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APPENDIX

APPENDIX

1. Bacterial Media

1.1 LB Medium (per liter)

10g Bacto®-tryptone

5g Bacto®-yeast extract

5g NaCl

Adjust pH to 7.0 with NaOH.

1.2 LB Plates with Ampicillin

Add 15g agar to 1 liter of LB medium. Autoclave. Allow the medium to cool to 50 °C before adding ampicillin to a final concentration of 100 µg/ml. Pour 30-35 ml of medium into 85 mm petri dishes. Let the agar harden. Store at 4 °C for up to one month or at room temperature for up to one week.

1.3 LB Plates with Ampicillin/IPTG/X-Gal

Make the LB plates with ampicillin as above; then supplement with 0.5 mM IPTG and 80 µg/ml X-Gal and pour the plates. Alternatively, 100µl of 100 mM IPTG and 20 µl of 50 mg/ml X-Gal may be spread over the surface of an LB ampicillin plate and allowed to absorb for 30 minutes at 37 °C prior to use.

1.4 SOC Medium (100ml)

2.0 g Bacto®-tryptone

0.5 g Bacto®-yeast extract

1 ml 1M NaCl

0.25 ml 1M KCl

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

1 ml 2M glucose, filter sterilized

Add Bacto®-tryptone, Bacto®-yeast extract, NaCl and KCl to 97 ml distilled water. Stir to dissolve. Autoclave and cool to room temperature. Add 2 M Mg^{2+} stock and 2 M glucose, each to a final concentration of 20 mM. Bring to 100 ml with sterile, distilled water. The final pH should be 7.0.

2. *Pichia pastoris* Media

2.1 Low Salt LB (Luria-Bertani) Medium

1 % Tryptone

0.5 % Yeast Extract

0.5 % NaCl

Adjutst to pH 7.0 with NaOH.

For 1 liter, dissolve 10 g tryphone, 5 g yeast extract and 5 g NaCl in 950 ml deionized water. Adjust the pH of the solution to 7.5 with NaOH and bring the volume up to 1 liter. Autoclave for 20 minutes at 15 lb/sq. in. Let cool to ~55 °C and add desired antibiotics at this point. Store at room temperature or at +4 °C.

2.2 Yeast Extract Peptone Dextrose Medium - YPD or YEPD (1 liter)

1 % Yeast Extract

2 % Peptone

2 % Dextrose (glucose)

Dissolve 10 g yeast extract and 20 g of peptone in 900 ml of water. Note: Add 20 g of agar if making YPD slants or plates. Autoclave for 20 minutes on liquid cycle. Add 100 ml of 10X D (20% Dextrose). The liquid medium is stored at room temperature. YPD slants or plates are stored at +4 °C. The shelf life is several months.

2.3 Yeast Extract Peptone Dextrose Medium – YPDS + Zeocin™ Agar (1 liter)

1 % Yeast Extract

2 % Peptone

2 % Dextrose (glucose)

1 M Sorbitol

2 % Agar

100 µg/ml Zeocin™

Dissolve 10 g yeast extract, 20 g peptone and 182.2 g sorbitol in 900 ml of water. Note: Add 20 g of agar and autoclave for 20 minutes on liquid cycle. Add 100 ml of 10X D (20% Dextrose). Cool solution to ~ 60 °C and add 1.0 ml of 100 mg/ml Zeocin™. Store YPDS or plates containing Zeocin™ at +4 °C in the dark. The shelf life is one to two weeks.

2.4 Buffered Glycerol-Complex Medium and Buffered Methanol-Complex Medium – BMGY and BMMY (1 liter)

1 % Yeast Extract

2 % Peptone

100 mM Potassium phosphate, pH 6.0

1.34 % YNB

4×10^{-5} % Biotin

1 % Glycerol or 0.5 % methanol

Dissolve 10 g yeast extract and 20 g peptone in 700 ml of water. Autoclave for 20 minutes on liquid cycle. Cool to room temperature, then add 100 ml 1 M potassium phosphate buffer (pH 6.0), 100 ml 10X YNB, 2 ml 500X B (0.02% Biotin), and 100 ml 10X GY (10% Glycerol) and mix well. For BMMY, add 100 ml

10X M (5% Methanol) instead of glycerol. Store media at +4 °C. The shelf life of this solution is approximately two months.

3. Buffer

3.1 1X Equilibration/Wash Buffer (pH 7.0)

50 mM	Sodium Phosphate pH 7.0
300 mM	NaCl

3.2 1X Equilibration Buffer (pH 8.0)

50 mM	Sodium Phosphate pH 8.0
300 mM	NaCl

3.3 1X Elution Buffer (pH 5.0)

50 mM	Sodium Phosphate pH 5.0
300 mM	NaCl

3.4 Alkaline Lysis Solution I

50 mM	Glycose
25 mM	Tris-Chloride, pH 8.0
10 mM	EDTA, pH 8.0

3.5 Alkaline Lysis Solution II

0.2 N	NaOH
1 % (w/v)	SDS

3.6 Alkaline Lysis Solution III

60 ml	5 M Potassium Acetate
11.5 ml	Glacial Acetic Acid
28.5 ml	dH_2O

3.7 STE BUFFER

10 mM	Tris-Cl pH 8.0
0.1 M	NaCl
1 mM	EDTA pH 8.0

3.8 Tris-Glycine Buffer (1X)

25 mM	Tris-Cl
250 mM	Glycine

3.9 10X Tris EDTA (TE) pH 8.0

100 mM	Tris-Cl, pH 8.0
10 mM	EDTA, pH 8.0

3.10 1X Phosphate-Buffered Salive (PBS)

137 mM	NaCl
2.7 mM	KCl
10 mM	Na_2HPO_4
2 mM	KH_2PO_4

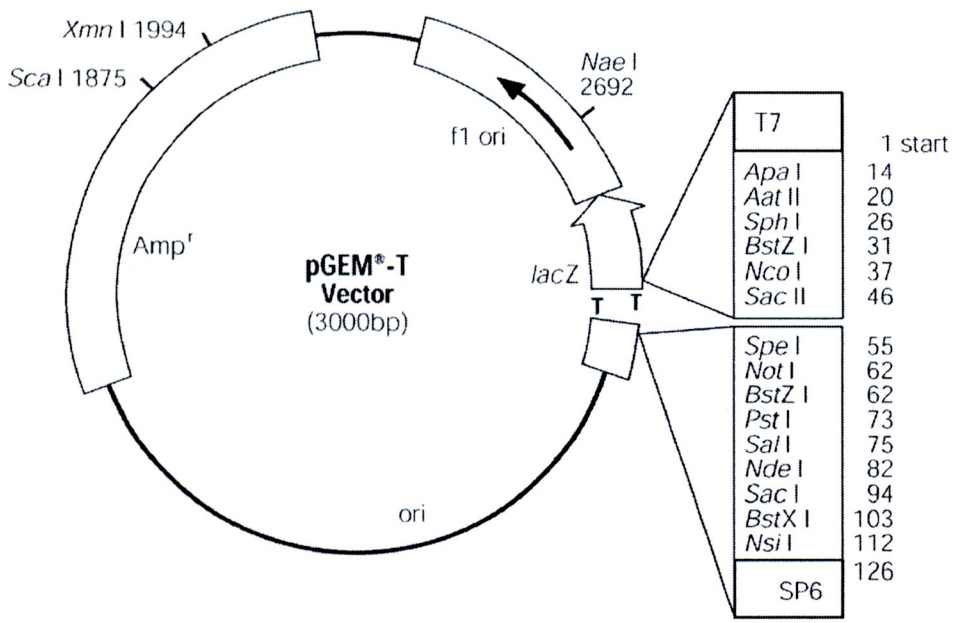
Dissolve 8 g of NaCl, 0.2 of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 800 ml of dH_2O. Adjust pH to 7.4 with HCl. Add dH_2O to 1 liter and sterilize by autoclaving for 20 minutes at 15 psi (1.05 kg/cm^2) on liquid cycle. Store the buffer at room temperature.

3.11 Blotting Transfer Buffer pH 8.3

20 mM	Tris-Cl
150 mM	Glycine
20 % v/v	Methanol

4. Vector

4.1 pGEM®-T Vector Circle Map and Sequence Reference Points.

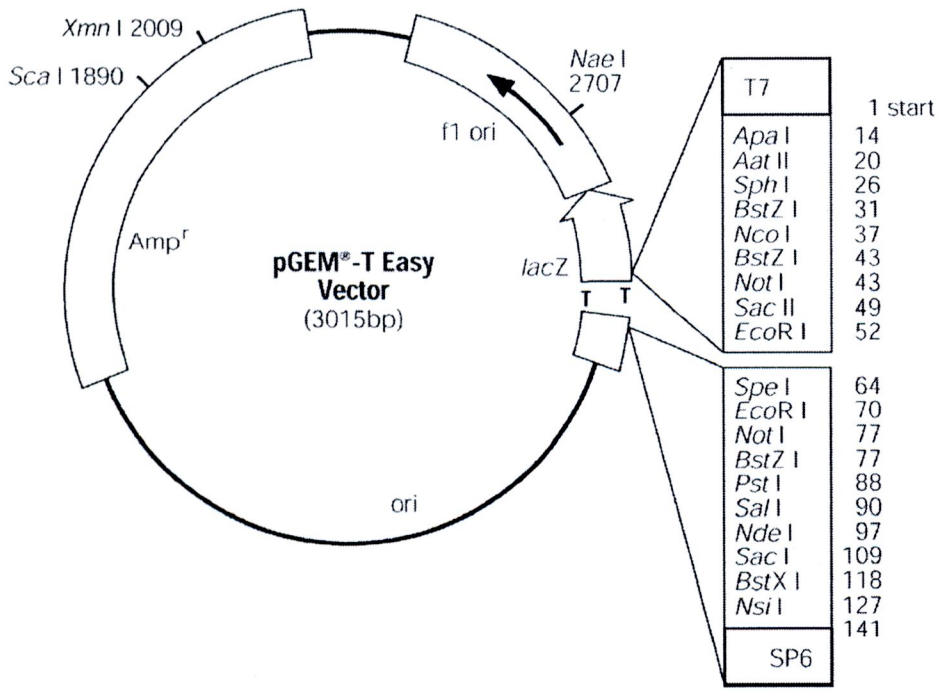


pGEM®-T Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–113
SP6 RNA polymerase promoter (–17 to +3)	124–143
SP6 RNA polymerase transcription initiation site	126
pUC/M13 Reverse Sequencing Primer binding site	161–177
lacZ start codon	165

lacoperator	185–201
β-lactamase coding region	1322–2182
phage f1 region	2365–2820
lacoperon sequences	2821–2981, 151–380
pUC/M13 Forward Sequencing Primer binding site	2941–2957
T7 RNA polymerase promoter (–17 to +3)	2984–3

4.2 pGEM®-T Easy Vector Circle Map and Sequence Reference Points.

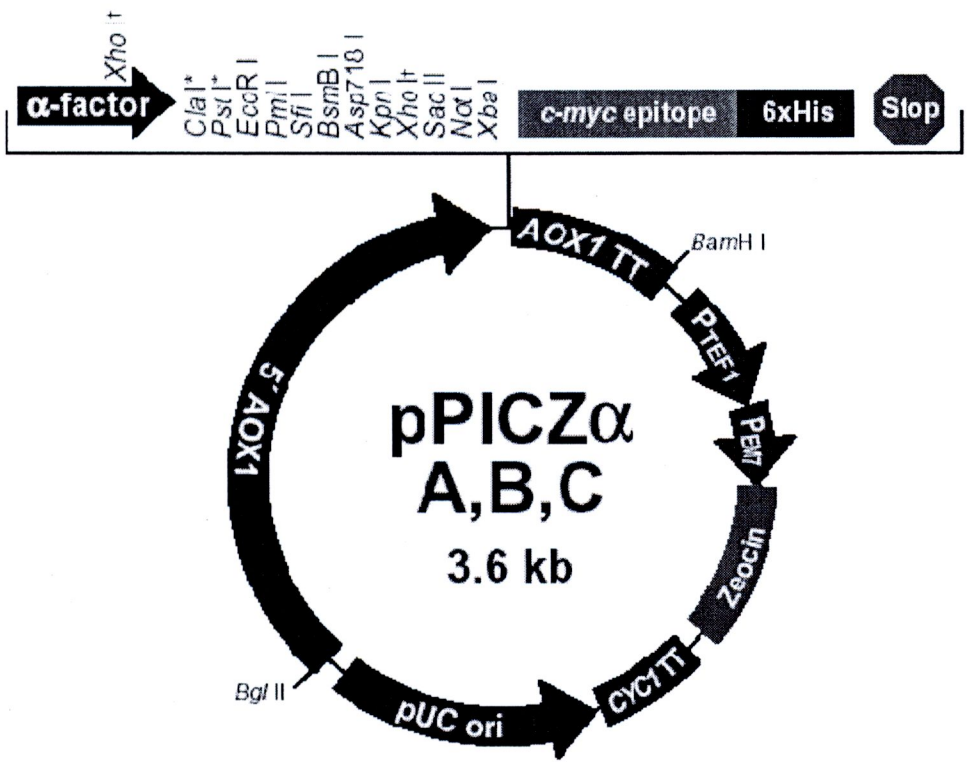


pGEM® -T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
lacZ start codon	180

lacoperator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lacoperon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3

4.3 Map of pPICZα A, B, C.



5. Others

5.1 DAB /$NiCl_2$ Visualization Solution

5 ml	100 mM Tris-C pH 7.5
120 µl	DAB stock (40 mg/ml in H_2O, stored in 100 µl aliquots at –20 °C
25 µl	$NiCl_2$ stock (80 mg/ml in H_2O, stored in 100 µl aliquots at –20 °C

Mix just before use.

5.2 12 % Gel (5 ml) Resolving Gels for Tris-Glycine SDS-Polyacrylamide Gel Electrophoresis

1.6 ml	H_2O
2.0 ml	30 % acrylamide mix
1.3 ml	1.5 M Tris, pH 8.8
0.05 ml	10 % SDS
0.05 ml	10 % ammonium persulfate
0.002 ml	TEMED

5.3 5 % Stacking Gel (1 ml)

0.068 ml	H_2O
0.17 ml	30 % acrylamide mix
0.13 ml	1.0 M Tris, pH 6.8
0.01 ml	10 % SDS
0.01 ml	10 % ammonium persulfate
0.001 ml	TEMED
0.1 %	SDS

5.4 2X SDS Gel-Loading Buffer

100 mM	Tris-Cl, pH 8.8
4 % w/v	SDS
0.2 % w/v	bromphenol blue
20 % v/v	glycerol
200 mM	dithiothreitol or β-mercaptoethanol

BIOGRAPHY

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