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 µl 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 RI* (Gibco BRL, USA) uses with React 6 Buffer
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