Appendix

Preparation of reagents

10X MOPS
0.2M MOPS pH 7.0, 20mM Sodium acetate, 10mM EDTA pH 8.0

Filter sterilized with 0.45μm filter.

DEPC water
0.1% diethylpyrocarbonate was added to 1ltr double distilled water in a fume hood and mixed well. After incubating it for 1hr at 37°C it was autoclaved.

Preparation of bacterial culture media

LB medium (Luria Broth)

10 gm of Tryptone, 5gm of Yeast, 5gm of Sodium chloride (Hi Media) were dissolved in 1liter of dw. Media were sterilized by autoclaving for 20 minutes at 15 lb/sq.in.

LB Agar
15gm of agar powder, 10gm of Tryptone, 5gm of Yeast, 5gm of Sodium chloride, (Hi media) were dissolved in dw. Media were sterilized by autoclaving for 20 minutes at 15lb/sq.in. LB agar was allowed to cool to 45°C and poured (~30 ml per plate) in 90 mm disposable petri plates (Tarsons) along with appropriate antibiotics and allowed to solidify.

Antibiotics

Ampicillin
100mg/ml ampicillin stock was prepared in autoclaved dw and sterilized by filtration through 0.22 μm filter. 100μl aliquots were stored by freezing at –20°C.

Kanamycin
50mg/ml kanamycin stock was prepared in autoclaved distilled water and sterilized by filtration through 0.22 μm filter. 100μl aliquots were stored by freezing at –20°C.
**Stock solution of commonly used reagents**

1M Tris

121.1gm of Tris base was dissolved in 800ml of dw and pH set (6.8, 7.4, 8.0) with concentrated HCl. Volume was made up to 1liter and autoclaved.

0.5M EDTA

186.1gm of disodium EDTA.-2H2O was added in 800ml of dw, stirred vigorously on a stirrer, pH set to 8.0 with NaOH (~20 gm of NaOH pellets) and volume made up to1liter and autoclaved.

3M sodium acetate

204.5gm of C2H3O2Na. 3H2O was dissolved in 400ml of dw, pH set to 5.3 with glacial acetic acid, volume made up to 500 ml and autoclaved.

10% SDS

10gm of electrophoresis grade SDS was dissolved in 70ml of dw, heated at 60°C to dissolve and the volume made up to 100ml.

Ethidium Bromide (10 mg/ml)

10mg of ethidium bromide was dissolved in 1ml dw, stored in a opaque bottle.

30% Acrylamide Stock

29.2gm of acrylamide and 0.8gm of bis-acrylamide were dissolved in 50ml of ddH2O. Volume was made up to 100ml, the solution filtered through Whatman no. 1 paper, degassed and stored in an opaque bottle.

Calcium Chloride (0.1 M)

1.47gm of CaCl2.2H2O was dissolved in 100ml of ddH2O and sterilized by autoclaving.

IPTG (1M)

238mg of IPTG was dissolved in 1ml of dw, filter sterilized and stored at -20°C in 50μl aliquots.
Sodium Phosphate (1M)

Monobasic
138gm of NaH$_2$HPO$_4$.H$_2$O was dissolved in 800ml of dw and volume made up to 1liter.

Dibasic
268gm of Na$_2$HPO$_4$.7H$_2$O was dissolved in 700ml of dw and volume made up to 1liter.

Ammonium persulfate (10%)
To 1gm of ammonium persulfate, 10ml of dw was added and the solution stored for several weeks at 4°C.

10 X TAE buffer (Tris acetate, EDTA)

4.84gm of Tris base in 80ml of dw was dissolved and 1.2ml of glacial acetic acid and 2ml of 0.5 EDTA pH 8.0 were added. Final volume was made up to 100ml.

Phosphate Buffer Saline (PBS)

8gm of NaCl, 2gm of KCl, 1.44gm of Na$_2$HPO$_4$ and 0.2gm of KH$_2$PO$_4$ were dissolved in 800ml of dw. pH was set to 7.4 with HCl. Final volume was made up to 1 liter and sterilized by autoclaving at 15lb/ sq.in for 20 minutes and stored at room temperature.

SDS-PAGE electrophoresis buffer

3gm of Tris base, 14.4gm of glycine and 1gm of SDS were dissolved in 1 liter of dw.

Protein transfer buffer

5.8gm of Tris base, 2.9gm of glycine and 0.33gm of SDS were dissolved in 500ml of dw. 200 ml of ethanol was added and the final volume was made up to 1 liter.

2X SDS-PAGE sample buffer

The composition of sample buffer is as follows

- Tris-Cl (pH6.8) 100mM
- DTT 200mM
- SDS 4%
Bromophenol blue  0.2%
Glycerol  20%
β-mercaptoethanol  10%

10X Ligation buffer
Tris.Cl pH 7.8  500mM
MgCl$_2$  100mM
DTT  100mM
ATP  10mM

10X Amplification buffer
Tris.Cl pH 8.3  100mM
MgCl$_2$  15mM
KCl  500mM
Gelatin  0.1%

DNA loading dye (6X)
0.2gm bromophenol blue, 0.2gm of xylene cyanol and 30ml of glycerol were dissolved and volume set to 100 by autoclaved dw.

SDS-PAGE reagents
Composition of resolving gel (12%) 10 ml
30% acrylamide solution  4.0 ml
1.5M Tris-Cl pH 8.8  2.5 ml
dw  3.3ml
10% SDS  100μl
10% APS  100μl
TEMED  10μl
Composition of stacking gel (5%) (5.0 ml)

- 30% acrylamide solution: 0.83 ml
- 1.0M Tris.Cl pH 6.8: 0.68 ml
- dw: 3.4 ml
- 10% SDS: 50 μl
- 10% APS: 50 μl
- TEMED: 5 μl

Staining solution

1 gm of coomassie blue was dissolved in 450 ml of methanol. 100 ml of glacial acetic acid was added and the volume made up to 1 liter by double distilled water, filtered through Whatman no.1 filter and stored at room temperature.

Destaining solution

Methanol: water: acetic acid were mixed in the ratio of 45:45:10 and stored at room temperature.

Electroporation Buffer

0.25 gms HEPES, 0.403 gms NaCl, 186 mg KCl, 6.2 mg Na₂HPO₄, 54 mg glucose, pH 7.4 added to 100 ml dw and sterilized by filtration.

Vectors used in the study

pGEM®-T Easy TA cloning Vector

The pGEM®-T Easy Vector has been linearized at base 60 with EcoRV and a T added to both 3’-ends. The EcoRV site will not be recovered upon ligation of the vector and insert.
The promoter and multiple cloning sequence of the pGEM®-T Easy Vector. The top strand shown corresponds to the RNA synthesized by T7 RNA polymerase. The bottom strand corresponds to the RNA synthesized by SP6 RNA polymerase.

pGEM®-T Easy Vector Map and Sequence Reference Points
<table>
<thead>
<tr>
<th>Component Description</th>
<th>Position</th>
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<tbody>
<tr>
<td>T7 RNA polymerase transcription initiation site</td>
<td>1</td>
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<tr>
<td>multiple cloning region</td>
<td>10–128</td>
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<tr>
<td>SP6 RNA polymerase promoter (−17 to +3)</td>
<td>139–158</td>
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<td>139–158</td>
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<tr>
<td>SP6 RNA polymerase transcription initiation site</td>
<td>141</td>
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<tr>
<td>pUC/M13 Reverse Sequencing Primer binding site</td>
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<tr>
<td>T7 RNA polymerase promoter (−17 to +3)</td>
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</tr>
</tbody>
</table>

To calculate the appropriate amount of PCR product (insert) to include in the ligation reaction, use the following equation. $\text{ng of vector} \times \frac{\text{kb size of insert}}{\text{insert:vector molar ratio}} = \text{ng of insert}$
pPROExHTc  Vector Map

- 

- HpaI (4515)
- ORF frame 2
- ApaI (4220)
- lacI
- BclI (4023)
- pSEX_3 Primer
- F1 origin

- rbcL terminator
- rbcL_T1 terminator
- rbcL_T2 terminator
- AmpR promoter

- Ampicillin
- ORF frame 1
- FspI (1525)

- trc promoter
- M13_pUC_rev_primer
- NarI (257)
- Ncol (340)
- BarHI (346)
- EcoRI (353)
- StuI (363)
- SalI (369)
- SacI (379)
- SpeI (381)
- NotI (389)
- EagI (399)
- BstBI (398)
- XbaI (403)
- PstI (415)
- Xhol (418)
- KpnI (434)
- HindIII (438)
- pTrcHis_rev_primer
- pBAD_rev_primer

- pBR322_origin

- 4780 bp

- 3084
- 3955
- 3069
- 706
- 1106
- 1554
- 2789
- 2391
- 1992

pPROEX HTc
pGEX4T
pKSNeo

pKSNEO vector MCS with primer sequencing

**MCS_PKSNEO_VECTOR**

GTCAGCATCGCCGCCGCGGCATGGGTGCTCGCACCTTTCCATTCTCTCC
GTACGCGTTGCGCCTGCTACTCTGCTCACTCTTCTCCCTCCTCTCTCTCCGCA
TCTGCACCTTGTGTGTTCTGTGTGTGCTCAGCGCCACACGTACTCTACACGCTTT
CTCTGATGCCTCCTCCTCGGCCCTGCCTGAAACGATGACGTGCACGCCGCCACTC
GCTCAGCCGCTCCCATCCACATGCATTCTGCAC(TCTACAGC (McsInt)
GACTCTAAGCGGTATAGTCATCCTCTCTCCTCAACCACCACACTCGCTTCTTCTG
CTCTACCTATTACTCGCCAGCCACATATCTGCTATAACCTGCCTCC
CCCACCCGCTCCCACAC( Neo Fp) ATCCGCCACCGCTACGCAAGCGTCTG
ACTCTAGACTAGTGGAATCCCCCCGGGCTGCAATGCGATCGATGACTCTAGCTC
AGATGCACCTCTCCACGACATGCGCCGGAGGCGGAGATGAAGGCGAGCAG (NeoR)
CGACCCCTTT

NeoFp: CACATATCTGCTATAACCTGC
NeoRp: GCATCTAGATCATCGATCGC
McsInt: ATGCATTCTGCAC(TCTACAGC