

# Agarose Gel Electrophoresis of Low Molecular Weight DNA

## Reagents

Agarose  
ethidium bromide, 5ug/ml  
70% Ethanol  
Proteinase K, 1mg/ml (in DI H<sub>2</sub>O)  
DNase-free RNase A, 1mg/ml (in DI H<sub>2</sub>O)

### 10XTBE

108 g Tris base  
55 g boric acid  
960 ml H<sub>2</sub>O  
40 ml 0.5M EDTA, pH 8.0

### 6X gel loading buffer

30% glycerol  
0.25 g bromophenol blue  
0.25 g xylene cyanol  
70 ml DI H<sub>2</sub>O

### DNA extraction buffer

0.2M Phosphate-Citrate buffer, pH 7.8  
(made of 192 parts 0.2M Na<sub>2</sub>HPO<sub>4</sub>  
and 8 parts 0.1M citric acid , pH 7.8)

## Procedure

1. Wash 1-2X10<sup>6</sup> single cells in cold PBS. Re-suspend cells in 200ul cold PBS and vortex.
2. In a glass 12X75mm tube vortex 4mls ice cold 70% ethanol and slowly add the 200ul cells/PBS. Incubate at -20°C for 24 – 72 hours.
3. Pellet cells by centrifugation and remove all ethanol.
4. Add 50ul DNA extraction buffer to cell pellet, vortex and incubate at 37°C for 30'.
5. Transfer to a microfuge tube and pellet cells at 1,500xg for 10'. Remove and retain 40ul of the supernatant containing LMW-DNA. Discard pellet.
6. Add 5ul DNase-free, RNase and incubate at 37°C for 30'.
7. Add 5ul proteinase K and incubate at 37°C for 30'.
8. Add 5ul gel loading buffer and load entire sample into well of a 0.8% agarose gel in 1X TBE. Run a control lane of DNA molecular weight standards from 100bp – 1,000bp.
9. Apply 2V/cm for 16-20 hours and stain with ethidium bromide to photograph the characteristic 'laddering' associated with low molecular weight DNA found in apoptotic cells undergoing DNA fragmentation.

## References

Gong, J., Traganos, F., and Darzynkiewicz, Z. (1994) **A Selective Procedure for DNA Extraction from Apoptotic Cells Applicable for Gel Electrophoresis and Flow Cytometry** *Analyt. Biochem.*, 218: p 314-319

Robinson, J. Paul, Mng. Ed.: *Current Protocols in Cytometry* 1998, John Wiley & Sons, Inc. New York, NY p. 7.5.10