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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 1 month or at room temperature for up to 1 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 tc 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 P otassium phosphate, pH 6.0

1.34 % YNB

$4 \times 10^{-5}$ % 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 | $KH_2PO_4$ |

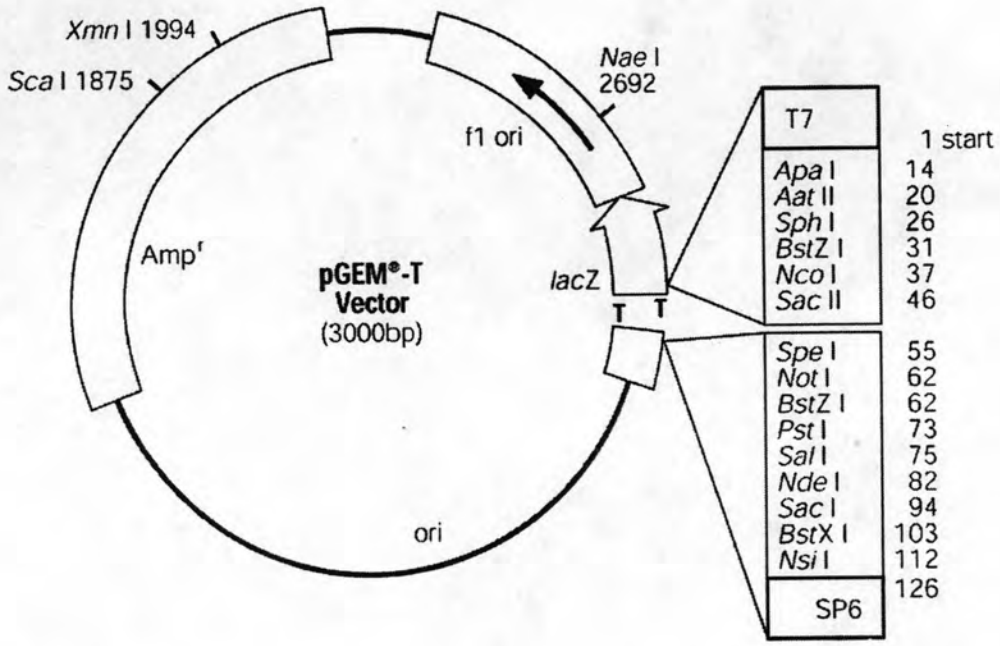
Dissolve 8 g of NaCl, 0.2 of KCl, 1.44 g of $Na_2HPO_4$ and 0.24 g of $KH_2PO_4$ in 800 ml of $dH_2O$. Adjust pH to 7.4 with HCl. Add $dH_2O$ to 1 liter and sterilize by autoclaving for 20 minutes at 15 psi (1.05 $kg/cm^2$) on liquid cycle. Store the buffer at room temperature.

### 3.11 Blotting Transfer Buffer pH 8.3

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

## 4. Vector

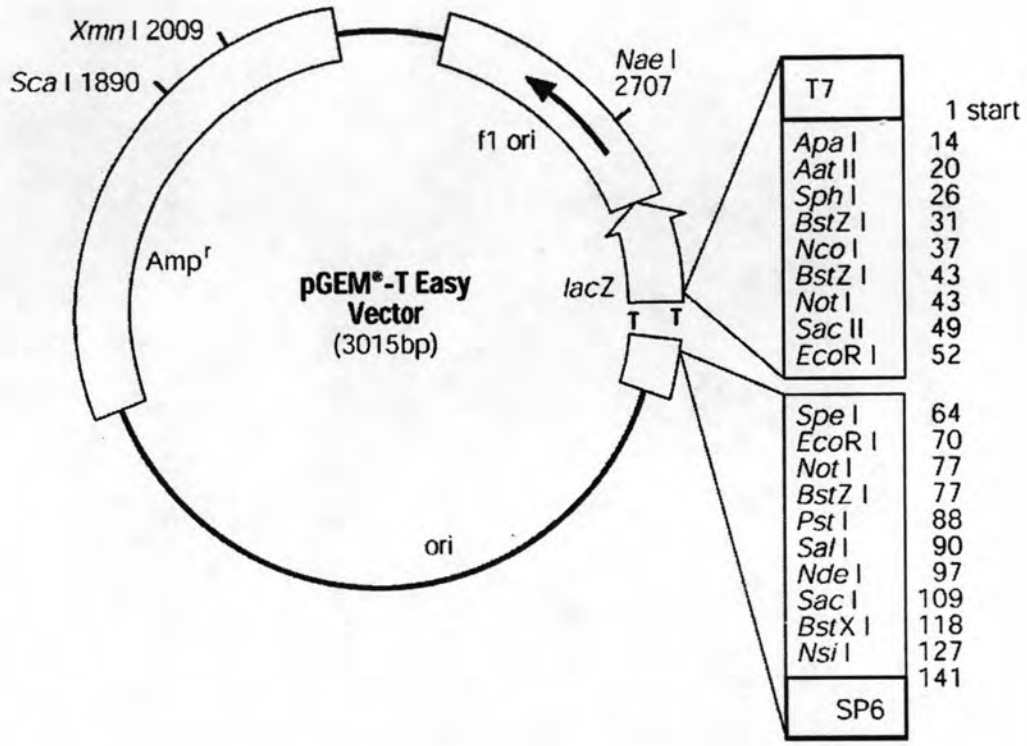
### 4.1 pGEM®-T Vector Circle Map and Sequence Reference Points.




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 |

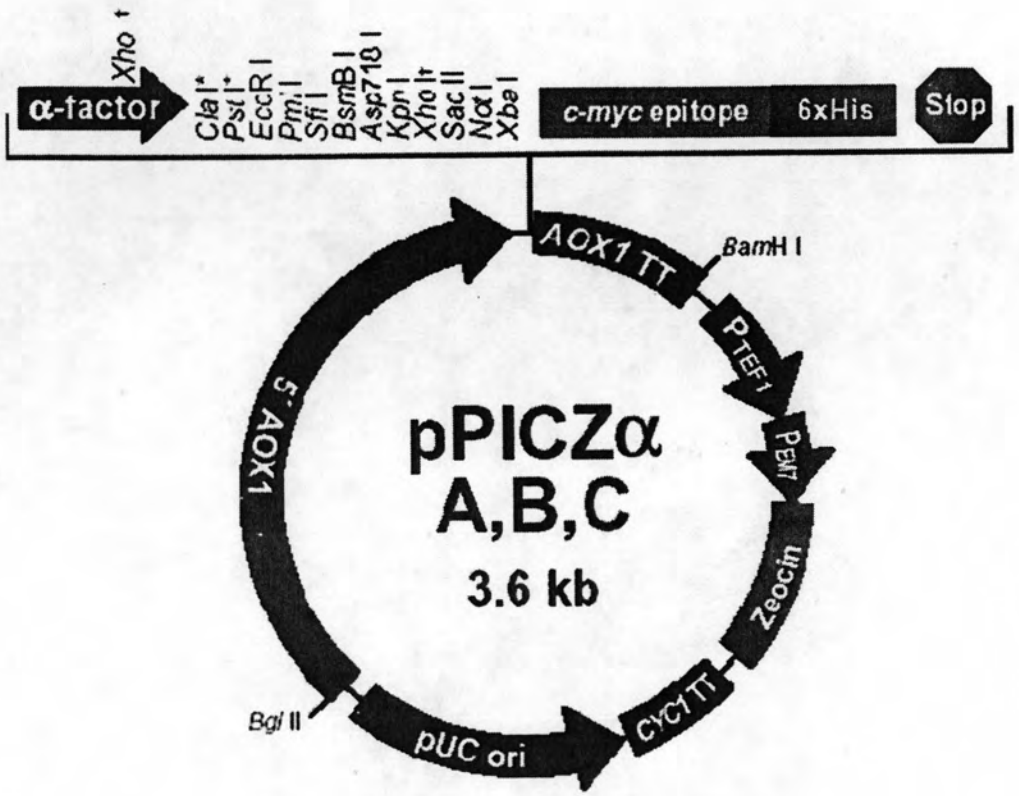
## 4.2 pGEM®-T Easy Vector Circle Map and Sequence Reference Points.




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

Miss Chaunchom Maunpasitporn was born on May 18, 1967, in Chainat province, Thailand. She obtained the Bachelor's Degree from the Department of Medical Technology at Cheingmai University in 1989 and the Master's Degree from the Department of Science in Biotechnology at Chulalongkorn university in 1998. She has worked at Hematology Department of Mediciene Faculty of Medicine, Chulalongkorn University as a medical technologist since 1992. In 2003 She continued her study for Doctor's Degree in Biomedical Science, Faculty of Graduate School, Chulalongkorn University.