APPENDIX E

Bactrial media

1. Luria-Bertani (LB) broth

Commercial LB powder (Merck, Darmstadt, Germany) (25 g) was dissolved in 1,000 ml of DW. After autoclaving, the broth was kept at 4°C.

2. LB-G broth

Commercial LB powder (Merck, Darmstadt, Germany) (25 g) was dissolved in 1,000 ml of DW. After autoclaving, the broth was allowed to cool down to about 55-60°C and 55.6 ml of 2 M glucose was added. The broth was kept at 4°C.

3. LB agar plate

LB agar (Merck) was prepared by dissolving LB agar powder (37 g) in 1,000 ml of DW. After autoclaving, the medium was allowed to cool down to 55-60°C before pouring into petridishes (23-25 ml per plate).

3. Ampicillin LB agar (LB-A agar)

One liter of LB agar was prepared as described above. After autoclaving and cooling down to 55-60°C, ampicillin (sterilized by membrane filtering) was added to the preparation to yield a final concentration of 100 μg/ml. Homogeneous agar was poured into 100-mm petridishes (23-25 ml/plate). The plate was stored at 4°C.

4. Minimal medium plates

The followimg stock solution were prepared:

$MgCl_2 \cdot 6H_2O$ (1 M)

$MgCl_2 \cdot 6H_2O$ (20.33 g) was dissolved in distilled water to a final volume of 100 ml and autoclave.

$CaCl_2 \cdot 2H_2O$ (1 M)

$CaCl_2 \cdot 2H_2O$ (14.7 g) was dissolved in distilled water to a final volume of 100 ml and autoclave.

Thiamine Hydrochloride (1 M)

Thiamine hydrochloride (33.73 g) was dissolved in 100 ml of DW and sterilized using a 0.22 μm filter.

Glucose (20%)

D-(+)-glucose (anhydrous) (20 g) was dissolved in 100 ml of DW to a final volume of 100 ml and sterilize by filtration through a 0.22 μm filter. Do not autoclave.

Minimum medium plate (MM)

Na_2HPO_4 (dibasic) (6 g), KH_2PO_4 (3 g), and NH_4Cl (1 g) were dissolved in 500 ml of DW. The pH was adjusted to 7.4 with NaOH.

In a separate 1 liter bottle, 15 g of Bacto-agar was dissolved in 500 ml of DW.

Both solution were sterilized by autoclaving. After the preparations were cooled down to 50–60°C, they were mixed. One ml each of 1 M $MgCl_2 \cdot 6H_2O$, 1 M $CaCl_2 \cdot 2H_2O$, and 1 M Thiamine hydrochloride, and 5 ml of 20% glucose were added. The preparation was immediately poured into petridishes.

5. SOBAG agar plate

SOBAG agar plates were prepared by dissolving Bacto-tryptone (20 g), Bacto-yeast extract (5 g), NaCl (0.5 g), and Bacto-agar (15 g) in 900 ml of DW. After autoclaving, the preparation was cooled down to 50–60°C. Sterile 1 M $MgCl_2$ (10 ml), sterile 2 M glucose (55.6 ml), and filter-sterilized 20 mg/ml ampicillin (5 ml) were added. The preparation was immediately poured into petridishes.

6. YT medium (2x)

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.

7. YT-AG medium (2x)

This medium is made by adding ampicillin and glucose to the 2x YT broth to the concentration of 100 μg/ml and 2%, respectively.

8. YT-AK medium (2x)

This medium is made by adding ampicillin and kanamycin to the 2x YT broth to the concentration of 100 μg/ml and 50 μg/ml, respectively.

9. YT-AI medium (2x)

This medium is made by adding ampicillin and IPTG to the 2x YT broth to the concentration of 100 μg/ml and 1mM, respectively.

10. PEG/NaCl

PEG/NaCl was prepared by dissolving polyethylene glycol 8000 (200 g) and NaCl (146.1 g) in 1 liter of DW. The solution was heated to dissolve and autoclaved to sterilize.

11. TES buffer (1x)

The buffer was prepared by dissolving Tris-base (4.85 g), EDTA (0.037 g), and sucrose (34.2 g) in 150 ml of DW. The pH of the preparation was adjusted to 8.0 by using HCl or NaOH. The volume of solution was adjusted to 200 ml and filtered through 0.2 μm filter. The solution was stored at 2–8°C.