APPENDIX H

Reagents for molecular cloning

1. Reagents for plasmid DNA extraction

1.1 Solution I (50 mM glucose, 10 mM EDTA in 25 mM Tris-HCl, pH 8.0)

To prepare solution I, 0.9 g of glucose, 0.33 g of Tris and 0.37 g of EDTA were dissolved in 80 ml of UDW and the pH was then adjusted to 8.0 with 1 M HCl or 1 N NaOH. The solution was sterilized by autoclaving and then stored at 25°C.

1.2 Solution II (0.2 M NaOH, 1% SDS)

This solution was prepared by mixing 0.2 ml of 5 N NaOH and 0.5 ml of 10% SDS in 4.3 ml of UDW. This solution was prepared immediately before use.

1.3 Solution III (3 M potassium acetate, pH 5.2)

The solution was prepared by dissolving 29.44 g of potassium acetate in 80 ml of DW. The pH was adjusted to 5.2 with glacial acetic acid. The volume was then made up to 100 ml with UDW before autoclaving, then stored at 25°C.

2. Reagents for recombinant protein expression and periplasmic protein extraction

2.1 1 M IPTG

Stock 1 M IPTG (Isopropyl β-D-1-thiogalactopyranoside) (Amersham Biosciences, USA) was prepared by dissolving 1.2 g of IPTG in 5 ml of UDW. The solution was sterilized by filtering through 0.22 μm Millipore membrane and stored at -20°C in small aliquots.

2.2 1x TES buffer, pH 8.0

This buffer consisted of 0.2 M Tris-HCl, pH 8.0, 0.5 mM EDTA and 0.5 M sucrose. The following ingredients were dissolved in 900 ml of DW:

Tris-base	24.23	g
$EDTA2H_2O$	0.19	g and
Sucrose	171.20	g

The pH of the preparation was adjusted to 8.0 with concentrate HCl. The volume was made up to 1,000 ml with UDW. The buffer was filtered through 0.22 μm Millipore membrane and stored at 4°C.

2.3 1/5x TES buffer, pH 8.0

One volume of 1x TES, pH 8.0 was added to four volumes of UDW.

3. Reagents for DNA electrophoresis (agarose and polyacrylamide)

3.1 5x TBE buffer (per one liter)

To prepare 5x TBE buffer, the buffer was prepared by mixing the following ingredients:

Tris-base	52	g
Boric acid	27.5	g
EDTA·$2H_2O$	4.65	g and
UDW	700	ml

The solution was adjusted pH to 8.3 with concentrate HCl before the volume was made to 1,000 ml. This buffer was sterilized by autoclaving.

3.2 Working 0.5x TBE buffer

5x TBE (100 ml) was added to 900 ml of UDW. This solution can be reused three times.

3.3 Ethidium bromide buffer

To prepare stock ethidum bromide solution, a tablet of ethidium bromide was dissolved in 1 ml of UDW to make 10 mg/ml concentration. Fifty microliters of the stock solution was then added to 100 ml of the buffer to make of 0.5 μg/ml working concentration. The solution was kept protected from light.

3.4 10x loading dye

Ten times concentrate loading dye (10x) consisted of 50% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanole FF.

3.5 50x TAE buffer (Tris-acetate/EDTA electrophoresis buffer)

To prepare 50x TAE buffer, following ingredients were mixed:

Tris-base	242	g
EDTA·$2H_2O$	18.16	g
Glacial acetic acid	57.1	ml and
UDW	700	ml

The pH of this buffer was adjusted to 8.3 with glacial acetic acid and the volume was made up to 1,000 ml and sterilized by autoclaving.

3.6 1% agarose gel preparation

Agarose (Amersham Biosciences) (0.3 g) was added to 30 ml of either 1x TAE or 1x TBE buffer and dissolved by heating. Molten agarose was allowed to cool down to 50-60°C at 25°C before poring in casting apparatus.

3.7 12% polyacrylamide gel preparation

The solution was prepared from mixing following ingredients:

30% acrylamide	4.8	ml
UDW	4.8	ml
5x TBE	2.4	ml
10% APS	200	µl and
TEMED	10	µl

The reagents were gently mixed before loading in gel casting apparatus, overlaid with UDW and allowed to polymerize for at least 20 minutes at 25°C.

4. Reagents for preparing competent *E. coli* cells and *E. coli* transformation

4.1 Preparation of competent cells for electroporation

4.1.1 1 mM HEPES, pH 7.0

To prepare this solution, 0.260 g of HEPES was dissolved in 1,000 ml of UDW. Sterilization was done by 0.22 µm Millipore membrane filtration.

4.1.2 10% glycerol

Ten milliliters of glycerol was added to 90 ml of UDW and sterilized by autoclaving.

4.2 Preparation of competent cells

4.2.1 0.1 M $MgCl_2$

This solution was prepared by dissolving 9.52 g of $MgCl_2$ in 1,000 ml of UDW. The sterilization was done by autoclaving.

4.2.2 0.1 M CaCl2

This solution was prepared by adding 11.1 g of $CaCl_2$ in 1,000 ml of UDW. The sterilization was done by autoclaving.

4.3 *E. coli* transformation

4.3.1 SOB medium

SOB medium was prepared by dissolving 20 g of tryptone, 5 g of yeast extract and 0.5 g of NaCl in 950 ml of UDW. Ten milliliters of 250 mM KCl was then added. The final volume was made up to 1,000 ml with UDW. After autoclaving, the broth was stored at 4°C.

4.3.2 SOC medium

To prepared SOC medium, 5 ml of a sterile 2 M $MgCl_2$ was added to SOB medium. Glucose (2 M) was then added to a final concentration of 20 mM.

4.4 5% X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)

X-Gal (Amersham BioSciences) (0.5 g) was dissolved in 10 ml of dimethylformamide (DMF) to make 5% (w/v). The preparation was kept in small aliquots with light protection at -20°C.

5. Reagents for total RNA manipulation

5.1 Diethylpyrocarbonate (DEPC) treated water

DEPC treated water was prepared by adding 1 ml of DEPC (Invitrogen, USA) in the 1 liter of UDW. The preparation was mixed by inverting and was incubated in fume hood for overnight, then autoclaving was performed.

5.2 70% ethanol in DEPC treated water

Ethanol (70 ml) was added to 30 ml of DEPC treated water. This reagent was kept at -20°C.

6. Reagents for DNA manipulation

6.1 Tris-EDTA (TE) buffer, pH 8.0

This buffer contained 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA.

6.2 3 M sodium acetate, pH 5.2

To prepare this solution, sodium acetate (40.8 g) was dissolved in 50 ml of DW, then pH was adjusted to 5.2 with concentrate acetic acid. The volume was made up to 100 ml with UDW.