APPENDIX A

CHEMICALS AND EQUIPMENTS

1. CHEMICALS/MATERIALS AND ANTIBODIES

All chemicals used in this study were analytical grade reagents

- Acrylamide (BDH Laboratory Supplies, UK)
- Agarose (electrophoresis grade) (Sigma-Aldrich Co.,USA)
- Ammonium persulphate (Amersham Biotech, Sweden)
- Aprotinin (Sigma, St. Louis, Mo, USA)
- Bis-acrylamide (BDH Laboratory Supplies, UK)
- Chloramphenicol (Sigma-Aldrich Co., USA)
- Developer and replenisher (Kodak, NY, USA)
- EDTA (Sigma-Aldrich Co.,USA)
- Ethanol (Merck, Darmstadt, Germany)
- Ethidium bromide (Sigma-Aldrich Co.,USA)
- Fetal calf serum (Gibco, Grand Island, NY, USA)
- FITC-sheep anti-mouse Igs (Silenus, Melbourne, Australia)
- Glacial acetic acid (BDH Laboratory Supplies, UK)
- Glycerol (Sigma-Aldrich Co.,USA)
- HCl (Merck, Darmstadt, Germany)
- HRP- rabbit-anti-mouse Igs antibody (Zymed, CA, USA)
- IPTG (Amresco, Solon, OH, USA)

- Iodoacetamide (Sigma, St. Louis, Mo, USA)
- Kanamycin (Sigma-Aldrich Co.,USA)
- Methanol (Merck, Darmstadt, Germany)
- MOPS (Amresco, USA)
- NaCl (BDH Laboratory Supplies, UK)
- NaOH (BDH Laboratory Supplies, UK)
- Nonidet P-40 (Sigma, St. Louis, Mo, USA)
- Paraformaldehyde (Fluka, Buchs, Switzerland)
- Phenylmethylsulfonyl fluoride (Sigma, St. Louis, Mo, USA)
- Polyvinylidene-fluoride membrane (PALL, East Hill, NY, USA)
- RPMI-1640 (Gibco, Grand Island, NY, USA)
- SDS (Sigma-Aldrich Co.,USA)
- Skimmed milk (Difco Laboratories, Detroit, MI, USA)
- TEMED (Sigma-Aldrich Co.,USA)
- Tetracycline (Sigma-Aldrich Co.,USA)
- Tris Base (Sigma-Aldrich Co.,USA)
- Tryptone (Life Technologies, Scotland)
- Tween20 (Fluka, Buchs, Switzerland)
- Yeast extract (Life Technologies, Scotland)

2. MOLECULAR REAGENTS AND KITS

- 1 kb DNA and Protein marker (Fermentas, MA, USA)
- dNTPs (Fermentas, MA, USA)
- DNA Ligase (Fermentas, MA, USA)

- Primers (Life Technology, USA)
- ProofStart DNA polymerase (QIAGEN, Hilden, Germany)
- QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany)
- QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany)
- Restriction enzymes (Fermentas, MA, USA)
- T4 ligase (Fermentas, MA, USA)
- Taq DNA polymerase and 10X reaction buffer (Roche Molecular Biochemicals, Germany)

3. EQUIPMENTS

- 37°C incubator (JP Selecta, Barcelona, Spain)
- Biological safety cabinet (NUAIR Fembrook Lane, Plymouth, MN 55447)
- FACS Calibur flow cytometer (Beckton Dickinson, USA)
- Fluorescent microscope (Olympus, USA)
- Electrophoretic power supply, ECPS 3000/150 (Amersham Pharmacia Biotech, Sweden)
- Microcentrifuge (Eppendorf AG, Hamburg, Germany)
- Microplate Reader EL340 (BIO-TEK Instruments, USA)
- MRX-150 refrigerated microcentrifuge (TOMY, Japan)
- PCR Mastercycler personal (Eppendorf, USA)
- RT6000 D refrigerated centrifuge (Sorvall, USA)
- UV160 Spectrophotometer (Shimadzu, Japan)
- UV Transilluminator (Hoefer Scientific Instruments, USA)

4. MICROORGANISMS

- *Escherichia coli* Origami B (Novagen, Madison, WI)

  [F^- *ompT hsdS*$_B$ (r_B^- m_B^-) *gal dcm lacY1 ahpC gor522*::Tn10 *trxB* (Kan^R, Tet^R)]

- *Escherichia coli* XL-1 Blue (Stratagene, USA)

  [*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac*F' [*proAB*$^+$, *lac*I^q *lac*ZΔM15 Tn10 (tet^r)]]

APPENDIX B

Reagent preparations

1. 10X Tris-acetate/EDTA electrophoresis buffer (TAE)

Tris-base	48.40	gm
Glacial acetic acid	11.42	ml
0.5M EDTA (pH 8.0)	20	ml

Dissolved all ingredients in distilled water (DW) and filled up to 1,000 ml and kept at 4 °C.

2. 1% agarose gel

Agarose gel	0.5	gm
1X TAE buffer until the volume	50	ml

Melt by microwave oven until the agarose was completely dissolved.

3. Ethidium bromide working solution (10 mg/ml)

Ethidium bromide	1.0	gm
DW until the volume	100	ml

Kept in the dark bottle and stored at room temperature.

4. 6X gel loading buffer

Bromphenol blue	0.25	%
Glycerol	30	%

Mix thoroughly and kept at -20 °C

5. 1 mM Phosphate buffer saline (PBS), pH 7.2

NaCl	8.00	gm
KCl	0.20	gm
Na_2HPO_4	1.15	gm
KH_2PO_4	0.20	gm
Dissolved all ingredient in DW until the volume	900	ml

Adjusted the pH to 7.2 with 1N HCl or 1N NaOH.

Added DW until the volume was reached 1000 ml and stored at 4 °C

6. Reagents for SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

6.1 1.5 M Tris-HCl pH 8.8

Tris-base	18.15	gm
Deionized DW	75	ml

Adjusted the pH to 8.8 with concentrated HCl.

Adjusted the volume to 100 ml with deionized DW and stored at 4 °C.

6.2 0.5 M Tris-HCl pH 6.8

Tris-base	6.0	gm
Deionized DW	80	ml

Adjusted the pH to 6.8 with concentrated HCl.

Adjusted the volume to 100 ml with deionized DW and stored at 4 °C.

6.3 Running buffer

Tris-base	1.51	gm
Glycine	7.20	gm
Sodium dodesyl sulphate	0.50	gm

Dissolved in 500 ml DW and kept at 4 °C.

6.4 Blotting buffer

Tris-base	3.03	gm
Glycine	14.41	gm
Sodium dodesyl sulphate	0.5	gm
Dissolved all gradients with DW	700	ml
Methanol	200	ml

Dissolved all ingredients in DW and filled up to 1,000 ml and kept at 4 °C.

6.5 Copolymerization of 4% stacking gel (5 ml)

Stock acrylamide 30%	0.665	ml
Gel buffer pH 6.8	1.25	ml
SDS 10%	0.05	ml
DW	3.01	ml
Ammonium persulfate 10%	0.1	ml
TEMED	0.01	ml

6.6 Copolymerization of 12% Separatinging gel (10 ml)

Stock acrylamide 30%	4.0	ml
Gel buffer pH 8.8	2.5	ml
SDS 10%	0.1	ml
DW	3.3	ml
Ammonium per sulfate 10%	0.1	ml
TEMED	0.01	ml

7. Reagent for using in ELISA

7.1 0.05% Tween20 in PBS (Washing buffer)

1 mM PBS	500	ml
Tween 20	0.25	ml

Mixed well and stored at room temperature.

7.2 Carbonate/bicarbonate coating buffer

Na_2CO_3	1.59	gm
$NaHCO_3$	2.93	gm
NaN_3	0.20	gm

Dissolved all ingredients in DW and filled up to 1,000 ml and kept at 4 °C.

8. Reagent for using in plasmid mini-preparation

8.1 3 M Sodium Acetate pH 7.0

NaAcet. $3H_2O$	40.8	gm

Adjust pH to 7.0 with NaOH/HCl

Dissolved in 100 ml DW and kept at 4 °C.

8.2 Potassium Acetate

Potassium Acetate	29.4	gm
Glacial acetic acid	11.5	ml

Dissolved in 100 ml DW and kept at 4 °C.

8.3 10 M NaOH

NaOH	200	gm

Dissolved in 500 ml DW and kept at 4 °C.

8.4 10% SDS

SDS	5	gm

Dissolved in 50 ml DW and kept at 4 °C.

Stored at room temperature.

8.5 7.5 M NH_4Acetate

NH_4Acetate	57.8	gm

Dissolved in 100 ml DW and kept at 4 °C.

8.6 1 M glucose buffer

D-glucose	18.02	gm

Dissolved all ingredients in DW and filled up to 100 ml

Autoclave and keep at 4 °C.

8.7 0.5 M EDTA pH 8.0

EDTA	37.22	gm
DW	100	ml

Adjust pH to 8.0, add DW to 200 ml and keep at 4 °C.

8.8 10X GLUCOMIX

1 M glucose buffer	50	ml
0.5 M EDTA pH 8.0	20	ml
1 M Tris pH 8.0	25	ml
DW	5	ml

Autoclave and keep at 4 °C.

8.9 1X glucomix-lysozyme solution

10X GLUCOMIX	300	µl
lysozyme stock (50 mg/ml in DW)	300	µl
DW	2.4	ml

Keep on ice or stored at 4°C for 7 days.

9. Super Broth

Tryptone	30.0	gm
Yeast extract	20.0	gm
Morpholinepropanesulphonic acid (MOPS)	10.0	gm

Dissolved all ingredients in 1,000 ml distilled water.

Sterilized by autoclave and keep at 4°C.

10. Tris lysis buffer pH 8.2

Tris base	6.06 gm
NaCl	5.9 gm
EDTA	0.74 gm
NaN_3	0.2 gm
Distilled water	1000 ml

11. Lysis buffer

Phenylmethylsulfonyl fluoride (100 mM in acetone)	100 µl
Iodoacetamide (0.5 M in distilled water)	100 µl
Aprotinin (1 mg/ml in PBS)	100 µl
10% NP-40 (in Tris lysis buffer)	1 ml
Tris lysis buffer pH 8.2	8.7 ml

Mix well, aliquot and stored at -20°C

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