CHAPTER 9

References

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Appendices

APPENDIX A

Buffers, Solutions, Reagents and Culture media

Agarose diffusion assay (ADA) medium

1.0% (w/v)	Type II agarose (Sigma A-6877)
0.01% (w/v)	Polygalacturonic acid (PGA) (Sodium polypectate, Sigma P-1879)
0.5% (w/v)	Ammonium oxalate (Sigma A-8545)

For 100 ml of buffer with pH 4.6:

26.7 ml	0.1 M Citric acid
23.2 ml	0.2 M Na_2HPO_4

ADA (Modified)

0.8%	Agarose
0.5%	PGA
100 mM	Sodium acetate buffer (pH 4.7)

Develop plate with 6 N HCl for a few minutes until zones appear.

Antibiotic stocks

Ampicillin	100 mg in 1 ml dH_2O
Cefotaxime	250 mg in 1 ml dH_2O
Gentamycin	50 mg in 1 ml dH_2O
Kanamycin	50 mg in 1 ml dH_2O
Rifampicin	25 mg in 1 ml 100% methanol

All antibiotics dissolved in dH_2O were filter-sterilised through 0.2 µm sterile filters. Rifampicin was made fresh before use. Antibiotic stocks were stored in aliquots at -20°C.

Ca^{2+} /Mn^{2+} solution for the preparation of competent *E. coli* cells

40 mM	NaAc
100 mM	$CaCl_2$
70 mM	$MnCl_2.4H_2O$

Adjust to pH 5.5 with HCl, be careful not to over-acidify the solution as precipitation will occur. Filter-sterilise and store at 4°C.

Citrate / Phosphate buffer

For 100 ml of buffer with the following pH:

pH 4.6	pH 6.0	
26.7 ml	17.9 ml	0.1 M Citric acid
23.3 ml	32.1 ml	0.2 M Na_2HPO_4
50 ml	50 ml	dH_2O

2% CTAB DNA extraction buffer

2% (w/v)	CTAB (Hexadecyl trimethyl ammonium bromide)
1.4 M	NaCl
20 mM	EDTA
100 mM	Tris (pH 8.0)
0.2% (v/v)	β-mercaptoethanol, added just before use.

2% CTAB DNA extraction buffer with PVP

2% (w/v)	CTAB (Hexadecyl trimethyl ammonium bromide)
1.4 M	NaCl
20 mM	EDTA
100 mM	Tris (pH 8.0)
0.2% (v/v)	β-mercaptoethanol
1% (w/v)	PVP (Polyvinyl pyrrolidone) (Sigma PVP-40)

10% CTAB

0.7 M	NaCl
10% (w/v)	CTAB

Dellaporta DNA extraction buffer

100 mM	Tris (pH 8.0)
0.5 M	NaCl
50 mM	EDTA (pH 8.0)
0.07% (v/v)	β-mercaptoethanol

Denaturation solution

0.4 N	NaOH
0.6 M	NaCl

Depurinating solution

0.25 M HCl

DIG Blocking buffer

1% (w/v) Blocking reagent (Roche Diagnostics) in maleic acid buffer.

DIG Detection buffer

0.1 M Tris-HCl
0.1 M NaCl
pH 9.5

DIG Maleic acid buffer

0.1 M Maleic acid
0.15 M NaCl
pH 7.5

DIG Washing buffer

0.3% (v/v) Tween 20 in maleic acid buffer.

Ethidium bromide

Dissolve 10 mg ethidium bromide powder in 1 ml dH_2O. Cover tube with aluminium foil and store at 4°C.

0.1 N HCl/ 70% Ethanol

For 600 ml:

6 ml 10 N HCl
420 ml Ethanol
174 ml dH_2O

2× LB Medium for competent cells

Composition per l:

20 g Tryptone
10 g Yeast extract
1 g NaCl

Adjust the pH to 7.0 and autoclave. Before use, add $1/100^{th}$ volume 20% sterile glucose.

Luria-Bertani (LB) Medium

Composition per l:

10 g Tryptone

5 g Yeast extract

5 g NaCl

For LB agar, add 15 g Bacto-agar.

Minimal salts medium

Composition per 100 ml:

0.2 ml 1 M $MgSO_4.7H_2O$

1 ml 0.001% $MnSO_4.H_20$

2.5 ml 1 M KNO_3

1 ml 0.01% $ZnSO_4$

1 ml 0.0015% $CuSO_4$

1 ml 0.01% Fe $SO_4.7H_2O$

91.5 ml Citrate-PO_4 buffer (pH 6.0)

Add 1% (w/v) pectin (Sigma P-9135, washed with 0.1 N HCl/ 70% Ethanol and dried) to the citrate-phosphate buffer (pH 6.0) and autoclave. Add the filter-sterilised salts just before use.

Miniprep Solution I

25 mM Tris-HCl, pH 8.0

10 mM EDTA

50 mM Glucose

Miniprep Solution II

0.2 N NaOH

1% SDS

Miniprep Solution III

2.5 M Potassium acetate

2.5 M Acetic acid (MW = 60.05g/mol; 1 l = 1.05 kg ∴ 3.574 ml per 25 ml solution III)

pH 5.4 with Acetic acid

Modified soil extract agar (MSEA)

Composition per l:

a. Soil extract

Boil 1 kg of soil in 1 litre of water for 30 min. Filter through filter paper. Use 24 ml of filtrate per litre of MSEA medium.

b. Agar

12 g Agar nr. 3

2 g Poligalacturonic acid (PGA)

1.5 g KH_2PO_4

4.0 g K_2HPO_4

c. Salts

0.2 g KH_2PO_4

0.1 g KCl

0.1 g $MgSO_4.7H_2O$

0.002 g $FeSO_4.7H_2O$

0.4 g $NaNO_3$

Mix a, b and c. Add 1 ml Tergitol and 966 ml dH_2O. Stir while heating to dissolve. Autoclave for 20 min.

d. Antibiotics

0.006 g Biotin

0.06 g Chloramphenicol

0.06 g Tetracycline hydrochloride

0.06 g Streptomycin

Dissolve in 10 ml methanol. Filter-sterilise and add to cooled medium.

1× MS media

1× MS salts (M5519 from Sigma; or CN2230 from Highveld Biologicals)

3% (w/v) Sucrose

0.8% (w/v) Agar

Adjust pH to 5.9 before adding agar, autoclave. Add the appropriate antibiotics after cooling.

Neutralisation solution

0.5 M Tris (pH 7.5)

1.5 M NaCl

1% PAHBAH reagent

5% *p*-hydroxybenzoic acid hydrazide (PAHBAH) in 0.5 M HCl, store at -20°C.

Just before use, mix 1 volume of 5% PAHBAH in 0.5 M HCl with 4 volumes of 0.5 M NaOH to give a final PAHBAH concentration of 1%.

0.42% PGA (in a sodium phosphate/ citric acid buffer, pH 4.6)

For 10 ml:

42 mg PGA (Sodium polypectate, Sigma P-1879)

2.33 ml 0.2 M $NaHPO_4$

2.67 ml 0.1 M Citric acid

5 ml dH_2O

Aliquot and store at -20°C.

Potassium phosphate buffer pH 5.8

Per 100 ml of buffer:

0.85 ml 1 M K_2HPO_4

9.15 ml 1 M KH_2PO_4

Rindite

7 vol. 2-Chloro-ethanol

3 vol. 1,2 Dichloro-ethanol

1 vol. Carbon tetrachloride

Place 300 µl of this mixture per kg potatoes on a piece of cotton wool. Seal in plastic bag with potatoes for 48 hr. Remove potatoes from bag and place at 25°C.

RNase A (10 mg/ml)

Dissolve 10 mg RNAse A in 1 ml 1× TE buffer, pH 8.0. Heat to 100°C for 10 min. Allow to cool slowly to room temperature. Store at -20°C.

20× SSC

3 M NaCl

0.3 M Sodium citrate

pH 7.4

Stringency wash buffer I

2× SSC

0.1% SDS

Stringency wash buffer II

0.5× SSC

0.1% SDS

50× TAE

Composition per l:

242 g Tris hydroxy methyl aminoethane (Tris)

57.1 ml Glacial acetic acid

100 ml 0.5 M EDTA (pH 8.0)

For 0.5× TAE: 20 ml 50× TAE and 1980 ml dH_2O.

1× TE buffer (pH 8.0)

10 mM Tris-HCl (pH 8.0)

1 mM EDTA (pH 8.0)

1× TNE buffer

10 mM Tris-HCl (pH 8.0)

1 mM EDTA (pH 8.0)

0.2 M NaCl

pH 7.4

5% X-gal

Dissolve 50 mg 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) in 1 ml 100% dimethylformamide (DMF). Store in dark at -20°C. Plate out 35 µl per petridish.

APPENDIX B

Primers used in this study

Primer	Length (bp)	Sequence 5' - 3'	T_m[a] (°C)	% GC
AP-PGIP-INVR	25	AGG TTC TTG AGT TGG CTG AGG AAG T	74	48
AP-PGIP-L2	23	GCA GCC ATG GAA CTC AAG TTC TC	70	52
AP-PGIP-R	30	CCC GGA TCC ATC TGC AGT TGT GGC CAT TAC	94	57
GSTreverse	38	AAA CTG CAG[b] CCA TGT CGA C[c]TG TTA ATA CTG TGT TTT TC	54[d]	39
NPTII-L	21	GAG GCT ATT CGG CTA TGA CTG	64	52
NPTII-R	21	ATC GGG AGC GGC GAT ACC GTA	68	62
pBI121 Seq.primer 2	20	GAC GCA CAA TCC CAC TAT CC	62	55
PUC/M13-40F	17	GTT TTC CCA GTC ACG AC	52	53
PUC/M13R	17	CAG GAA ACA GCT ATG AC	50	47
SK	20	CGC TCT AGA ACT AGT GGA TC	60	50
T3	24	GCG CGA AAT TAA CCC TCA CTA AAG	70	46
T7	20	TAA TAC GAC TCA CTA TAG GG	56	40
U19F	19	GTT TTC CCA GTC ACG ACG T	58	53

a – T_m calculated using formula: T_m (°C) = 4(GC) + 2(AT)

b – *Pst*I restriction enzyme recognition site

c – *Sal*I restriction enzyme recognition site

d – T_m calculated using *Primer Designer Version 3.0* (Scientific and Educational Software).

APPENDIX C

Plasmid maps

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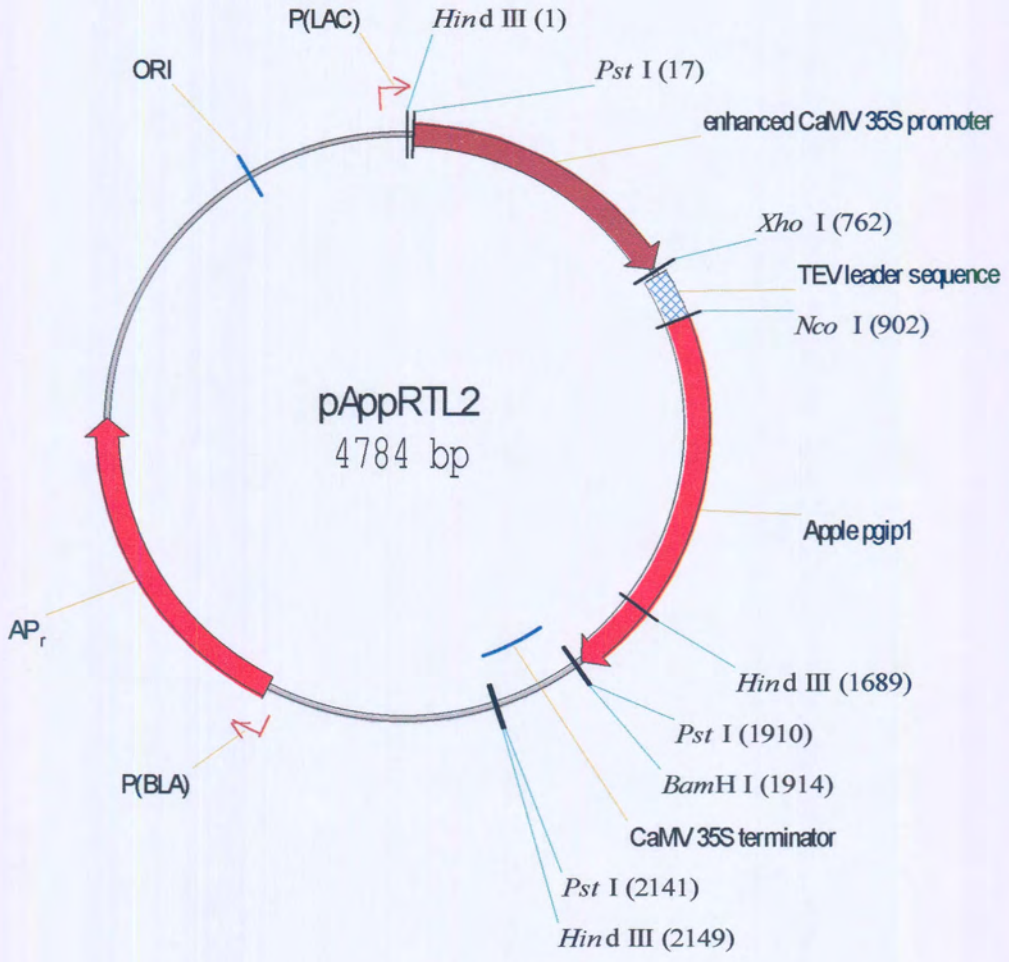

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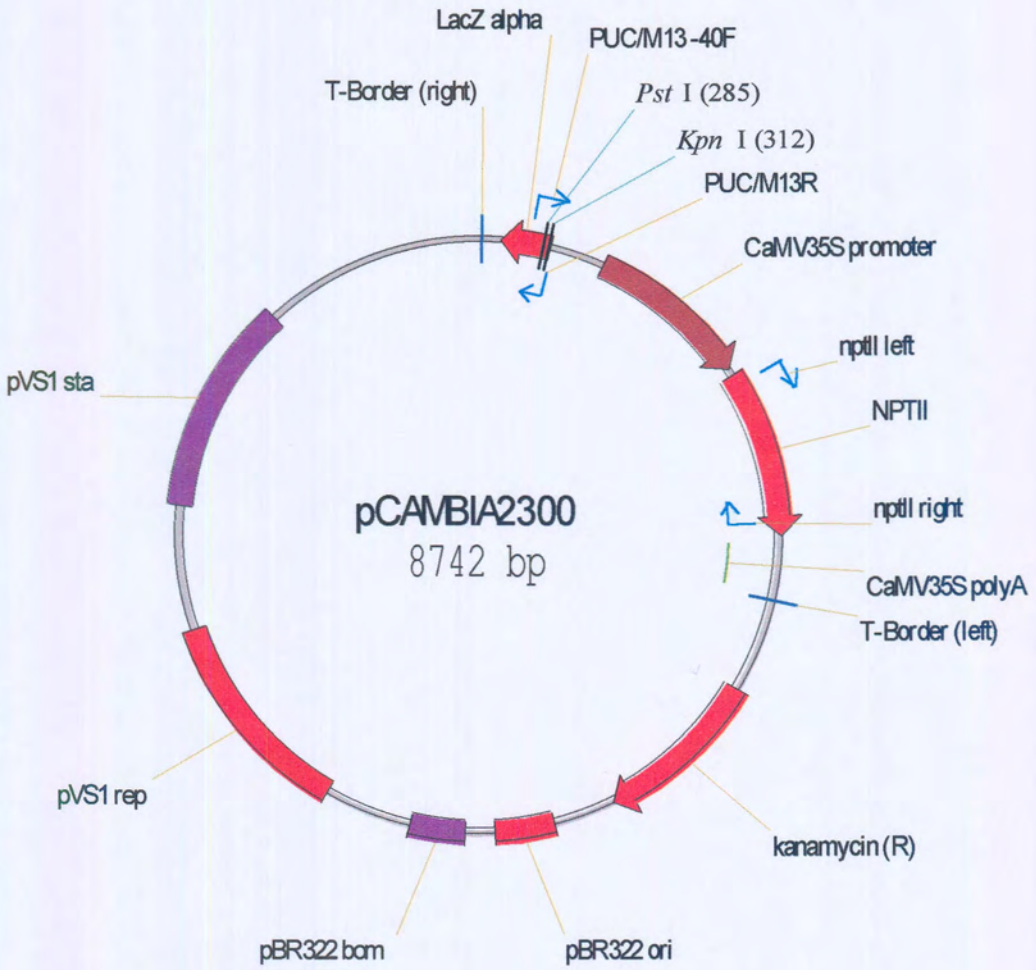

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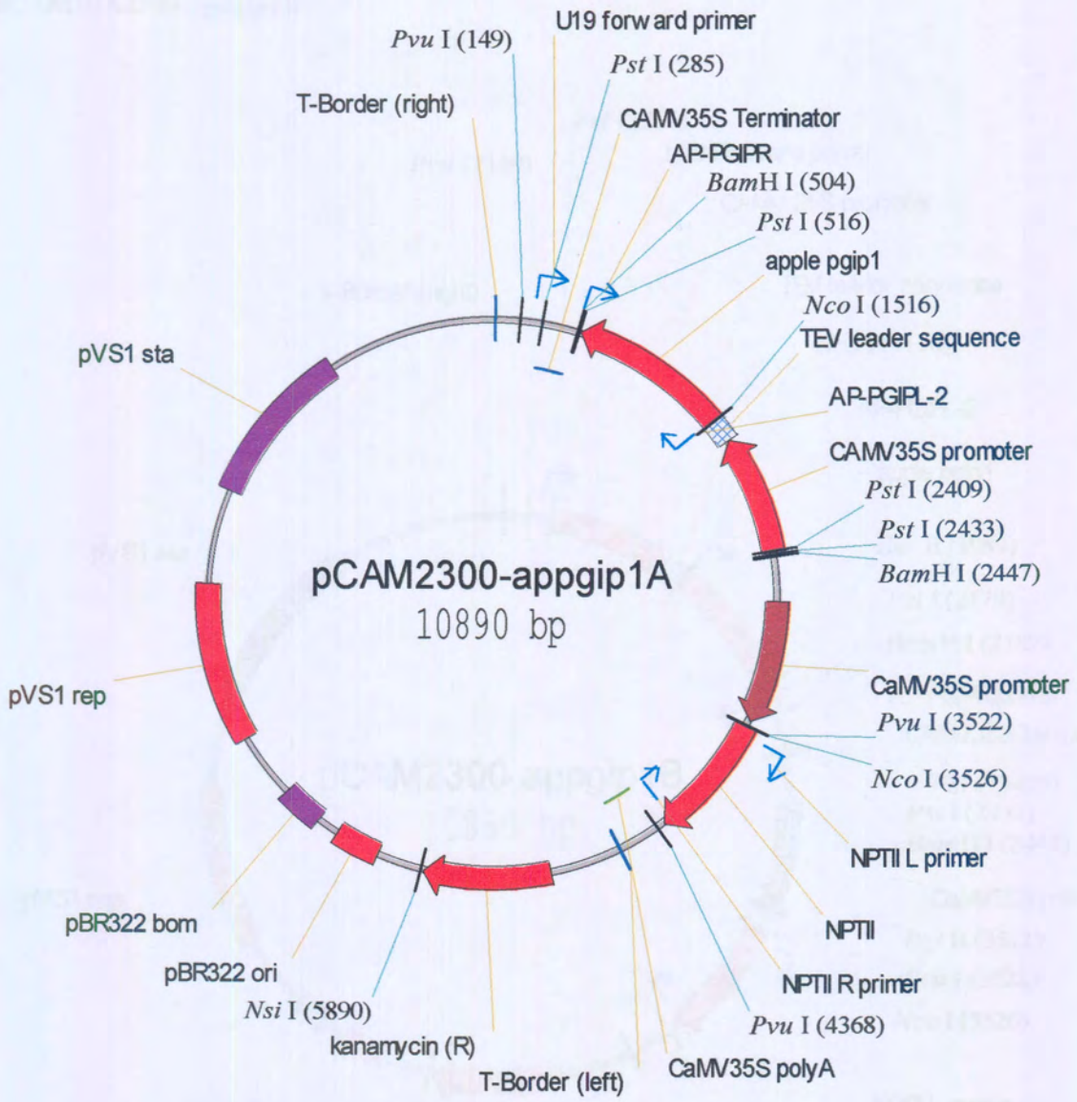

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