APPENDIX D

Reagents for molecular cloning

1. Reagents for DNA manipulation

1.1 Tris-EDTA (TE) buffer, pH 8.0

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

1.2 Sodium acetate, pH 5.2 (3 M)

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.

2. Reagents for DNA electrophoresis

2.1 TBE buffer (5×) (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.

2.2 Working 0.5× TBE buffer

One hundred ml of 5× TBE were added to 900 ml of UDW. This solution can be reused three times.

2.3 Ethidium bromide solution

To prepare stock ethidum bromide solution, a tablet of ethidium bromide (USB Corporation, Cleveland, Ohio, USA) 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 solution to make of 0.5 μg/ml working concentration. The solution was kept protected from light.

2.4 10× loading dye

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

2.5 TAE buffer (Tris-acetate/EDTA electrophoresis buffer) (50×)

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.

2.6 agarose gel preparation (1%)

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

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

3.1 Reagents for preparation of competent cells

Hepes, pH 7.0 (1 mM)

Hepes sodium salt (0.26 g) was dissolved in 900 ml of DW. The pH of solution was adjusted to 7.0. The volume of solution was adjusted to 1000 ml and filtered through 0.2 µm filter to sterile. The solution was stored at 4°C.

Hepes, pH 7.0 containing 10% glycerol (1 mM)

Hepes sodium salt (0.26 g) was dissolved in 800 ml of DW and glycerol (100 ml) was added. After completely dissolving, the pH of solution was adjusted to 7.0. The volume of solution was adjusted to 1,000 ml and filtered through 0.2 µm filter to sterile. The solution was stored at 4°C.

Glycerol (10%)

Glycerol (100 ml) was mixed with 900 ml of DW. The solution was filtered through 0.2 mm filter or autoclaved to sterile.

3.2 Reagents for *E. coli* transformation

2x YT-G medium

The medium was prepared by dissolving Bacto-tryptone (17 g), Bacto-yeast extract (10 g), and NaCl (5 g) in 900 ml of DW. After completely dissolving, the

volume was adjusted to 1 liter with DW. After autoclaving, the broth was kept at 4°C. 2 M Glucose (36%) was added to yield the final concentration of 2% glucose.

4. Reagents for plasmid DNA extraction

4.1 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.

4.2 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.

4.3 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 RT.