APPENDIX

1. Media

1.1 MRS (De Man Rogosa Sharpe) broth (Atlas, 1993)

Glucose	18.5	g
Pancreatic digest of gelatin	10.0	g
Beef extract	8.0	g
Yeast extract	4.0	g
Sodium acetate ($CH_3COONa\ 3H_2O$)	3.0	g
K_2HPO_4	2.0	g
Ammonium citrate	2.0	g
$MgSO_4.7H_2O$	0.2	g
$MnSO_4.4H_2O$	0.05	g
Polysorbate 80	1.0	g
Distilled water	1.0	L

pH 6.2±0.2 at 25°C

Add component to distilled or deionized water and bring volume to 1.0 L. Mix thoroughly. Distribute into tubes or flask. Autoclave for 15 min at 15 psi pressure 121°C

1.2 MRS (De Man Rogosa Sharpe) agar (Atlas, 1993)

Glucose	18.5	g
Agar	13.5	g
Pancreatic digest of gelatin	10.0	g
Beef extract	8.0	g
Yeast extract	4.0	g
Sodium acetate	3.0	g
K_2HPO_4	2.0	g
Ammonium citrate	2.0	g
$MgSO_4.7H_2O$	0.2	g
$MnSO_4.4H_2O$	0.05	g
Polysorbate 80	1.0	g
Distilled water	1.0	L

pH 6.2±0.2 at 25°C

Add component to distilled or deionized water and bring volume to 1.0 L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flask. Autoclave for 15 min at 15 psi pressure 121°C. Pore into sterile Petri dishes or leave in tube.

1.3 MRS (De Man Rogosa Sharpe) agar + $CaCo_3$

Glucose	18.5	g
Agar	13.5	g
Pancreatic digest of gelatin	10.0	g
Beef extract	8.0	g
Yeast extract	4.0	g
Sodium acetate	3.0	g
K_2HPO_4	2.0	g
Ammonium citrate	2.0	g
$MgSO_4.7H_2O$	0.2	g
$MnSO_4.4H_2O$	0.05	g
Polysorbate 80	1.0	g
$CaCO_3$	5.0	g
Distilled water	1.0	L

pH 6.2±0.2 at 25°C

Add component to distilled or deionized water and add $CaCo_3$ 5 g and bring volume to 1.0 L. Mix thoroughly. Gently heat while stirring and bring to boiling. Distribute into tubes or flask. Autoclave for 15 min at 15 psi pressure 121°C. Pore into sterile Petri dishes .

2. Chemical solution

2.1 Glycerol solution

Composition per 0.5 liter:

Glycerol	325	ml.
$MgSO_4$ $7H_2O$	6.024	g.
Tris HCl	1.514	g.
pH 8.0		

Add component to distilled/deionized water and bring volume to 0.5 L. Mix thoroughly. Distribute into flask. Autoclave for 15 min at 15 psi pressure 121°C.

2.2 0.85% NaCl

Composition per 0.5 liter:

NaCl	4.25	g.
H_2O	500	ml.

Add component to distilled/deionized water and bring volume to 0.5 L. Mix thoroughly. Distribute into each tube . Autoclave for 15 min at 15 psi pressure 121°C.

2.3 Buffer I

Composition

50 mM Tris base
1 mM EDTA base
6.7% Sucrose

Adjust pH 8.0 by using glacial acetic acid. Autoclave for 15 min at 15 psi pressure 121°C.

2.4 Buffer II

Composition

50 mM Tris base
0.25 mM Na_2-EDTA

Adjust pH 8.0 by using 10 M NaOH. Autoclave for 15 min at 15 psi pressure 121°C.

2.5 SDS (Sodium Dodecyl Sulfate) buffer

Composition

50 mM Tris base
20 mM NA_2-EDTA

Adjust pH 8.0 by using glacial acetic acid. Autoclave for 15 min at 15 psi pressure 121°C. Add 20% (w/v) SDS after autoclave.

2.6 Lysozyme buffer

Composition

2.5 mM Tris base

Adjust pH 8.0 by using glacial acetic acid. Autoclave for 15 min at 15 psi pressure 121°C. Add lysozyme 20 mg/ml after autoclave.

2.7 Tris HCl buffer

Composition

2.0 M Tris HCl

Adjust pH 7.0 by using 10 M NaOH. Autoclave for 15 min at 15 psi pressure 121˚C.

2.8 Sodium chloride solution (NaCl)

Composition

5.0 M NaCl

Autoclave for 15 min at 15 psi pressure 121˚C.

2.9 Sodium hydroxide solution (NaOH)

Composition

3.0 N NaOH

2.10 Phenol solution

Prepare phenol saturated in 3% NaCl . Keep at 20˚C in the dark.

2.11 Chloroform : Iso-amyl alcohol

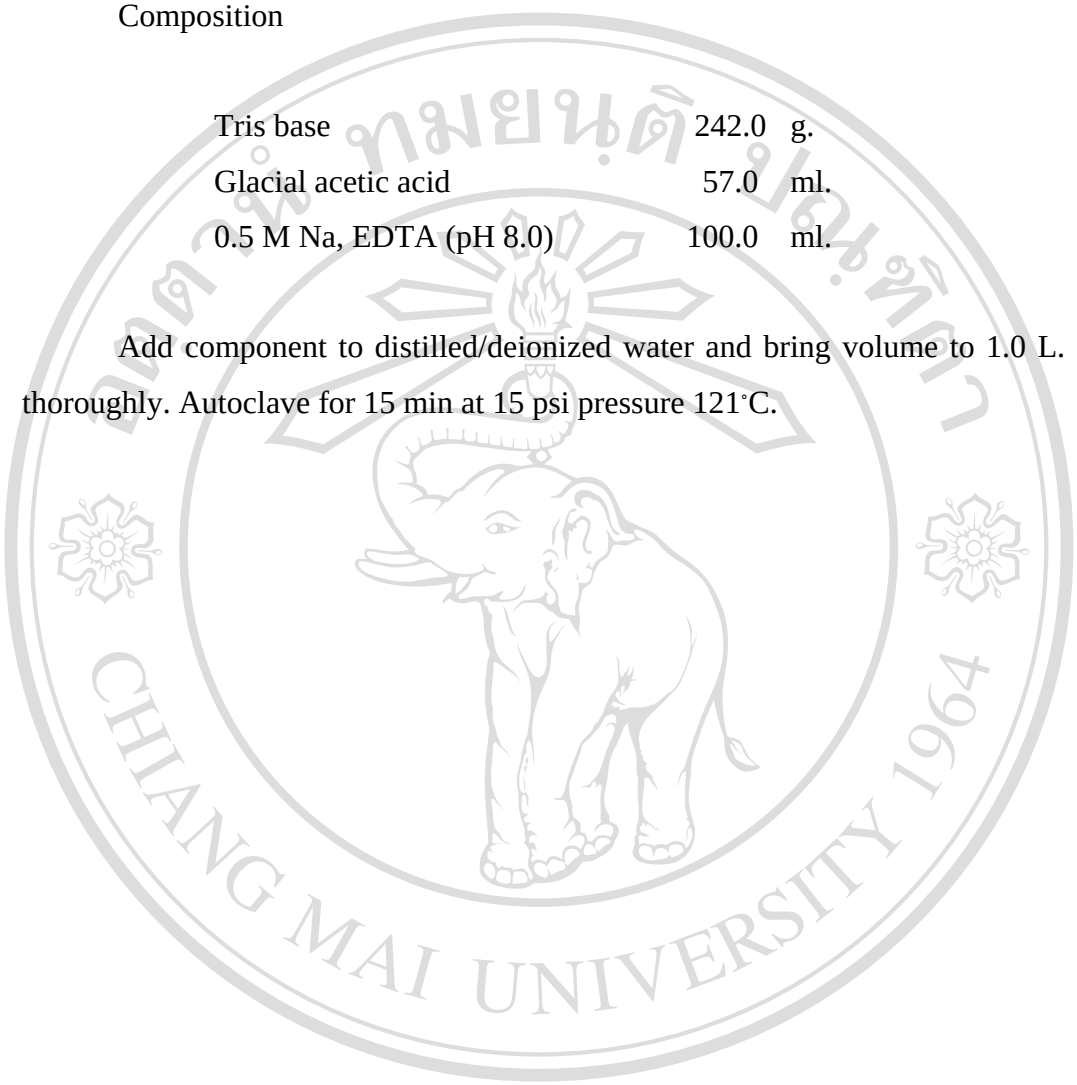
Mix chloroform solution and iso-amyl alcohol solution with ratio 24:1. The solution sensitive to the light, so keep at room temperature in dark.

2.12 50X TAE (Tris-acetate)

Composition

Tris base	242.0	g.
Glacial acetic acid	57.0	ml.
0.5 M Na, EDTA (pH 8.0)	100.0	ml.

Add component to distilled/deionized water and bring volume to 1.0 L. Mix thoroughly. Autoclave for 15 min at 15 psi pressure 121°C.



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