



APPENDIX J

ชำนักรหอสมุด

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 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.

2. Reagents for DNA electrophoresis

2.1 5× TBE buffer (per one liter)

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

Tris-base	52	g
Boric acid	27.5	g
EDTA•2H ₂ O	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

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

2.3 Ethidium bromide buffer

To prepare stock ethidium 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 buffer 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 50× TAE buffer (Tris-acetate/EDTA electrophoresis buffer)

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

Tris-base	242	g
EDTA•2H ₂ O	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 1% agarose gel preparation

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 Reagent for preparation of chemical competent cell TSS and transformation

TSS buffer

Bacto-tryptone	1.0	g
Bacto yeast extract	0.5	g
NaCl	0.5	g
Polyethylene glycol (PEG, MW 3350)	10	g

All above were dissolved in 70 ml of distilled water, then added 5 ml of dimethylsulfoxide (DMSO) (Sigma Chemical Co.) and 5 ml of 1 M MgCl₂. The pH was adjusted to 6.5 with HCl or NaOH. The final volume was adjusted to 100 ml with distilled water and sterilized by filtering through a 0.22 mm filter. The buffer was stored at 4°C and stable up to 6 months.

3.2 *E. coli* transformation

3.2.1 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.

3.2.2 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.

3.3 5% X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside)

X-Gal (USB Corporation) (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 .

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.