DNA extraction from nucleated blood – Phenol Chloroform for high quality intact DNA

Buffers:

PBS buffer

proteinase K (600mAU/ml, 40mAU/µg protein)

RNAse A (10mg/ml)

lysis buffer: buffer AL (from Quiagen kit or any other lysis buffer like Queen's lysis buffer).

Queen's lysis buffer
1.21 g Tris (0.01M Tris-Cl); 0.58g NaCl (0.01M NaCl); 20ml EDTA (0.5M) pH 8.0 (0.01M EDTA); 10g n-lauroylsarcosine (1% n-lauroylsarcosine)
Adjust pH to 8
Mix in 800ml ddH2O and bring vol. to 1L

TE buffer

Always cut pipet tips with clean (!) scissors.

1. Pipet 40 µl proteinase K into a 1.5 ml microcentrifuge tube. Add 15 µl non-coagulated blood. Adjust the volume to 220 µl with PBS.

2. Add 200 µl lysis buffer. Mix with vortex (!) and incubate at 56°C overnight using a rotor.

3. Add 5 µl (final conc. ~ 50 µg) RNAseA (Fermentas) and incubate at 37°C for 30 min.

4. Add 550 µl of Phenol-chloroform to each tube (to remove proteins). Shake gently, don't vortex so that the liquids are well mixed.

5. Centrifuge for 5 minutes at 13000rpm (first round), 10 minutes at 13000rpm (repeated round).

6. Pipette all of the supernatant, top layer (water+DNA) into a new 1.5 ml centrifuge tube.

7. If lots of protein is left (thick layer between phenol and water), redo step 4-6. **Always redo 4-6!**

8. Add 500 µl chloroform to each tube (to remove phenol). Shake gently, don't vortex so that the liquids are well mixed.

9. Centrifuge for 10 minutes at 13000rpm.

10. Carefully pipette the supernatant (water+DNA) into a new 1.5 ml centrifuge tube (e.g. 400 µl). Be careful not to get anything of the other layer of liquid transferred to the new tube! Note: The volume of the supernatant and the volumes correlated to in step 12-13 are not critical and exact. An approximate volume is OK. Usually the volume of the supernatant is ~400 µl.

11. Add 0.1 volumes of 3M NaAc to each tube containing the supernatant. For example, 40 µl to 400 µl supernatant.

12. Add 2 volumes of icecold isopropanol to each tube. For example, 800 µl for 400 µl supernatant in step 11.

13. Turn the tubes upside down (multiple times- do not vortex) so that everything gets mixed in the tube.

14. Incubate at -18°C one hour. 15 minutes in –70˚C is an option if such a freezer is available.

15. Centrifuge for 15 minutes at 13000rpm at 4° C. Carefully remove all liquid with a pipette.

    Wash the pellet twice with 70% Ethanol.

    Dissolve DNA (air dry, but not too dry!) in TE buffer.