SOP#125: QIAamp Investigator DNA extraction from dried blood spots

1. **PURPOSE:**

This protocol describes QIAamp Investigator the DNA extraction method from dried blood spots (DBS) collected with Whatman® 903 Protein Saver Cards using QIAamp DNA Investigator kit by QIAGEN . The blood collection protocol is described in SOP 123. Details of the QIAamp DNA Investigator kit can be found in the QIAamp DNA Investigator handbook (https://www.qiagen.com/us/resources/resourcedetail?id=26ef8f2c-7c2a-49e6-b2d2-39d4e130b3cc&lang=en)

2. **MATERIALS**

	Vendor	Cat#
QIAamp DNA Investigator Kit	Qiagen	56504
96-100% Ethanol	varies	N/A
Kimwipes	Kimtech	varies
Alcohol pad	varies	N/A
P1000 pipetteman and filter tips	varies	varies
P200 pipetteman and filter tips	varies	varies
P20 pipetteman and filter tips	varies	varies
1.5 ml eppendorf tubes (presterilized)	varies	varies
Eppendorf tube racks for 1.5 ml tubes	varies	varies
Marker pen	varies	N/A
3 mm hole punch	varies	N/A
tweezers	varies	N/A

Buffer ATL, AL, protease K, AW1 concentrate, AW2 concentrate, MinElute Columns, ATE buffer and 2 ml collection tubes are parts of the QIAmp DNA Investigator kit.
Complete AW1 buffer is made by adding 25 ml of 96-100% ethanol to the AW1 bottle containing 19 ml AW1 concentrate provided in the kit.
Complete AW2 buffer is made by adding 30 ml of 96-100% ethanol to the AW2 bottle containing 13 ml AW2 concentrate provided in the kit.

2.2 Equipment

- Eppendorf microfuge, model 5415D
- Scientific Industries Vortex Genie-2
- Eppendorf thermomixer
- Thermo scientific NanoDrop 2000c
- Biosafety cabinet (tissue culture hood), certified and prepared for sterile technique

3. **EXPERIMENTAL PROCEDURES**

NOTE: Steps 3.1 to 3.3 should be done in a biosafety cabinet

3.1. Use a 3 mm diameter punch, cut 6 punches from DBS card directly into 1.5 ml Eppendorf tube (aim for areas with lots of blood). Use a pair of tweezers to transfer the DBS punches into the prelabeled 1.5 ml Eppendorf tubes.

3.2. Clean the hole punch and tweezer after each sample by using an alcohol pad and drying the punch and tweezer completely before the next sample.
3.3. Add 280 µl Buffer ATL buffer and 20 µl proteinase K. Mix thoroughly by vortexing.
3.4. Place the 1.5 ml Eppendorf tube in a thermomixer and incubate at 56°C with shaking at 900 rpm for 1 h.
3.5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
3.6. Add 300 µl Buffer AL buffer and mix by vortexing for 10 s.
3.7. Place the 1.5 ml tube in Thermomixer and incubate at 70°C with shaking at 900 rpm for 10 minutes.
3.8. Briefly centrifuge the 1.5 ml Eppendorf tube to remove drops from the inside of the lid. Add 150 µl ethanol (96–100%), close the lid, and mix thoroughly by vortexing for 15 s.
3.9. Briefly centrifuge the 1.5 ml Eppendorf tube to remove drops from the inside of the lid.
3.10. Carefully transfer cell lysate to corresponding QIAamp MinElute Columns. Pipette towards the center of the spin column. Take care not to touch and wet the rim of the spin columns. Spin columns in a microfuge for 1 minute at 8,000 rpm.
3.11. Transfer spin columns to a clean set of collection tubes and discard old collection tubes with filtrate. Wash columns by adding 500 µl of Buffer AW1. Pipette towards the center of the spin column. Take care not to touch and wet the rim of the spin columns. Spin at 8,000 rpm in Eppendorf microfuge for 1 minute. Transfer the column to a clean collection tube and discard the old collection tubes with the filtrate.
3.12. Wash columns by adding 700 µl of Buffer AW2. Spin at 8000 rpm in a microfuge for 1 minutes. Transfer the spin columns to a clean set of collection tubes and discard the old collection tubes with the filtrate.
3.13. Add 700 µl of ethanol (96–100%). Spin at 8000 rpm in a microfuge for 1 minutes. Transfer the columns to a clean set of collection tubes and discard the old collection tubes with the filtrate. Centrifuge at 13,200 rpm for 3 min to dry the membrane completely. Transfer spin columns to clean, labeled 1.5 ml Eppendorf tubes. Open the lid of the QIAamp MinElute column, and incubate at at 56°C for 3 min.
3.14. Add 55 µl Buffer AE to spin columns. Let columns stand for 5 minutes. Spin columns down for 1 minute at 13,2000 rpm.
3.15. Store DNA at 4°C for DNA concentration and quality determination. Store the DNA at -80°C for future use.

4. REVISION HISTORY

DATE	AUTHOR	REVISION #	REVISION REASON
April 28, 2020	Jue Lin	A	Initial Release
September 13, 2020	Jue Lin	B	Minor changes