Human Genomic DNA Extraction from Frozen Blood Samples Through Salting Out Method

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BACKGROUND

There are several various procedures for the preparation of genomic DNA. They all start with some form of cell lysis, followed by deproteinization and recovery of DNA. The main differences between various approaches lie in the extent of deproteinization and in molecular weight of the DNA produced. The isolation procedure described here is relatively brief and relies on the powerful proteolytic activity of proteinase K combined with the denaturing ability of the ionic detergent SDS. Use of proteinase K for DNA purification was described by Gross-Bellard et al. (1972) and Enrietto et al., (1983). EDTA is included in the digestion buffer to inhibit DNases. This method takes only two hours (2 hr.) for the isolation of DNA Extraction from the blood (Frozen derbies cells).

REAGENTS

TE: 10 mM Tris-HCl, 1mM EDTA

TKM1: 10mM Tris-HCl pH 7.6; 10 mM KCl, 10 mM $MgC1_2$; and 2 mM EDTA

TKM2: 10 mM Tris-HCl pH 7.6, 10 mM KCl, 10 mM $MgCl_2$, 0.4 M NaCl; and 2 mM EDTA

Triton X-100: Fisher or SigmaMillipore

PROTOCOL

1) Take **250 µL** Blood Sample and Add 850 µL **TKM1, 45 µL TritonX-100** +Vertex +Incubate at 41 °C for 4 Minutes and Centrifuge at 9000rpm for 3 Minutes and Discard the Supernatant.
2) Add 850 µL **TKM1**, **40 µL TritonX-100** +Vertex +Incubate at 41 °C for 4 Minutes and Centrifuge at 9000rpm for 3 Minutes and Discard the Supernatant.
3) Add 300 **µL TKM2+40 µL (10%) SDS + Vertex + Incubate 42**°C for 6 -7 Minutes.
4) Directly Add NaCl (6M) 100 **µL + Vertex.**
5) **Centrifuge at 10,000rpm for 3 Minutes** Supernatant Transfer to a new tube.
6) Add 300 µL PCI /Isopropanol + Vertex + Centrifuge at 10,000 rpm for Minutes. (Cloudy appearance)
7) Aqueous Phase transfer to a new tube
8) Add equal Concentration of 100% Ethanol to new tube +Vertex+ Centrifuge at 10,000 rpm for 5 minutes.
9) Drying (5-10 minutes) + add 50 **µL TE buffer.**