HIRT DNA PREP FOR ANIMAL CELLS

Introduction:

A method to prepare episomal DNA from animal cells (transfected vectors, viruses)

Materials:

Hirt lysis buffer: 0.6% SDS, 10 mM EDTA pH 7.5
5 M NaCl
RNase A: 2 mg/ml
Phenol, saturated with 0.1 M Tris pH 7.4
5 M Sodium acetate pH 6.0

Procedure:

1. Wash 10 cm confluent plates with 1 x PBS at room temperature.
2. Add 2.0 ml. of Hirt lysis buffer. Let sit at room temperature 0-20 min.
3. Scrape lysate into 12 ml Falcon tubes. Pour into SW50.7 polyallomer tubes.
4. Add 1/4 volume 5 M NaCl, cover top with parafilm.
5. Store at 4°C for 8-20 hrs.
6. Spin at 4°C 18,000 rpm/40 min.
7. Pour supernatant into 12 ml Falcon tube, add 25x of RNase A (2 mg/ml). Incubate at 37°C for 60 min.
8. Phenol extract 2x.
   Ether extract 1x.
9. Add sodium acetate to 100 mM;
   Add 5 ml isopropanol, store overnight at -20°C.
10. Spin at 10 K for 30 min.
11. Rinse pellet with ice cold 70% ethanol; invert, air dry, resuspend pellet in TE.