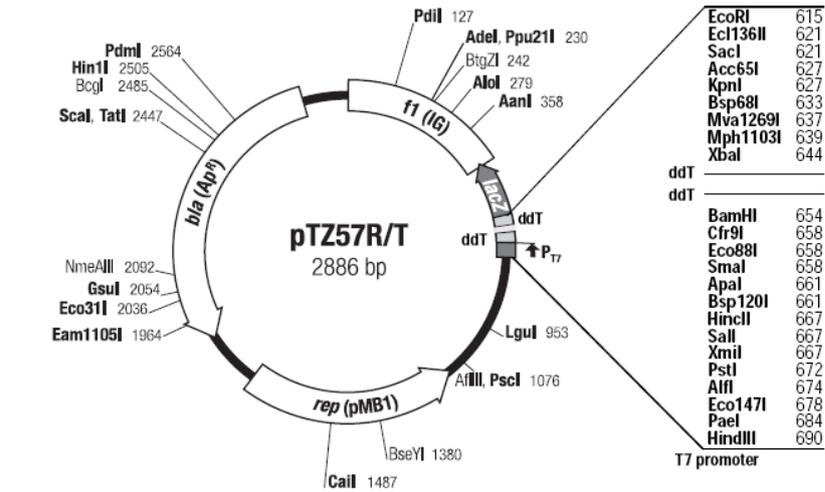


APPENDIX

APPENDIX A VECTOR FOR EXPERIMENT

Cloning vector



M13/pUC sequencing primer (-20), 17-mer (#S0100)

615 EcoRI Eco136II Acc65I Bsp68I Mva1269I
 SacI KpnI

5' G TAA AAC GAC GGC CAG TGA ATT CGA CCT CGG TAC CTC GCG AAT GCA
 3' C ATT TTG CTG CCG GTC ACT TAA GCT CGA GCC ATG GAG CGC TTA CGT
 LacZ ← Val Val Ala Leu Ser Asn Ser Ser Pro Val Glu Arg Ile Cys

XbaI 650 PCR product 651 BamHI Cfr9I Eco88I SmaI ApaI Bsp120I HincII SalI XmiI PstI
 TCT AGA T ddT AT CGG ATC CCG GGC CCG TCG ACT GCA
 AGA TCT A dA ddT TA GCC TAG GGC CCG GGC AGC TGA CGT
 Arg Ser Ile Pro Asp Arg Ala Arg Arg Ser Cys

AflI
 Eco147I PaeI HindIII 695
 GAG GCC TGC ATG CAA GCT TTC CCT ATA GTG AGT CCT ATT AGA GCT TGG CGT
 CTC CGG ACG TAC GTT CGA AAG GGA TAT CAC TCA GCA TAA TCT CGA ACC GCA
 Leu Gly Ala His Leu Ser Glu Arg Tyr His Thr Thr Asn Ser Ser Pro Thr

AAT CAT GGT CAT AGC TGT TTC CTG 5'
 TTA GTA CCA GTA TCG ACA AAG GAC 5'
 M13/pUC reverse sequencing primer (-26), 17-mer (#S0101)
 Ile Met Thr Met

Figure 40 Map of the pT57R/T cloning vector and DND sequence of MCS region

APPENDIX B BACTERIAL STRAINS

Escherichia coli DH5 α was used as host cells for amplification of recombinant plasmids (Madison, WI, USA)

Escherichia coli BL21 (DE3) pLysS was used as host cells for expression (Novagen, USA)

Table 3 Strains Genotypes

| Strain | Genotype |
|-----------------|--|
| DH5 α | F ⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ 80dlacZ Δ M15 Δ (lacZYA-argF)U169, hsdR17(r _K ⁻ m _K ⁺), λ - |
| BL21(DE3) pLysS | F ⁻ ompT hsdSB(rB ⁻ mB ⁻) gal dcm (DE3) pLysS (CamR) |

APPENDIX C LIST OF SOLUTIONS AND BUFFER

50X TAE buffer solution (1000 ml)

- a. 242 g Tris-base
- b. 57.1 ml Boric acid
- c. 100 ml 0.5 EDTA

50 mg/ml Ampicillin stock solution (50 ml)

- a. 2.5 g ampicillin sodium salt in 50 ml H₂O

20 mg/ml X-Gal stock solution (10 ml)

- a. 200 mg X-Gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside)
in 10 ml N, N-dimethylformamide

100 mM IPTG stock solution (50 ml)

- a. 1.2 g IPTG (isopropyl- β -D-thiogalactopyranoside)
in 50 ml H₂O

1.5 M Tris-HCl (pH 8.8), 100 ml

- a. 18.2 g Tris
- b. Add HCl to pH 8.8

0.5 M Tris-HCl (pH 6.8), 100 ml

- a. 6.0 g Tris
- b. Add HCl to pH 6.8

10% SDS (w/v), 100 ml

- a. 10 g SDS

10% Ammonium persulfate, 5 ml

- a. 0.5g ammonium persulfate

Electrophoresis buffer for nondenaturing (1 liter)

- a. 3.0 g Tris
 - b. 14.4 g Glycine
- pH 8.8

5X Sample buffer for nondenaturing (10 ml)

- a. 3.1 ml 1 M Tris-HCl (pH 6.8)
- b. 5 ml Glycine
- c. 0.5 ml 1% bromophenol blue
- d. 1.4 ml H₂O

Electrophoresis buffer for denaturing (1 liter)

- a. 3.0 g Tris
 - b. 14.4 g Glycine
 - c. 1 g SDS
- pH 8.8

Staining solution (250 ml)

- a. 0.25 g Coomassie brilliant blue R-250
- b. 125 ml Methanol
- c. 25 ml Acetic acid
- d. 100 ml H₂O

Destaining solution (500 ml)

- a. 200 ml Methanol
- b. 50 ml Acetic acid
- c. 250 ml H₂O

5X Sample buffer for denaturing (10 ml)

- a. 0.6 ml 1 M Tris-HCl (pH 6.8)
- b. 5 ml Glycine
- c. 2 ml 10% SDS
- d. 0.5 ml 2-mercaptoethanol
- e. 1 ml 1% bromophenol blue
- f. 0.9 ml H₂O

4.5% stacking gel (3.075 ml)

- a. 450 µl 30% acrylamide
- b. 750 µl Tris-HCl pH 6.8
- c. 50 µl 10% ammonium persulfate
- d. 20 µl 10% SDS
- e. 5 µl TEMED
- f. 1.8 ml H₂O

10% separating gel (10 ml)

- a. 3.3 ml 30% acrylamide
- b. 3.3 ml Tris-HCl pH 8.8
- c. 50 µl 10% ammonium persulfate
- d. 133 µl 10% SDS
- e. 5 µl TEMED
- f. 3.2 ml H₂O

1 M Sodium phosphate dibasic (Na₂HPO₄)

- a. 14.196 g Na₂HPO₄
- b. 100 ml H₂O

1 M Sodium Phosphate, Monobasic (NaH₂PO₄)

- a. 13.8 g NaH₂PO₄
- b. 100 ml H₂O

1 M Sodium Phosphate Buffer, pH 7 (100 ml)

- a. 57.7 ml 1 M Na_2HPO_4
- b. 42.3 ml 1 M NaH_2PO_4

1X Phosphate Buffered Saline (PBS Buffer)

- a. 8g of NaCl
- b. 0.2g of KCl
- c. 1.44g of Na_2HPO_4
- d. 0.24g of KH_2PO_4

Luria-Bertani (1000 ml)

- a. 10 g of tryptone
- b. 5 g of yeast extract
- c. 10 g of NaCl, and 1 liter

Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4 (500 ml)

- a. 10 ml 1 M sodium phosphate
- b. 14.61 g NaCl
- c. 1.7 g Imidazole

Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4 (500ml)

- a. 10 ml 1 M sodium phosphate
- b. 14.61 g NaCl
- c. 17 g Imidazole

20 mM sodium phosphate, 0.5 M NaCl, 1 mM CaCl_2 (50 ml)

- a. 1.0 ml 1 M sodium phosphate
- b. 1.461 g NaCl
- c. 0.006 g CaCl_2