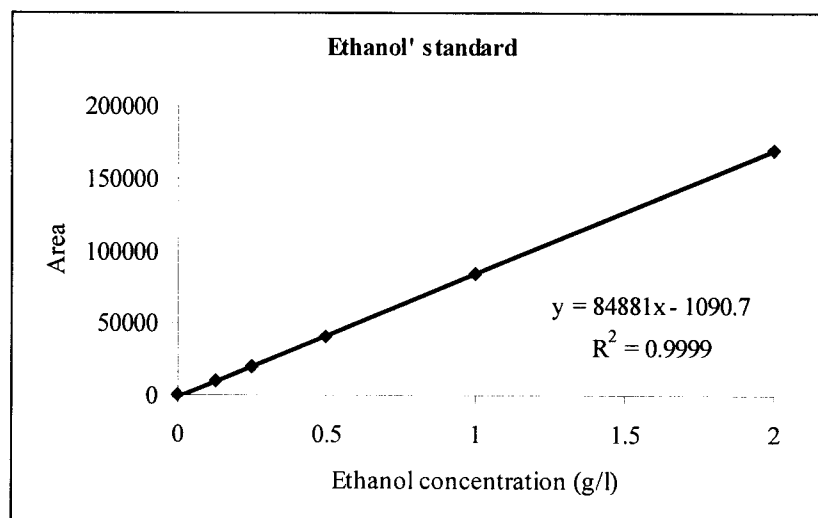
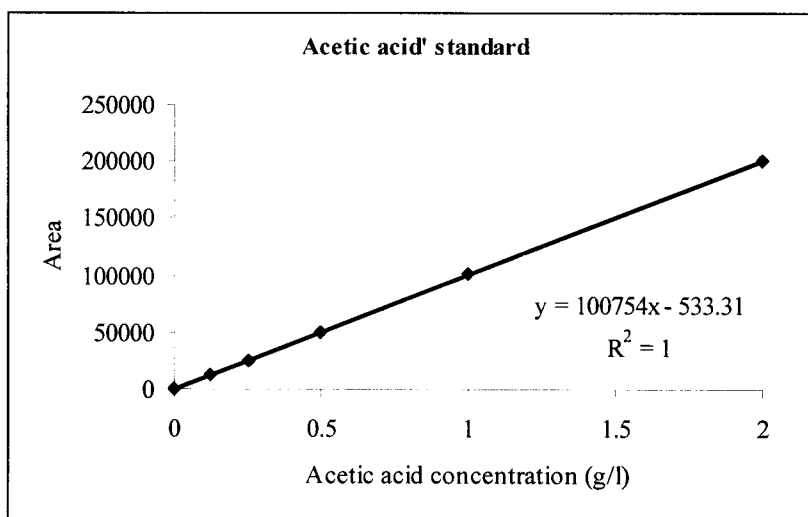
Appendix D

Chromatogram standard

Chromatogram standard for HPLC analysis







Appendix E

Primer Design

Xylose reductase primers design

	5	15	25	35	45	55	65	75	85	95
<i>C. shehatae</i>	-----									
<i>C. tenuis</i> X	-----									
<i>K. lactis</i> X	CTCGAGCTTG	AAACTTGGAA	AACACGGCA	CTACTGACOG	GTTTCTCAAT	GCCCTGCTTT	GAATATGGGA	CGCTGCTACT	ACTGCTCTAC	TGTCCTTCTG
<i>P. guillier</i>	-----									
<i>P. stipitis</i>	-----									
	105	115	125	135	145	155	165	175	185	195
<i>C. shehatae</i>	-----									
<i>C. tenuis</i> X	COGTTTCTCC	TCTACCAACC	TCTOGCAGCC	GGGGCOGTAC	GTCTCTCACC	GAATCTCTCA	CGAATCTCT	CACOGCACOG	CCAACCCAC	GCTGGACCTG
<i>K. lactis</i> X	AGTCCGTGGC	ACAGCGTGAC	TACTCCATGC	CCCTGGACTG	AACCACTGCC	TTTTCAACAA	CGGAATAACA	COGTGTAATA	AGAGGGOGAA	AAGTATCGTA
<i>P. guillier</i>	-----									
<i>P. stipitis</i>	-----									
	205	215	225	235	245	255	265	275	285	295
<i>C. shehatae</i>	-----									
<i>C. tenuis</i> X	CTGCCACTCC	GCACCATCAC	CCCATGAACC	GCACCGAATT	TCTCCACAA	CAACT-TCAC	CACCACCAOG	CCAGTAACCT	CATCCACAG	ACCACTCCAA
<i>K. lactis</i> X	AAATATCGAC	ACCGGGACTT	ACGTTCAAGA	GCATCGGATA	AATGAGGGAA	CAACCGTTAC	TCACATAACA	ATTATTGTTG	CTACTGTGTC	TTCTTCTGTG
<i>P. guillier</i>	-----									
<i>P. stipitis</i>	-----									
	305	315	325	335	345	355	365	375	385	395
<i>C. shehatae</i>	-----									
<i>C. tenuis</i> X	ACAACCACAA	ACGCACCACC	AGACGCTACA	AATTCCCATT	TTTCACCCAC	AACTAAACAC	TCACTCAACA	CCCACACCCC	-ATACCGCTC	CCATGACCTC
<i>K. lactis</i> X	TTGCCTTTTT	TTTCTTTTAC	TGTTTTCTTA	GCAAATCGTA	GCTOGCAACA	TAGCAATATC	AGCACTAGCA	TCOGGAATCA	AGGTTCCATT	ATGTGGGAAA
<i>P. guillier</i>	-----									
<i>P. stipitis</i>	-----	-----	-----	-----	GATCCACAGA	CACTAATTGG	TTCTACATTA	TTGTGTTC	GACACAAACC	CCAGCGTTGG

	405 415 425 435 445 455 465 475 485 495
<i>C. shehatae</i>	-----
<i>C. tenuis</i> X	ATCTCCCGCT CCAAGTCCCA CCTTTCACCT TCCTCACOG-----TCCTCA CCTTCCTCCA CCCTCTTTCC CCATT-AGGC TGAAAAATTA TGGAACAAT
<i>K. lactis</i> X	GAGCGAAGGC AAATTAATAC AGTGTGGCTT GATTGGGAAG TCACTGCACG CACTOGTCTT TCAGCTGGAA TAGCAATATT GTGTACAATA TGTAATGAGT
<i>P. guillier</i>	GAACGCAATC GGATTCCCGT TGTAACATCG GAGTCGAGT-----TGTGCG GCTTAGTCAG CTGGAGCTAT TGATGGTGGG TTTCAACTCA GGOGACAAGG
<i>P. stipitis</i>	CGGTTTCTGT CTGCGTTCCT CCAG-CACCT TCTTGCTCA-----ACCCCA GAAGG-TGCA CACTGCAGAC ACACA-TACA TAOGAGAACC TGGAACAAT

	505 515 525 535 545 555 565 575 585 595
<i>C. shehatae</i>	-----
<i>C. tenuis</i> X	ATCGGT---- TGCGTGGT-----TTTGCT C-COGCCACG ACCTCCCATG GTTAGCTCCA TCCTGGCATG GGTGAAAAAT GGGGTATATA AGTCGGTGGG
<i>K. lactis</i> X	TTAGGCCCTC TTCGCAATA ATGGTGAAGC AAAATAGAGA ATCTACGGAA AACTTGTTAG TTAGATTTTA GTATATAA-G GGAGTGCAA CCTCTACAAG
<i>P. guillier</i>	CTACGC---- TTAAGTGGCA CCACAACGCA AGAAGTTGGC CAATTGAGTG GTCTACACCG TTTCTTGGTT OGCAATAA-G TCTCCATTTT OGCAGCTGGA
<i>P. stipitis</i>	ATCGGT---- GTCGGTGACC GAAATGTGCA A-ACCCAGAC ACGACTAATA AACCTGGCAG CTCCAATACC GCCGACAA-- CAGGTGAGGT GACCGATGGG

	605 615 625 635 645 655 665 675 685 695
<i>C. shehatae</i>	TTCCCCCAG ATATOGTATA AGCTGGAAAG AAGTATCAGT ACCAGTCTAC AGTTGTGTCT AATCCCAGCT TCTTTCTGTA ATCTACTAAC TACATCCACA
<i>C. tenuis</i> X	TTCCCCCAA AGATC--AGA AGTTAGGAAT TGAT----- ----TATCC AGT-AAGTAC CAGGCTAGCT T-TTCCTGTA ATCTACATTA TACATACACA
<i>K. lactis</i> X	TAGCAATTGA TTTTGTACC ATTTGGCAAC TTGTGTT--- ACCACCAACA AGGCAACGAT TGTATTAGTG ATAATATAGC ACGCTAAAGA TAATATOGTA
<i>P. guillier</i>	GTGCGGATTT ACCTTCCCTG GAACAATAGT CGGAAT---- ATGCGACTTG CGGGGATCGC TCCACTATAT AAGAAGGCGC ATCCCCAGAA AATATTTCATC
<i>P. stipitis</i>	GTGCCAATTA ATGTC-TGAA AATTGGGGTA TATAAATATG GCGATTCTCC GGAGAATTTT ----TCAGTT TTCTTTTCAT TTCTCCAGTA TTCTTTTCTA

	705 715 725 735 745 755 765 775 785 795
<i>C. shehatae</i>	ATGAGCCCAA GCCCAATTCC AGCTTTCAAG TTGAACAACG GCCTTGAAAT GCCATCCATC GGTTTTCGGCT GTTGGAAGCT CGACAAATCT ACOGCOGCG
<i>C. tenuis</i> X	ATGAGCGCAA GT---ATCCC AGACATCAA TTGAGCTCCG GCCACTTAAT GCCTTCCATC GGTTTTCGGCT GTTGGAAGCT CGCCAACGCT ACOGCOGCTG
<i>K. lactis</i> X	GAGATGACGT ACTTAGCAGA AACAGTTACT TTAAACAATG GCGAAAAGAT GCGGCTAGTC GGCTTAGGTT GCTGGAAGAT GCCCAACGAC GTTTGTGCOG
<i>P. guillier</i>	ATCAATT--- -----GCTAT GTCTATCAAG TTAAACTCTG GATATGACAT GCCCTCGGTG GGTTTTCGGCT GCTGGAAGGT CGACAATGCC ACCTGTGCOG
<i>P. stipitis</i>	TACAACATA CTACAATGCC TTCTATTAAG TTGAACTCTG GTTACGACAT GCCAGCGTC GGTTTTCGGCT GTTGGAAGGT OGAAGTCGAC ACCTGTCTG

	805 815 825 835 845 855 865 875 885 895
<i>C. shehatae</i>	ACCAGGTCTA CAACGCCATC AAGGCOGGTT ACAGATTGTT OGAAGGTGCC GAGGACTACG GTAACGAACA AGAAGTCGGT GAAGGTGTCA AGAGAGCCAT
<i>C. tenuis</i> X	AACAAGTCTA CCAAGCCATC AAGGCOGGTT ACAGATTGTT OGAAGGTGCC GAGGACTACG GTAACGAAAA GGAAGTCGGT GACGGTGTCA AGAGAGCCAT
<i>K. lactis</i> X	ACCAAATTTA OGAAGCCATT AAGATCGGAT ATOGTTTATT CGATGGTGCC CAAGATTACG CCAACGAGAA AGAAGTTGGA CAGGGTGTCA ACAGAGCCAT
<i>P. guillier</i>	ACACCATCTA CAATGCCATC AAGGTGGGAT ACAGATTATT TGACGGAGCC GAGGATTACG GTAACGAAAA GGAAGTGGGA GATGGTATTA ATAGAGCACT
<i>P. stipitis</i>	AACAGATCTA COGTGCTATC AAGACCGGTT ACAGATTGTT OGAAGGTGCC GAAGATTACG CCAACGAAAA GTTAGTTGGT GCOGGTGTCA AGAAGGCCAT

XR-F Primer 5' GGYTTNGGYT GYTGGAAG 3'

	905	915	925	935	945	955	965	975	985	995
<i>C. shehatae</i>	CGACGAAGGT	ATTGTCACCA	GAGAGGAGAT	CTTCCTCACC	TCCAAGTTGT	GGAACAACCTA	CCAACGACCCA	AAGAACGTCG	AAACCGCCTT	GAACAAGACC
<i>C. tenuis</i> X	CGACGAAGGT	CTTGTCAAGA	GAGAAGAGAT	CTTCCTCACC	TCCAAGTTGT	GGAACAACCTA	CCAACGACCCA	AAGAACGTCG	AGACCGCCTT	GAACAAAACC
<i>K. lactis</i> X	CAAAGAAGGG	CTTGTTAAGA	GAGAGGATTT	AGTTGTTGTC	TCCAAGCTAT	GGAACAGTTT	CCACCATCCG	GACAAOCTAC	CTOGTGCCTT	GGAAAGAACT
<i>P. guillier</i>	CGATGAGGGC	TTGGTTGCCA	GAGATGAGCT	TTTOGTTGTT	TCCAAGCTCT	GGAACTCGTT	CCATGACCCC	AAAAACGTTG	AAAAGGCGTT	GGACAAAACA
<i>P. stipitis</i>	TGACGAAGGT	ATOGTCAAGC	GTGAAGACTT	GTTCCCTTACC	TCCAAGTTGT	GGAACAACCTA	CCAACGACCCA	GACAAOCTCG	AAAAGGCCTT	GAACAGAACC
	1005	1015	1025	1035	1045	1055	1065	1075	1085	1095
<i>C. shehatae</i>	CTCAAGGACC	TTAAGGTGGA	CTACGTTGAC	TTGTTCTTGA	TCCACTTCCC	AATTGCCTTC	AAGTTTGTCC	CAATCGAGGA	GAAATATCCA	CCAGGATTCT
<i>C. tenuis</i> X	CTCGCOGACC	TTAAGGTTGA	CTACGTTGAC	TTGTTCTTGA	TCCACTTCCC	AATTGCCTTC	AAGTTCTGTC	CAATCGAGGA	GAAGTACCCA	CCAGGATTCT
<i>K. lactis</i> X	CTTTCOGATT	TGCAATTGGA	CTATGTTGAC	ATATTCTACA	TCCACTTCCC	ATTGGCCTTC	AAGCCTGTGC	CATTGATGGA	GAAGTATCCT	CCAGGTTTCT
<i>P. guillier</i>	TTGAGOGACT	TGAAGGTGGA	CTACCTTAC	TTGTTCTTGA	TCCACTTCCC	AATTGCCTTC	AAGTTTGTTC	CCTTCGAGGA	GAAATATCCT	CCAGGATTCT
<i>P. stipitis</i>	CTTTCGACT	TGCAAGTTGA	CTACGTTGAC	TTGTTCTTGA	TCCACTTCCC	AGTCACCTTC	AAGTTCTGTC	CATTAGAAGA	AAAGTACCCA	CCAGGATTCT
	1105	1115	1125	1135	1145	1155	1165	1175	1185	1195
<i>C. shehatae</i>	ACTGTGGTGA	CGGTGACAA-	-----	-CTTOGTCTA	CGAAGACGTC	CCAATCTTGG	AGACCTGGAA	GGCCCTCGAG	AAGTTGGTCA	AGGCCGGTAA
<i>C. tenuis</i> X	ACTGTGGTGA	CGGTAAACAA-	-----	-CTTOGTCTA	CGAAGACGTT	CCAATCTTGG	AGACCTGGAA	GGCCCTCGAG	AAGTTGGTTG	CTGCCGGTAA
<i>K. lactis</i> X	ACACOGGTAA	GGAAGACGAA	GCTAAAGGTC	ACATTGAAGA	AGAACAAGTA	CCACTATTGG	ACACTTGGAG	GGCTTTAGAA	AAACTAGTOG	ACCAAGGTAA
<i>P. guillier</i>	ACTGTGGAGA	TGGGGACAA-	-----	-GTTCCACTA	CGAGGACGTC	CCACTCATOG	ACACCTGGAG	AGCATTGGAG	AAGTTGGTGG	AGAAGGGTAA
<i>P. stipitis</i>	ACTGTGGTAA	GGGTGACAA-	-----	-CTTOGACTA	CGAAGATGTT	CCAATTTTAG	AGACCTGGAA	GGCTCTTGAA	AAGTTGGTCA	AGGCCGGTAA
	1205	1215	1225	1235	1245	1255	1265	1275	1285	1295
<i>C. shehatae</i>	GATTAGATCC	ATOGGTGTCT	CCAACTTCCC	AGGTGCTTTA	CTCTTGGACT	TGTTTCAGAGG	TGCCACCATC	AAGCCTGCTG	TTTTGCAAGT	TGAGCACCAC
<i>C. tenuis</i> X	GATTAAGTCC	ATOGGTGTCT	CTAACTTCCC	AGGTGCTTTG	CTCTTGGACT	TGCTCAGAGG	TGCTACCATC	AAGCCAGCTG	TCTTGCAAGT	TGAGCACCAC
<i>K. lactis</i> X	GATCAAGTCC	TTGGGTATTT	CCAACTTTTC	AGGTGCGTTG	ATCCAAGATT	TGCTACGTTG	TGCTCGTATC	AAGCCAGTTG	CCCTACAAAT	OGAGCATCAT
<i>P. guillier</i>	AATCAGATCC	ATTGGTATTT	CCAACTTTAG	TGGTGCCTTG	ATCCAGGACT	TGTTGAGAAG	TGCCAAAATC	AAGCCAGCAG	TGTTGCAGAT	CGAACACCAC
<i>P. stipitis</i>	GATCAGATCT	ATOGGTGTTT	CTAACTTCCC	AGGTGCTTTG	CTCTTGGACT	TGTTGAGAGG	TGCTACCATC	AAGCCATCTG	TCTTGCAAGT	TGAACACCAC
	1305	1315	1325	1335	1345	1355	1365	1375	1385	1395
<i>C. shehatae</i>	CCATACTTGC	AACAACCAAA	GTTGATTGAG	TACGCTCAAA	AGGTGGTAT	CACTGTCACC	GCTTACTCTT	CTTTCGGTCC	TCAATCTTTC	GTTGAGATGA
<i>C. tenuis</i> X	CCATACTTGC	AACAACCAAA	GTTGATCGAG	TTGCTCAAA	AGGCCGGTGT	TACCATCACC	GCCTACTCTT	CTTTCGGTCC	TCAATCTTTC	GTTGAGATGA
<i>K. lactis</i> X	CCATATTTGA	CACAGGAAAG	ATTGATTTAA	TACGTCGAAG	ATGCOGGCAT	TCAAGTGGTT	GCCTACTCCT	OGTTCGGTCC	AGTGTGTTTC	CTGGAATTGG
<i>P. guillier</i>	CCTTACTTGC	AGCAACCAAG	ATTGGTTGAG	TAOGTTCAAT	CTCAAGGCAT	OGCCATCACC	GCATACTGTT	CTTTCGGACC	CCAATCTTTC	GTGGAATTGG
<i>P. stipitis</i>	CCATACTTGC	AACAACCAAG	ATTGATCGAA	TTGCTCAAT	CCOGTGGTAT	TGCTGTCACC	GCTTACTCTT	OGTTCGGTCC	TCAATCTTTC	GTTGAATTGA

	1405	1415	1425	1435	1445	1455	1465	1475	1485	1495
C. shehatae	ACCAAGGTAG	AGCTTTGAAC	ACCCCAACCT	TGTTTOGAACA	TGAOGTCAIT	AAGGCTATTG	CTGCCAAGCA	CAACAAAGTC	CCAGCCGAGG	TTTTTGTGAG
C. tenuis X	ACCAAGGTAG	AGCTTTGAAC	ACCCCAACCT	TGTTTOGCACA	CGACACCATC	AAGGCTATTG	CTGCCAAGTA	CAACAAGACC	CCAGCTGAGG	TTTTTATTGAG
K. lactis X	AAAACAAGAA	AGCTCTTAAC	ACCCCTACTT	TGTTTGTAGCA	TGACACGATT	AAGTCTATCG	CTTCCAAGCA	CAAAGTCACT	CCACAACAAG	TTCTGTGTAG
P. guillier	ACCACCCTOG	TGTCAAGGAT	GTCAAGCCAT	TGTTTOGAGCA	CGACGTCATC	AAGTCCGTTG	CTGGCAAAGT	CAAGAAGACC	CCAGCACAGG	TGTTGTGAG
P. stipitis	ACCAAGGTAG	AGCTTTGAAC	ACTTCTCCAT	TGTTTOGAGAA	CGAAACTATC	AAGGCTATCG	CTGCTAAGCA	GGTAAGTCT	CCAGCTCAAG	TCTTGTGAG
	1505	1515	1525	1535	1545	1555	1565	1575	1585	1595
C. shehatae	ATGGTCCGCT	CAAAGAGGCA	TAGCTGTCAT	TCCAAAGTCT	AACCTTCCAG	AGAGATTAGT	TCAAAAACAGA	AGTTTCAACG	A--CTTCGAG	TT-GACCAAG
C. tenuis X	ATGGGCGGCC	CAAAGAGGTA	TOGCTGTCAT	TCCAAAGTCT	AACCTCCCAG	AGAGATTAGT	CCAAAACAGA	AGTTTCAACA	C--CTTCGAC	TT-GACCAAG
K. lactis X	ATGGGCCACA	CAAAATGGCA	TTGCCATCAT	TCCAAAATCA	TCAAAGAAGG	AAAGATTGCT	GGATAACTTG	AGGATCAACG	ATGCATTGAC	TTTGACCGAT
P. guillier	ATGGGCCACT	CAAAGAGGAC	TTGCCGTGAT	TCCCAAGTOG	AACAATCCCG	ATAGGTTGTT	GAGCAACTTG	AAGGTGAACG	A--CTTTGAT	TT-GTCGCAA
P. stipitis	ATGGTCTTCC	CAAAGAGGCA	TTGCCATCAT	TCCAAAGTCC	AACACTGTCC	CAAGATTGTT	GGAAAACAAG	GACGTCAACA	G--CTTCGAC	TT-GGACGAA
	1605	1615	1625	1635	1645	1655	1665	1675	1685	1695
C. shehatae	GAGGACTTTG	AGGAAATCTC	CAAGTTGGAC	ATCAACTTGA	GATTCAACGA	CCCATGGGAC	TGG---GACA	ACA---TTCC	AATCTTCGTT	TAAGCGTGCT
C. tenuis X	GAGGACTTGG	AGGAAATCGC	CAAGTTGGAC	ATGGGCTTGA	GATTCAACGA	CCCATGGGAC	TGG---GACA	ACA---TTCC	AATCTTCGTT	TAAGCGTGCT
K. lactis X	GATGAATTGA	AGCAAATTTT	GGGCTTGAAT	CAAAATATCA	GATTCAATGA	CCCTTGGGAA	TGGCTAGACA	ACGAATTCCC	AACTTTCATC	TAATTACACT
P. guillier	GAAGACTTCC	AAGAAATCTC	CAAGTTGGAC	ATTGAATTGA	GATTCAACAA	TCCTTGGGAC	TGG---GACA	AGA---TTCC	AACTTTCATC	TAAGTTGGCC
P. stipitis	CAAGATTTGG	CTGACATTGC	CAAGTTGGAC	ATCAACTTGA	GATTCAACGA	CCCATGGGAC	TGG---GACA	AGA---TTCC	TATCTTCGTC	TAAGAAGGTT
					XR-R Primer					
					CTAAGTTRYT RGGWACCCTK AC					
					5'CA KTCCCAWGR TYRTGAATC 3'					
	1705	1715	1725	1735	1745	1755	1765	1775	1785	1795
C. shehatae	TGTTGCAGAA	GAAGAAG-AA	TGCGACAAA	AATGCOGA-A	TCAATG----	-GGTTCAATG	TCATAATTAG	AAACTATATA	TAAATACTAT	T----AATTT
C. tenuis X	TGC-----A	GAAGACG-GA	CGGTGGCAA	A-TGCOGA-A	TCAATG----	-GGTTCAATG	TCATAATTAG	AAACTACATA	TAAATACTAT	T----AATTT
K. lactis X	AATGCAGATG	GAATTCACGA	CATAATTGAT	GCCACTCACC	TATATATTCC	TGCTCT----	-----	-----	-----	-----
P. guillier	TTT----ATA	GAAAATC-AC	CAAAATTTAT	GTTGCTAACA	TATACGTT-G	TAAACCTATA	TAGOGTCCAA	CACTGGCTGG	GGCCAATGT	TCAACAACCT
P. stipitis	GCTTTATAGA	GAGGAAA-TA	AAACCTAATA	TACATTGA-T	TGTACATTTA	AAATTGAATA	TTGTAGCTAG	CAGATTGCGA	AATTTAAAAT	GGGA-AGGTG
	1805	1815	1825	1835	1845	1855	1865	1875	1885	1895
C. shehatae	AGCCCATTAT	TTATAACCTG	CTTTCITTTG	GTAGA-ACTA	-ACACCATAA	GCCCAGTAAT	AACC----CG	AAATATCTCT	GAAAA-ATCC	CAATTTTTTT
C. tenuis X	AGCCCATTAT	TTG-AATCTT	GTTTAATTCT	GTAGA-ACTA	-TCACCATAA	CCCCAACAAA	TATC----CC	TATTCTTACT	GAATA-AT--	CAACCTTTTT
K. lactis X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
P. guillier	GAGGCGTGAA	ACAAAGTCAT	CCOGATTGAT	GTATGCTCOG	CACATTCTTC	TCTTTCCGGT	GAAC---TCG	GTTCTOGAGT	GGAAACATCT	CCAGTTGTG
P. stipitis	ATTCTATCOG	TACGAATGAT	CTCTATGTAC	ATACACGTTG	-AAGATAGCA	GTACAGTAGA	CATCAAGTCT	ACAGATCATT	AAACATATCT	TAAATTGTAG

	1905	1915	1925	1935	1945	1955	1965	1975	1985	1995
<i>C. shehatae</i>	TT---TCTTG	ATCTCTCTCA	CCACATCACC	AGCATCAT--	AACAACACCA	AAATCACCCC	ATTTCATCCAT	CATCAACACC	CATAAAACAA	TATAACCATC
<i>C. tenuis</i> X	TG---TCITA	ATCCTTCTCG	TCCCATCATC	ACCATCAC--	AACCGAGT--	ACCTTAC---	-----	-----	-----	-----
<i>K. lactis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. guillier</i>	AA---AATAA	GGCATT--GC	CCAGGACGCA	ACTGGTAG--	AACAAT----	-----	-----	-----	-----	-----
<i>P. stipitis</i>	AAAACATAAA	ACTTTTCAAT	TCAAACCATG	TCTGCCAAGG	AATCAAATGA	GATTTTTTTTC	GCAGCCAAAC	TTGAATCCAA	AAATAAAAAA	CGTCATTGTC
	2005	2015	2025	2035	2045	2055	2065	2075	2085	2095
<i>C. shehatae</i>	AGATCAAACA	ACCAAACAAA	ATGCTTTTTCG	CCATACAAAA	A-----	-----	-----	-----	-----	-----
<i>C. tenuis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>K. lactis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. guillier</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. stipitis</i>	TGAAACAAC	CTATCTTATC	TTTCACCTCA	TCAATTCATT	GCATATCATA	AAAGCCTCOG	ATAGCATACA	AAACTACTTC	TGCATCATAT	CTAAATCATA
	2105	2115	2125	2135	2145	2155	2165	2175	2185	2195
<i>C. shehatae</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>C. tenuis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>K. lactis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. guillier</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. stipitis</i>	GTGCCATATT	CAGTAACAAT	ACCGGTAAGA	AACTTCTATT	TTTTTAGTCT	GCCTTAACGA	GATGCAGATC	GATGCAACGT	AAGATCAAAC	CCCTCCAGTT
	2205	2215	2225	2235	2245	2255	2265	2275	2285	2295
<i>C. shehatae</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>C. tenuis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>K. lactis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. guillier</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. stipitis</i>	GTACAGTCAG	TCATATAGTG	AACACCGTAC	AATATGGTAT	CTAOGTTCAA	ATAGACTCCA	ATACAGCTGG	TCTGCCCAAG	TTGAGCAACT	TTAATTTAGA
	2305	2315	2325	2335	2345	2355	2365	2375	2385	2395
<i>C. shehatae</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>C. tenuis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>K. lactis</i> X	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. guillier</i>	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
<i>P. stipitis</i>	GACAAAGTCG	TCTCTGTTGA	TGTAGGCACC	ACACATTCTT	CTCTTGCCCG	TGAACTCTGT	TCTGGAGTGG	AAACATCTCC	AGTTGTCAAA	TATCAAACAC

	2405	2415	2425	2435
C. shehatae	-----	-----	-----	-----
C. tenuis X	-----	-----	-----	-----
K. lactis X	-----	-----	-----	-----
P. guillier	-----	-----	-----	-----
P. stipitis	TGACCAGGCT	TCAACTGGTA	GAAGATTTTCG	TTTTTCGG

Appendix F
Research Publication

Research Publication

Chaowarat, M., Leelavatcharamas, V., Connerton, I.F., and Danvirutai, P. 2005. Acid of sugarcane bagasse for substrate preparation of xylitol production. The 1st International Conference on “Fermentation Technology for Value Added Agricultural Products”.(22-25 March 2005), Khon Kaen, Thailand. [Poster Presentation; Proceeding]