APPENDIX G

Reagents for plasmid DNA extract

1. Tris-HCl, pH 8.0 stock solution (2 M)

The solution was prepared by dissolving 242.28 g of Tris base in a volume of UDW. The pH was adjusted to 8.0 with HCl. The final volume was brought up to 100 ml with UDW and sterilized by autoclaving at 151 lb/inch^2, at 121^{o}C for 15 min.

2. Glucose stock solution (2 M)

The solution was prepared by dissolveing 36 g of glucose in 100 ml of UDW. The solution was sterilized by filtering as above and stored at -20^{o}C

3. EDTA stock solution (0.5 M)

The solution was prepared by dissolving 18.6 g of EDTA in a volume of UDW. The pH was adjusted to 8.0 with NaOH. The final volume was brought up to 100 ml with UDW and sterilized by autoclaving at 151 lb/inch^2, at 121^{o}C for 15 min.

4. Solution I (25 mM Tris pH 8, 50 mM glucose, 10 mM EDTA)

The buffer contained the following reagents:

2 M Tris-HCl, pH 8.0	1.25 ml
2 M glucose	2.50 ml
0.5 M EDTA, pH 8.0	200 ml

All components were diluted with UDW to final volume of 100 ml and mixed until solution becomes homogeneous.

5. Solution II (Alkaline solution)

This solution was prepared freshly before use by mixing 1 ml of 1 N NaOH, 8 ml of DW and 1 ml of 10% SDS solution.

6. Solution III (2.7 M potassium acetate, pH 4.8)

To prepare the solution, 26.49 g of potassium acetae (UNILAB, Australia) were dissolved in 70 ml of DW. The pH was adjusted to 4.8 with acetic acid. The final volume was brought up to 100 ml with UDW.

7. 70% ethanol

The solution was prepared by adding 70 ml of absolute ethanol to 30 ml of DW. The solution was kept at -20^{o}C.

8. TE buffer (10 mM Tris pH 8.0, 1 mM EDTA)

The buffer was prepared by diluting 0.5 ml of 2.0 M Tris-HCl pH 8.0 and 0.2 ml of 0.5 M EDTA in UDW to final volume of 100 ml and then sterilized by autoclaving.