DNA Laddering

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I. Protocol

1. Harvest cells
   Optional: wash plate with 37°C PBS (gently, so as not to lose cells); check on microscope, after aspirate PBS or media
   Place plate on ice
   Lyse with DNA ladder buffer 4°C
   150 ul/60 mm plate
   Scrape, transfer to eppendorf
   Rotate 4°C 20 min

2. Microcentrifuge 15 min, 4°C
   To pellet chromatin
   Transfer sup

3. Phenol/CHCl3 extract
   Add equal volume phenol/CHCl3
   Mix by pipetting
   Microfuge 2 min; transfer sup
   Will often get a very large interface

4. Optional: re-extract organic
   Add 300-500 ul TE8.0; mix; spin; pool sups

5. Ethanol Precipitate
   In eppendorf tube or 15 ml tube, depending on total sup volume
   Add 1/10 vol. 3 M NaOAcetate, 2 vol. ethOH
   –20°C overnight

6. Recover DNA
   Centifuge EtOH ppt:
   If in: 15 ml tubes: Sorvall SA600, 8,000 RPM, 10 min, 4°C epp tubes: microfuge 15 min, 4°C
   If was in 15 ml tube, probably best to resuspend in small volume (e.g., 500 ul TE), then re-EtOH ppt in epp tube, so pellet will be tight
   80% EtOH wash: add ≈500 ul; vortex; re-spin
   Speed-vac dry
   Resuspend in TE 8.0: ≈25 ul/eppendorf; room temp ≈30 min

7. RNase
   Add 1ul 1 ug/ul RNase A; room temp 30 min

8. Gel electrophoresis
   Add 5X loading buffer (*omit bromophenol blue: can interfere with band visibility; may be OK to add xylene cyanol)
   Run on 1.2% agarose gel, in TAE buffer

II. Reagents

DNA Laddering Buffer:

	40 ml
0.5% Triton X-100	800 ul 25%
5 mM Tris pH 7.4	200 ul 1M
20 mM EDTA	1.6 ml 500 mM
	37.4 ml dH2O

III. Notes

Reference: Hockenberry et al. Nature