

APPENDIX

1. 50x Tris-acetate/EDTA electrophoresis buffer (TAE)

Tris base	242	gm
Gracial acetic acid	57.1	ml
0.5 M EDTA (pH 8.0)	100	ml

Dissolved all ingredients in distilled water and filled up to 1,000 ml. Sterilized by autoclave and kept at room temperature.

2. 10x Tris-borate/EDTA electrophoresis buffer (TBE)

Tris base	108	gm
Boric acid	27.5	gm
0.5 M EDTA (pH 8.0)	40	ml

Dissolved all ingredients in distilled water and filled up to 1,000 ml. Sterilized by autoclave and kept at room temperature.

3. 1% agarose gel

agarose gel	1.0	gm
0.5x TAE	100	ml

Melted by microwave oven and added 4 μ l working ethidium bromide.

4. working ethidium bromide (10mg/ml)

Dissolved 1.0 gm ethidium bromide in 100 ml distilled water. Kept in dark bottle at 4°C.

5. 6x gel loading buffer

bromphenol blue	0.25	%
glycerol	30	%

Mix thoroughly and kept at -20°C.

6. 1 Kb DNA marker

1 Kb Plus DNA Ladder (Gibco BRL, USA)	5	μl
6x gel loading buffer	10	μl
distilled water	45	μl

Mix thoroughly and kept at -20°C.

7. stock 20% acrylamide

acrylamide	5	gm
N'-N'-bis-acrylamide	5	gm

Dissolved in distilled water and filled up to 500 ml. Sterilized by filtration and kept at 4°C.

8. 6% polyacrylamide gel (for 2 minigel)

stock 20% acrylamide	4.5	ml
10x TBE	1.5	ml
distilled water	9.0	ml

mix well all ingredients. add 100 μl of 10% ammonium persulfate and 3 μl of TEMED, then gentle mix and fill up the cast of gel.

9. 10x PCR buffer

KCl	3.73	gm
Tris HCl	1.58	gm

Dissolved in 100 ml of distilled water. Sterilized by autoclave and kept at -20°C.

10. 50 mM MgCl₂

Dissolved 476 gm MgCl₂ in 100 ml distilled water. Sterilized by autoclave and kept at 4°C.

11. DEPC-treated distilled water

Add 100 µl diethylpyrocarbonate (DEPC) to 100 ml distilled water, mix and let stand overnight at 37°C. Finally autoclave before use.

12. 0.1 M sodium citrate in 10% ethanol

sodium citrate dihydrate	14.71 gm
absolute ethanol	50.0 ml

Dissolved all ingredients in distilled water and filled up to 500 ml.

13. 0.1 M HEPES

Dissolved 1.3 gm HEPES (sodium salt) in 50 ml distilled water. Sterilized by autoclave and kept at 4°C.

14. Restriction endonuclease enzyme use and 10x buffer

- *Rsa* I (Gibco BRL, USA) uses with React 1 Buffer
- *Dde* I (Boehringer Mannheim, Germany) uses with SuRE/Cut™ Buffer H
- *Hae* III (Gibco BRL, USA) uses with React 2 Buffer
- *Hinf* I (Biolabs, USA) uses with NEBuffer 2
- *Eco* RII (Gibco BRL, USA) uses with React 6 Buffer

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