APPENDIX

APPENDIX

1. The pET-21(+) vector

The pET-21(+) vector carry an C-terminal His-Tag sequence. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer.

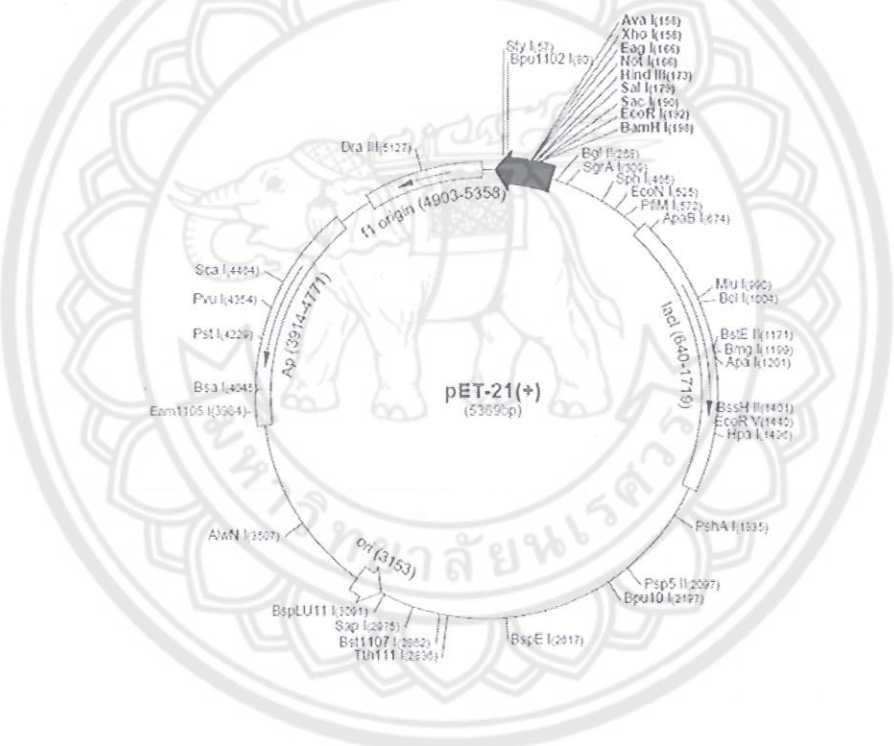

Figure 33 pET-21(+) cloning/expression region

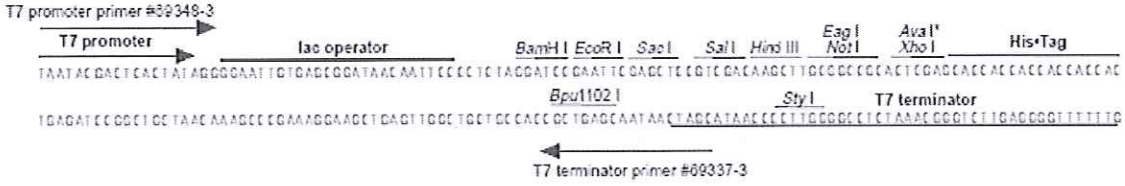

Figure 34 pET-21(+) sequence landmarks

T7 promoter	237-253
T7 transcription start	236
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag® coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	640-1719
pBR322 origin	3153
bla coding sequence	3914-4771
f1 origin	4903-5358

Preparation for media and reagents

1. **IPTG stock solution (0.1M)**

Dissolve 1.2 g IPTG (Isopropyl thio-β-D-galactoside) to final volume of 50 ml distilled water and store at 4° C.

2. **LB broth**

Dissolve 25 g of Luria broth base (LB) to 1 liter of distilled water. Autoclave the solution at 121°C for 15 min.

3. **LB plate with 100 ug/ml of ampicillin**

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
NaCl	5 g
Agar	15 g

Dissolve in 800 ml dH_2O. Adjust pH to 7.2 with NaOH and the volume to 1l with dH_2O. Sterilize by autoclaving at 121°C for 20 min. Allow the medium to cool to 50°C before adding ampicillin to a final concentration 100 µg/ml. Pour medium into petridishes.

4. **LB plate with100 µg/ml of ampicillin/IPTG/X-Gal**

Make the LB plates with ampicillin as above. Then spread 100 µl of 100 mM IPTG and 20 µl of 50 mg/ml X-Gal over the surface of the plates and allow to absorb for 30 min at 37° C before use.

5. Reagent for prepared competent cells

5.1 0.5 M EDTA (pH 8.0)

EDTA (disodium ethylene diamine tetraacetate ·2H2O) 186.1 g

Distilled water 800 ml

Adjust pH to 8.0 with NaOH (about 20 g)

Adjust the volume to 1 liter with dH2O. Sterilize by autoclaving at 121°C for 20 min.

5.2 10 mM TE pH 8.0

Tris (10 mM) 0.121 g

EDTA (1 mM) 0.3722 g

Water 80 ml

Adjust pH to 8.0 with HCl and the volume to 100 ml. Sterilize by autoclaving at 121°C for 20 min.

5.3 5X TBE buffer

Tris-base 54.0 g

Boric acid 27.5 g

0.5 M EDTA, pH 8.0 20 ml

Adjust volume with distilled water to 1 liter.

6. Solutions for protein

6.1 1.5 M Tris pH 8.0

1.5 M Tris 18.17 g

Adjust pH to 8.0 with HCl and the volume to 100 ml with dH_2O.

6.2 1.0 M Tris pH 6.8

1 M Tris 12.11 g

Adjust pH to 6.8 with HCl and the volume to 100 ml with dH_2O.

6.3 SDS-gel loading buffer (5X stock)

2.5 M Tris 0.30 g

10% SDS 1.0 g

0.5% Bromophenol blue 0.05 g

50% glycerol 5 ml

Adjust pH to 6.8 with HCl and the volume to 8 ml with dH_2O. Before use add 20 ml of β-mercapthoethanol to 80 ml of solution mixture.

6.4 Tris-Glycine electrode buffer (5X stock)

250 mM Tris	30.29 g
1.25 M glycine	144 g
0.5% SDS	5 g

Adjust pH to 8.3 with HCl and the volume to 1liter with dH_2O.

6.5 10% separation gel SDS-PAGE (10 ml)

1.5 M Tris (pH 8.0)	2.5 ml
dH_2O	2 ml
10% SDS	0.1 ml
30% acrylamide solution	3.3 ml
10% ammonium persulfate	0.1 ml
TEMED	0.004 ml

6.6 4% stacking gel SDS-PAGE (5 ml)

1.0 M Tris (pH 6.8)	1.25ml
dH_2O	3.4 ml
10% SDS	0.05 ml
30% acrylamide solution	0.65 ml
10% ammonium persulfate	0.05 ml
TEMED	0.004 ml

6.7 10% separation gel Native-PAGE (10 ml)

1.5 M Tris (pH 8.0)	2.5 ml
dH_2O	2 ml
30% acrylamide solution	3.3 ml
10% ammonium persulfate	0.1 ml
TEMED	0.004 ml

6.8 4% stacking gel Native-PAGE (5 ml)

1.0 M Tris (pH 6.8)	1.25ml
dH_2O	3.4 ml
30% acrylamide solution	0.65 ml
10% ammonium persulfate	0.05 ml

TEMED	0.004 ml

6.9 Destaining solution for Coomassie Stain

Methanol	400 ml
Water	500 ml
Glacial acetic acid	100 ml

6.10Staining solution with Coomassie brillant blue for protein

Coomassie brilliant blue R-250	1 g
Methanol	400 ml
Water	500 ml
Glacial acetic acid	100 ml

Filter the solution through a Whatman No. 1 filter to remove any particulate matter.

6.11 10X M PBS (1.5 M NaCl, 2.7 mM KCl, 10mM $Na_2HPO_4 2H_2O$, 2 mM KH_2PO_4 pH 7.4)

KCL	2 g
NaCl	80 g
$Na_2HPO_4 2H_2O$	14.4 g
KH_2PO_4	2.4 g

Adjust pH to 7.4 with NaOH and the volume to 1 liter with dH2O. Sterilize by autoclaving at 121°C

7. Solutions for protein purification

7.1 Binding buffer (20 mM sodium phosphate, 500 mM NaCl, 40 mM imidazole, pH 7.4)

sodium phosphate	2.8 g
NaCl	29.22 g
Imidazole	0.82 g

Adjust pH to 7.4 and the volume to 1 liter with dH2O. Sterilize by autoclaving at 121°C

7.2 Washing buffer (20 mM sodium phosphate, 500 mM NaCl, 60 mM imidazole, pH 7.4)

sodium phosphate	2.8 g
NaCl	29.22 g
Imidazole	1.23 g

Adjust pH to 7.4 and the volume to 1 liter with dH2O. Sterilize by autoclaving at 121°C

7.3 Elution buffer (20 mM sodium phosphate, 500 mM NaCl, 250 mM imidazole, pH 7.4)

sodium phosphate	2.8 g
NaCl	29.22 g
Imidazole	5.125 g

Adjust pH to 7.4 and the volume to 1 liter with dH2O. Sterilize by autoclaving at 121 °C