Human blood and umbilical cord DNA extraction.v1

Blood genomic DNA

Use kit from Machery-Nagel – NucleoSipn Blood QuickPure from Clonetech

1. Prewarm sufficient amount of buffer BE at 70°C in a heating block.
2. Pipette 25 µl proteinase K (concentration 20 mg/ml stored at -20°C) and 200 µl whole blood (collected in EDTA blood collection tube) into a 1.5 µl-microcentrifuge tube.
3. Add 200 µl lysis buffer BQ1 and vortex vigorously for 10-20 sec.
4. Incubate at 70°C for 15 min in a heating block.
5. Add 200 µl 100% ethanol and vortex.
6. Transfer the entire contents to a supplied column in a collection tube and centrifuge for 1 min @ 11,000 x g at room temperature (10,823 rpm in Eppendorf centrifuge 5424). Discard the collection tube, which contains dark colored liquid. The column contains the genomic DNA.
7. Place the column in a new collection tube and pipette 350 µl buffer BQ2 and centrifuge for 1 min @ 11,000 x g to wash the column. Discard the flow through and re-use collection tube.
8. Pipette 200 µl buffer BQ2 and centrifuge for 3 min @ 11,000 x g to dry the column..
9. Transfer the column to clean microcentrifuge tube. Pipette 25 µl prewarmed buffer BE (70°C) directly onto the column and incubate at room temp for 3 min. Centrifuge for 1 min @ 11,000 x g to elute the DNA.
10. Repeat - Pipette 25 µl prewarmed buffer BE (70°C) directly onto the column and incubate at room temp for 3 min. Centrifuge for 1 min @ 11,000 x g to elute more DNA from the column.
11. Measure DNA concentration on nanodrop (range should be 200-400 ng/ul).

Umbilical cord genomic DNA

1. Slice a cross-section of an umbilical cord ~0.5 cm width.
2. Identify the vein (The vein is larger in diameter, has a thinner vessel wall and is more elastic than the arteries. It is easy to identify because there are two arteries and the vein is the odd one out) and the two arteries.
3. Carefully dissect each vessel from the surrounding tissue and cut a small piece of connective tissue for DNA extraction.
4. Place each tissue sample in a tube with 750 µl genomic DNA lysis buffer and 10 µl proteinase K (concentration 20mg/ml stored at 4°C).
5. Incubate at 55°C overnight and complete regular genomic DNA isolation as per protocol online.
6. Measure DNA concentration on nanodrop (arteries/vein range 120-250 ng/ul; connective tissue 30-120 ng/ul).

Store remaining blood and umbilical cord in -80°C freezer in styrofoam rack.