Shalev Lab Protocols for U24 DNA Extraction Study

Summary: Explicit protocols for each DNA extraction method are provided below. For Gentra PureGene, extractions were conducted using factory protocols for 300uL whole blood without the optional RNase digestion step. For QIAmp, extractions were conducted following the Spin Protocol for Blood or Body Fluids from the QIAmp DNA Blood Mini Kit. QIASymphony extractions were performed in the lab of Dr. Sue Patrick at Hershey Medical Center following in accordance with manufacturer instructions.

Puregene DNA Purification from Whole Blood (TLH 10/07/2020)

*Whole blood samples should be thawed quickly in a 37 degree Celsius water bath with mild agitation and stored on ice before beginning the procedure.

1. Add 900 uL RBC Lysis Solution into a 1.5 mL tube
2. Add 300 uL of whole blood sample, mix by inverting 10 times.
3. Incubate for 1 minute at room temperature. Invert at least once during the incubation.
   *If blood is fresh (within an hour of withdrawal), increase incubation time to 3 min. to ensure complete RBC lysis.
4. Centrifuge for 30 seconds at 14,500 x g to pellet the white blood cells.
5. Carefully discard the supernatant by pipetting or pouring, leaving approximately 10 uL of the residual liquid and the white blood cell pellet.

6. Vortex the tube vigorously to resuspend the pellet in the residual liquid.
7. Add 300 uL Cell Lysis Solution, and pipet up and down to lyse the cells or vortex vigorously for 10 seconds.
   - If cell clumps are visible, incubate at 37 degrees until the solution is homogenous
8. Add 100 uL Protein Precipitate Solution, vortex for 20 seconds at a high speed.
9. Centrifuge for 1 min at 14,500 x g
10. Add 300 uL isopropanol into a clean 1.5 mL tube, and add the supernatant from the previous step by carefully pouring.

11. Mix by gently inverting the tube 50 times, until the DNA is visible as threads or a clump.
12. Centrifuge for 1 minute at 14,500 x g
13. Carefully discard the supernatant, and drain the tube by inverting on a clean paper towel, making sure the pellet stays in the tube.

14. Add 300 uL of 70% ethanol and invert several times to wash the DNA pellet
15. Centrifuge for 1 minute at 14,500 x g
16. Carefully discard the supernatant. Drain the tube on a clean paper towel. Air dry the pellet for 5 minutes.
17. Add 100 uL DNA hydration solution, vortex 5 seconds to mix.
18. Incubate at 65°C for 5 minutes
19. Incubate at room temperature overnight with gentle shaking. Samples can then be centrifuged briefly and transferred to a storage tube.

DNA Purification from Whole Blood (Spin Protocol) using QIAmp DNA Blood Mini Kit (TLH 10/07/2020)

1. Pipet 20 uL Qiagen Protease into the bottom of a 1.5 mL tube
2. Add 200 uL sample to the 1.5 mL tube.
3. Add 200 uL Buffer AL to the sample. Mix by pulse-vortexing for 15 seconds.
4. Incubate at 56°C for 10 minutes
5. Briefly spin the 1.5 mL tube to remove drops from the inside of the cap.
6. Add 200 uL 100% ethanol to the sample, and mix again by pulse vortexing for 15 seconds. After mixing, briefly spin the tubes again to remove drops from inside the lid.
7. Carefully apply the mixture from step 6 to the QIAmp Mini spin column in a 2 mL collection tube without wetting the rim. Close the cap, and centrifuge at 6000 x g for 1 minute.
8. Place the spin column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
9. Carefully open the spin column and add 500 uL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g for 1 min.
10. Place the spin column in a clean 2 mL collection tube, and discard the collection tube containing the filtrate.
11. Carefully open the spin column and add 500 uL Buffer AW2 without wetting the rim. Close the cap and centrifuge at 20,000 x g for 3 minutes.
12. Place the spin column in a new 2 mL collection tube and discard the old collection tube containing the filtrate. Centrifuge at 20,000 x g for 1 minute.
    a. This helps to eliminate buffer carryover
13. Place the spin column in a clean 1.5 mL tube and discard the tube containing the filtrate. Carefully open the column and add 200 uL Buffer AE or distilled water. Incubate at room temp for 1 minute, and then centrifuge at 6000 x g for 1 minute to elute the DNA.
14. Discard spin column, Nano-drop sample for nucleic acid concentration, and store in freezer.