Appendix B

Protein extraction buffer:

50 mM Tris pH 7.5:

Tris base	6.055 g

Adjust the pH to 7.5 with HCl and bring up to 1000 ml with distilled water.

Preparation of staining solution:

Tris-A buffer	35.0 ml
2M Malic acid pH 7.0	5.0 ml
0.5 M $MgCl_2$	0.3 ml
1% NAD	2.0 ml

Before used: add

1% NBT	1.0 ml
1% PMS	0.5 ml
1% MTT	1.0 ml

incubate with the gel or cellulose polyacetate in the dark for 30-60 min or until blue bands appear

Preparation of 2M Malic acid pH 7.0

Malic acid	536.4 g
NaOH in flask	320.0 g

Note: Malic acid was placed on ice and NaOH was slowly added. Adjust the pH to 7.0 with NaOH. Bring up volume to 2000 ml with distilled water.

Preparation of Tris-A buffer (0.2M Tris-HCl pH 8.0)

Tris base	24.22 g

Adjust the pH to 8.0 with HCl and bring up to 1000 ml with distilled water

Preparation of 0.5 M $MgCl_2$

$MgCl_2 \cdot 6H_2O$	101.65 g

Bring up to 1000 ml with distilled water

Preparation of 1% NAD

NAD	1 g

Bring up to 100 ml with distilled water

Preparation of 1% NBT

NBT	1 g

Bring up to 100 ml with distilled water

Preparation of 1% PMS

PMS	1 g

Bring up to 100 ml with distilled water

Preparation of 1% MTT

MTT	1 g

Bring up to 100 ml with distilled water

DNA extraction buffer:

(100 mM EDTA (pH 8.0), 10 mM Tris (pH 7.5), 1% SDS, 1μg/ml Proteinase K)

Preparation of 1M EDTA (pH 8.0):

EDTA	372.2 g
(disodium ethylenediaminetetraacetate·$2H_2O$)	
Distilled water	800 ml

Adjust the pH to 8.0 with NaOH and bring up to 1000 ml

Preparation of 1 M Tris (pH 7.5):

Tris base	24.22 g

Adjust the pH to 7.5 with HCl and bring up to 1000 ml with distilled water

Preparation of 1 % SDS:

Sodium dodesyl sulfate (SDS)	1 g

Bring up to 100 ml with distilled water

Preparation of 1 mg/ml Proteinase K

Proteinase K	1 mg

Bring up to 1 ml with distilled water

Preparation of Phenol

1. Liquified phenol should be stored at –20^{o}C, As need, remove the phenol from the freezer, allow it to warm to room temperature, and then melt it at 68^{o}C. The hydroxyquinoline was added to a final concentration of 0.1%. (Hydroxyquinoline is an antioxidant, a partial inhibitor of Rnase, and a weak chelator of metal ion). In addition, its yellow colour provides a convenient way to identify the organic phase.
2. To the melted phenol, add an equal volume of buffer (usually 0.5M Tris-HCl, pH 8.0). Stir the mixture for 15 min, when the two phase have separate as much as possible of the upper (aqueous) phase using a glass pipette attached to a vacuum line equipped with traps.
3. Add an equal volume of 0.1M Tris-HCl, pH 8.0 to the phenol. Stir mixture and remove upper phase as described in step 2. Repeat the extractions until the pH of phenolic phase is >7.8 (as measured with pH paper)
4. After the phenol is equilibrated and the final aqueaus phase has been removed, add 0.1 volume of 0.1 M Tris-HCl pH 8.0 containing 0.2 % β-mercaptoethanol.

   The phenol solution may be stored in this form under 100 mM Tris-HCl, pH 8.0 in light-tight bottle at 4^{o}C for periods of up to 1 month.

Preparation of 2M NaCl

NaCl	116.88 g

Add 800 ml of distilled water and bring to a final volume of 1000 ml.

Preparation of TE pH 8.0 (10 mM Tris-HCl, pH 8.0 and 1mM EDTA (pH 8.0)

Combine 1.22 g of Tris-base and 0.4 g of Na_2-EDTA-$2H_2O$ with 500 ml of distilled water. Adjust the pH to 8.0 with HCl while stirring. Bring to a final volume of 1000 ml with distilled water.

Solutions for electrophoresis

50XTAE: Electrophoresis buffer

Tris-base	242 g
Glacial acetic acid	57.1 ml
0.5M EDTA pH 8.0	100 ml

Combine the ingredients with distilled water and bring up to volume 1000 ml. Working solution in the gel and the buffer is 0.5X.

Sample buffer (25% (v/v) glycerol, 60 mM EDTA, 0.25% (w/v) Bromophenol blue)

Glycerol	25 ml
EDTA	60 mM
Bromophenol blue	0.25 g

Bring up to 100 ml with distilled water. Combined with samples for electrophoresis.

Solution for competent cell and transformation preparation

LB (Luria-Bertani)broth (supplement with 80 μg/ml ampicillin)

1% (w/v) tryptone or peptone	10.0 g

0.5%(w/v) yeast extract	5.0 g
NaCl	5.0 g

Bring up to 1000 ml with distilled water and then sterilize by autoclaving. When the medium was cooled to 50^{o}C, 0.8 ml of ampicillin (100mg/ml) was added.

LB (Luria-Bertani)broth (supplement with 10 μg/ml tetracyclin)

1% (w/v) tryptone or peptone	10.0 g
0.5%(w/v) yeast extract	5.0 g
NaCl	5.0 g

Bring up to 1000 ml with distilled water and then sterilize by autoclaving. When the medium was cooled to 50^{o}C, 1 ml of (100mg/ml) tetracyclin was added.

Ampicillin (100mg/ml)

Ampicillin	100 mg

Dissolve in 1ml of sterile distilled water. Store at -20 ^{o}C

Tetracyclin (10 mg/ml)

Tetracyclin	10 mg

Dissolve in 1 ml of absolute ethanol (0.5 ml) and sterile distilled water (0.5 ml).

0.1 M $MgCl_2$

$MgCl_2 \cdot 6H_2O$	20.33 g

Bring up to 1000 ml with distilled water and then sterilize by autoclaving.

0.1M $CaCl_2$

$CaCl_2 \cdot 2H_2O$	14.7 g

Dissolve in 1000 ml of distilled water and then sterilize by autoclaving.

LB agar (supplement with 80 μg/ml ampicillin)

1% (w/v) tryptone or peptone	10.0 g
0.5%(w/v) yeast extract	5.0 g
NaCl	5.0 g
1.8 % Agar	18.0 g

Bring up to 1000 ml with distilled water and then sterilize by autoclaving. When the medium was cooled to 50^{o}C, 0.8 ml of ampicillin (80mg/ml) was added. The medium was poured into glass plate.

LB agar (supplement with 10 μg/ml tetracyclin)

1% (w/v) tryptone or peptone	10.0 g
0.5%(w/v) yeast extract	5.0 g
NaCl	5.0 g
1.8 % Agar	18.0 g

Bring up to 1000 ml with distilled water and then sterilize by autoclaving. When the medium was cooled to 50^{o}C, 1 ml of tetracyclin (10mg/ml) was added. The medium was poured into glass plate.

Solution for plasmid extraction:

STET buffer

Glucose	80 g
Tritron X-100	50 ml
Na_2EDTA	18.61 g
Tris-base	12.1 g

Adjust pH to 8.0 with HCl and bring up to 1000 ml. Sterilize by autoclaving

RNase A (10mg/ml)

RNase A	10 mg

Dissolve and bring up to 1 ml with sterile distilled water. Boil in water for 5 min. When the water was cooled to room temperature, then store the RNase A in -20 ^{o}C. It should boil again for 10 min before use.

Solution for sequencing

10XTBE

Tris base	107.8 g
Boric acid	55.0 g
Na_2EDTA	8.2 g

The pH should be approximately 8.3 at room temperature. Bring up to 1000 ml with distilled water. Filter with Whatman #1 filter paper.

IUB codes

A = adenosine	S = G or C (Strong-3H bonds)
C = cytosine	W = A or T (Weak-2H bonds)
G = guanosine	Y = C or T (pYrimidine)
T = thumidine	B = C, G or T
U = uracil	D = A, G or T
K = G or T (Keto)	H = A, C or T
M = A or C (aMino)	V = A, C or G
R = A or G (puRine)	N = aNy base

Geological time:

	Millions of years ago
Preambrian Time	
Archean Era	**4600-2500**
Proterozoic Era	**2500-570**
Phanerozoic Time	
Paleozoic Era	
Cambrian Period	**570-505**
Ordovician Period	**505-438**
Silurian Period	**438-408**
Devonian Period	**408-360**
Carboniferous Period	**360-286**
Permian Period	**286-245**
Mesozoic Era	
Triassic Period	**245-208**
Jurassic Period	**208-144**
Cretaceous Period	**144-66.4**
Cenozoic Era	
Tertiary Period	
Paleocene Epoch	**66.4-57.8**
Eocene Epoch	**57.8-38.6**
Oligocene Epoch	**38.6-23.7**
Miocene Epoch	**23.7-5.3**
Pliocene Epoch	**5.3-1.6**
Quarternary Period	
Pleistocene Epoch	**1.6-0.01**
Holocene Epoch	**0.01-0**