Genomic DNA Isolation from Dried Blood (Guthrie/PKU) Spots

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http://www.pharmacy.vcu.edu/biomarker/

Materials

- QIAamp DNA Mini Kit (Qiagen, Valencia, CA)
- Proteinase K (100ug/ml)
- RNase A (100 mg/ml)
- 10% Bleach (0.6% w/v NaOCl): 1 volume 6% w/v sodium hypochlorite and 9 volumes water.
- Stainless steel forceps and small surgical scissors
- Centrifuge capable of performing 8,000 x g
- Water bath or heat block for incubation at 56°, 70° and 85 °C
- Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA).
- Qubit dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA).

Protocol

- All centrifugation steps are carried out at room temperature.
- Equilibrate all buffers to room temperature before use.
- Keep in mind three different heat block/bath temperatures are needed throughout the protocol: 85°C, 56°C, and 70°C.
- WARNING: Be careful not to combine Buffer AW1 with bleach. This will produce HCN gas!

1. Cut a dry blood spot into small pieces using sterilized scissors; divide pieces between two 2-mL tubes, and process in parallel (see Note 1). Soak used scissors in 10% bleach (v/v) for 5 minutes, and wash twice with ddH2O before re-using.

2. Add 720 µl of Buffer ATL to each tube and vortex to saturate paper.

3. Incubate on heat block/bath at 85°C for 10 minutes. Cool for a few minutes at room temperature then briefly centrifuge to collect condensation.

4. Add 80 µl proteinase K solution, vortex, and incubate on heat block/bath at 56°C for 1 h. Vortex every 15 minutes while incubating. Following incubation briefly centrifuge to collect condensation.

5. Add 16 µl RNase A solution, vortex, and incubate 5 minutes at room temperature.
6. Add 800 μl Buffer AL, vortex immediately and vigorously, then incubate on heat block/bath at 70°C for 10 minutes. Following incubation briefly centrifuge to collect condensation.

7. Transfer 800 μl of lysate to new 2-ml tubes (four 2-ml tubes of lysate per blood spot). Take care not to aspirate paper fragments during transferring.

8. Add 400 μl of 100% ethanol to each tube, mix thoroughly by vortexing, and pulse spin to bring the liquid down from the cap.

9. Transfer 600 μl of the mixture from step 8 to a new QIAamp Mini spin column and centrifuge at 8000 x g for 1 minute. Discard flow-through and place the column back into the collection tube. Repeat until all the lysate for the sample have been passed through the column (see Note 2).

10. Add 600 μl Buffer AW1 to the column without wetting the rim and centrifuge 8000 x g for 1 minute. Discard the flow-through and place the column in a new 2-ml collection tube.

11. Add 600 μl Buffer AW2 to the column and centrifuge at full speed (20,000 x g) for 30 seconds. Discard the flow-through.

12. Add 600 μl Buffer AW2 to the column and centrifuge at full speed (20,000 x g) for 2 minutes. Carefully remove the column and place into a new 1.5-ml tube. Discard the collection tube and flow-through.

13. Add 50 μl Buffer AE. Incubate at room temperature for 3 min then centrifuge at 8000 x g for 1 minute. Load an additional 50 μl of Buffer AE to the column and centrifuge at 8000 x g for 1 minute. Eluate contains isolated gDNA. Evaluate the concentration of the genomic DNA extracted by Qubit dsDNA HS Assay. A yield of 500-1000 ng of gDNA can be expected for a single ~13mm blood spot.

Notes

1. We have found that increasing the volume of lysis buffers results in increased yields of gDNA from dry blood spots. Dividing the material from a ~0.5 in dia. blood spot between two 2-ml microcentrifuge tubes allows full saturation and submersion of the filter paper.

2. If using material from several smaller blood spots the use of two spin columns may help prevent clogging by paper fibers and increase gDNA yields. Load two (2400 μl total) tubes of lysate/ethanol mixture from step 8 into separate columns.