

## VIII. APPENDIX

### 1. 50X Tris-acetate buffer (TAE)

Tris base	242 gm
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Glacial acetic acid	57.1 ml
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Dissolved all ingredients in EDTA(0.5M, pH 8.0) 100 ml, then sterilized by autoclave and kept at room temperature.

### 2. 1X TAE

50X TAE	20 ml
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Distilled water	980 ml
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### 3. Loading buffer 6X

Bromphenol blue	0.25 %
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Glycerol	30 %
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Mixed thoroughly and kept at  $-20^{\circ}\text{C}$

### 4. Ethidium bromide (10 mg/ml)

Ethidium bromide	1.0 gm
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Dissolved in distilled water 100 ml, then kept at  $4^{\circ}\text{C}$  in a dark bottle.

## 5. 1% Agarose

Agarose	1.0 gm
0.5X TAE	100 ml

Dissolved by heating until homogenous solution occurred before using.

## 6. 1 Kb ladder

Stock 1Kb	5 $\mu$ l
6X Loading buffer	10 $\mu$ l
Dissolved in distilled water	45 $\mu$ l

## 7. 20X TBE

Tris base 1M	60.55 gm
Boric acid 1M	30.85 gm
EDTA 20mM	20 ml

Dissolved all ingredients in distilled water and filled up to 500 ml, then kept at room temperature.

## 8. 10XTBE

20X TBE	50 ml
Distilled water	50 ml

## 9. 1XTBE

20X TBE	50 ml
Distilled water	950 ml

## 10. 40% Acrylamide:bis(19:1)

Acrylamide	380 gm
bisacrylamide	20 gm
Distilled water	up to 1,000 ml

## 11. 4% Acrylamide solution

Urea	31.50 g
deionized H <sub>2</sub> O	40.00 ml
10x TBE	3.75 ml
40% acrylamide: bis (19:1)	7.50 ml

Filter the acrylamide solution through a 0.2 micron filter

Note: Acrylamide is highly toxic. Always wear gloves when working with acrylamide solutions.

## 12. Fix/Stop Solution (10% Acetic acid) for silver staining

Glacial acetic acid	50 ml
Distilled water	450 ml

## 13. Staining Solution for silver staining

Silver nitrate (AgNO <sub>3</sub> )	0.4 gm
37% formaldehyde (H <sub>2</sub> CO)	600 $\mu$ l
Distilled water	400 ml

## 14. Developer solution for silver staining system

37% formaldehyde ( $\text{H}_2\text{CO}$ )	600 $\mu\text{l}$
10mg/ml sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ )	80 $\mu\text{l}$
Sodium carbonate ( $\text{Na}_2\text{CO}_3$ )	12 gm
Distilled water	400 ml

## 15. 10% Ammonium persulfate

Ammonium persulfate	0.1 gm
Distilled water	1 ml

## 16. Developer solution for APC film

Developer solution (stock)	75 ml
Distilled water	225 ml

## 17. Fixing solution for APC film

Fix A	75 ml
Fix B	8 ml
Distilled water	217 ml

**IX. CURRICULUM VITAE**

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