

## APPENDIX

### 1. Lowenstein-Jensen egg medium (L-J medium)

#### Solution 1

- L-J medium powder, Difco-BBL      9.4 gm
- Deionized water                              150 ml

The solution 1 was autoclaved and allowed it cool at room temperature.

#### Solution 2

- Homogenized whole egg                      200 ml

Mixed solution 1 and 2 together and stand for 30 minutes, then dispensed 6 ml. into sterilized McCartney bottle, slanted and coagulated by hot air oven 80°C for 120 minutes. Stored at 2-8 °C

### 2. Sheep blood agar

#### Solution 1

- Blood agar base powder, Difco-BBL
- Deionized water

The solution 1 was autoclaved and allowed it cool at 50-60 °C in water bath.

#### Solution 2

- Whole sheep blood

Mixed solution 1 and 2 together, then dispensed 20-25 ml into sterilized plastic disc, coagulated at room temperature for 60 minutes. Stored at 2-8 °C

### 3. MacConkey agar

- MacConkey agar powder, Difco-BBL
- Deionized water

The solution was autoclaved and allowed it cool at 50-60 °C in water bath, then dispensed 20-25 ml into sterile plastic disc, coagulated at room temperature for 60 minutes. Stored at 2-8 °C

#### 4. Sabouraud dextrose agar

- Sabouraud dextrose agar powder, Difco-BBL
- Deionized water

The solution was autoclaved and allowed it cool at 50-60 °C in water bath, then dispensed 8-10 ml into sterile screw cap tube, slanted and coagulated at room temperature for 60 minutes. Stored at 2-8 °C

#### 5. Reagents

##### 5.1 Reagent for Isolated of genomic DNA from mycobacteria

- 1xTE buffer (10 mM Tris/HCl, pH 8.0 and 1mM EDTA, pH 8.0)
- 10 mg/ml lysozyme
- 10% SDS/Proteinase K (5µl of 10 mg/ml proteinase K and 70 µl of 10% SDS)
- 5M NaCl
- CTAB/ NaCl solution (4.1 gm NaCl was dissolved in 80 ml distilled water. While stirring, 10 gm CTAB (N-cetyl-N,N,N,-trimethyl ammonium bromide) were added. The volume was adjusted to 100 ml with distilled water. The solution was stored at room temperature for no longer than 6 months. If necessary, the solution was heated to 65 °C for completely dissolve.
- 24:1 chloroform/ isoamyl alcohol
- Isopropanol
- 70% Ethanol

##### 5.2 Reagent for Polymerase Chain Reaction

- 5 U/µl FastStart Taq DNA Polymerase, Roche
- 10x PCR reaction buffer without MgCl<sub>2</sub> (500 mM Tris/HCl, 100 mM KCl, 50 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, pH 8.3)
- 25 mM MgCl<sub>2</sub>
- 5x GC-RICH solution
- 10 mM dNTP solution

##### 5.3 Agarose gel electrophoresis

- 50X Tris acetate buffer (242 gm Tris base, 57.1 ml Glacial acetic acid, 0.5M EDTA pH 8.0, Dissolve all ingredients in distilled water and fill up to 1000 ml. Autoclaved and keep at room temperature)
- 3% agarose gel (3 gm. LE agarose, 100ml of 0.5xTAE buffer)

- 6x loading buffer, Gibco BRL USA
- 1 kb Plus DNA Ladder (5 $\mu$ l of 1  $\mu$ g/  $\mu$ l DNA Ladder, 10  $\mu$ l 6x loading buffer and 45 distilled water)

## 6. Instruments

- Analytical Balance, Sartorius Germany
- Autoclave, Memmert Germany
- Automatic pipettes, Gilson Japan
- Biological safety cabinet class II, Gelman Science Australia
- Centrifuge, Kubota Japan
- Thermal cycler system PCT 100, MJ Research, Waltham, MA, USA
- Freezer -20<sup>0</sup>C, Sanyo Thailand
- Gel reader, Fotodyne USA
- Horizontal gel electrophoresis chamber, Mupid Tokyo Japan
- Hot Air Oven, Shel Lab USA
- Incubator, Memmert Germany
- Microcentrifuge, Clover Laboratories USA
- Microwave, Shape Japan
- pH meter, Pierce USA
- Refrigerator 4 <sup>0</sup>C, Sanyo Thailand
- UV spectrophotometer, Shimadzu Japan
- Vortex Genie-2<sup>TM</sup>, Scientific Industries USA
- Water bath, Memmert Germany

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