Phenol Chloroform Extraction of High Molecular Genomic DNA

Ingredients:

100 ml Extraction-Buffer (prepare freshly from Stock solutions):
100 mM NaCl
50 mM EDTA pH 8.0
50 mM Tris-HCl pH 7.5
1% Sarcosyl (N-Laurysarkosin Na Salt) Stock solution is 37%
7 mM ß-Mercaptoethanol

1. Precool the mortar and the pestle with liquid nitrogen. You can stick the mortar with water to the bench.
2. 0.5 g of Tissue (best is muscle) grind it in the presence of liquid nitrogen for about 10 min. The powder must be very fine. Add liquid nitrogen from time to time.
3. Transfer the powder into a clean 15 ml centrifuge tube, that contains 5 ml of the prewarmed (37°C) extraction buffer. Mix slowly but carefully by inversion.
4. Add 50 ml of Proteinase K c=10. Mix gentle by inverting slowly the tube approximately every 10 mintues and try to completely dissolve the remaining traces of tissue. Incubate at 45°C in a water bath for at least 30 min., or until the solution is becoming clearer and is getting even more viscous. If necessary, add more extraction buffer and Proteinase K.
5. Centrifuge for 5 minutes to eliminate remaining tissue if any with adaptors (Sorvall, Rotor SA, 10 000 rpm, room temperature).
6. Transfer the supernatant into a new tube. Add half a volume of equilibrated Phenol and mix gently but thoroughly through inversion for several minutes. Add half a volume of Chloroform/Isoamyl 24:1. Mix again gently but thoroughly.
7. Centrifuge for 15 min (Sorvall, Rotor SA, 10 000 rpm, room temperature).
8. Take carefully of the upper aqueous with a 1000ml Gilson (blue tip) cut off the end of the tips in order to avoid sheering of the high molecular weight DNA. After centrifugation the interphase has to be rather solid, the upper phase must be transparent, otherwise centrifuge again. Do not touch the interphase.
9. Repeat the phenol/chloroform extraction once again in exactly the same way (may not be necessary if the DNA is very clean).
10. Eliminate remaining Phenol rests by a final extraction with half volume choroform/isoamyl (24:1).
11. Precipitate the remaining aqueous phase by adding 1/10 vol 3 M NaOAc und 2 vol of Ethanol. The DNA should start to precipitate immediately, after mixing carefully the solution, in form of voluminous filamentous white mass.
12. Transfer the DNA with a glass rod to a large volume of the washing solution (10ml) the washing solution containing 70% EtOH.
13. Wash with 70% EtOH for upto 1 day.
14. Transfer the DNA with a glass rod, after eliminating excess liquid by draining, to an appropriate volume of TE (start with 400 µl, add more if DNA does not want to dissolve within a couple of hours).