

Extraction of Genomic DNA from Tissue Cell Suspensions (used for apoptosis DNA ladders)

1. Resuspend cells (from frozen pellet) in extraction buffer:
(For a pellet of cells, I used 1 ml of Extraction Buffer in an Eppendorf tube).
Extraction Buffer
10mM Tris-Cl (pH 8.0)
0.1 M EDTA (pH 8.0)
20 ug/ml pancreatic RNase A
0.5% SDS
+ 100 ug/ml Proteinase K
2. Very gently, resuspend cells by carefully flicking tube with finger (vigorous agitation will shear DNA).
3. Incubate at 55°C for 3 hours.
4. Cool to room temp.
5. Add equal volume of phenol equilibrated to pH 8.0 (Must be pH 8.0).
6. Gently mix two phases by slowly turning tubes over for 10 mins. (Do not vortex).
***Note: Do all of the following transfers of the DNA-containing supernatant with a wide-bore pipet tip. I use a blue-colored Eppendorf pipetman tip with the end cut off. Make the bore-size very large because the DNA will be viscous, and it can be easily sheared.*
- 6.5 Spin the tubes in an Eppendorf centrifuge on low speed (2x1000rpm) for 3mins or so to separate the phases.
7. Transfer the aqueous phase (DNA-containing) to new Eppendorf tubes.
8. Repeat the extraction using an equal volume of phenol+chloroform. Save supernatant with the wide-bore pipet.
9. Repeat extraction using an equal volume of chloroform. Save sup. as above.
10. Add 1/10 volume of 5M NaCl, and 1.5-2.0X volumes of 100% cold ethanol.
11. Put at -20°C for at least an hour. Spin on high in microfuge to precipitate DNA for 20 mins. Wash with cold 70% ethanol.
12. Resuspend in about 300 microliters of TE (pH 8.0) plus 0.25ul of a 10mgs/ml stock of RNase A.
13. Prepare a 1.5% agarose gel (minigel works) with wide lanes.

14. Load DNA using a wide-bore pipet tip. Run gel slowly...about 50-60 V for a couple of hours. Stain with ethidium bromide and see ladders!!