Isolation of High-molecular-weight (genomic) DNA from Mammalian Cells using Proteinase K and Phenol

1. Trypsinize cells if in a monolayer and count
2. Pellet cells by centrifuging 5 minutes at 500 x g at 4 degrees
3. Wash cells in ice cold PBS and re-pellet
4. Resuspend in 1 volume of digestion buffer. For concentrations of 3 x 10⁷ cells, use 0.3 mL digestion buffer. For larger number of cells use 1 mL digestion buffer/10⁸ cells
5. Incubate samples at 50°C for 12 – 18 hours while rotating
6. Extract with phenol/chloroform/isoamyl alcohol
7. Centrifuge 10 minutes at 1700 x g
8. Transfer aqueous layer to a new tube and add ½ volume of 7.5 M ammonium acetate and 2 volumes of 100% ethanol
9. Pellet DNA by spinning 2 minutes at 1700 x g
10. Wash with 70% ethanol
11. Decant ethanol and air dry
12. Resuspend DNA at 1 mg/ml in TE buffer. If necessary, shake gently at room temperature to 65°C to facilitate resuspension. (~ 1g mammalian cells yields 2 mg DNA)
13. Add 10% SDS to a final concentration of 0.1% and RNAse A to a final concentration of 1 µg/mL.
14. Incubate for 1 hour at 37°C
15. Phenol/chloroform extract and ethanol precipitate

**Digestion Buffer**

100 mM NaCl
10 mM Tris [pH 8.0]
25 mM EDTA [pH 8.0]
0.5% SDS
0.1 mg/mL proteinase K