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Supplemental Material to:

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High quality methylome-wide investigations through next-generation sequencing of DNA from a single archived dry blood spot

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(QIAamp DNA Mini Kit) Catalog Number 51104

The original protocol, DNA Purification from Dried Blood Spots, can be found in the QIAamp DNA Mini and Blood Mini Handbook (Second Edition, NOV 2007) from Qiagen.

Please note that this protocol has been modified to allow a complete dried blood spot to be processed in one reaction. All modifications from the original protocol are marked in red.

Important point before starting

■ All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

■ Prepare an 85°C water bath for use in step 2, a 56°C water bath for use in step 3, and a 70°C water bath for use in step 4.
■ Equilibrate Buffer AE or distilled water to room temperature for elution in step 10.
■ Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 17.
■ If a precipitate has formed in Buffer AL or Buffer ATL, dissolve by incubating at 56°C.

Procedure

1. Place 1 dry blood spot into a 2 ml microcentrifuge tube and add 180 µl x 4 = 720 µl of Buffer ATL.
Cut a dry blood spot into 4-6 pieces with clean scissors, wash scissors with 3% bleach and then ddH2O.

2. Incubate at 85°C for 10 min. Briefly centrifuge to remove drops from inside the lid.

3. Add 20 µl x 4 = 80 µl proteinase K stock solution. Mix by vortexing, and incubate at 56°C for 1 h. Briefly centrifuge to remove drops from inside the lid.
Note: The addition of proteinase K is essential. Vortex at 5-10 min intervals.
If RNA-free genomic DNA is required, **4 x 4 µl RNase A** stock solution
(100 mg/ml) should be added to the sample prior to the addition of Buffer AL.

4. Add 200 x 4 = 800 µl Buffer AL to the sample. Mix thoroughly by vortexing, and incubate at 70°C for 10 min. Briefly centrifuge to remove drops from inside the lid.
In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

5. Transfer 800 µl sample to a new 2 ml tube (now each sample will have two 2 ml tubes) Add 200 x 2 µl ethanol (96–100%) to each sample tube, and mix thoroughly by vortex. Briefly centrifuge to remove drops from inside the lid.
It is essential for the sample and ethanol to be mixed thoroughly.

6. Carefully apply 700 µl of the mixture from step 5 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Repeat this step until the entire sample is loaded into one column.
Dump the through flow and Place the QIAamp Mini spin column back to 2 ml collection tube and load the rest of sample to the column until finish, and discard the tube containing the filtrate.*
Close each QIAamp Mini spin column in order to avoid aerosol formation during the centrifugation.

7. Carefully open the QIAamp Mini spin column and add 700 µl Buffer AW1 without wetting the rim.
Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min.
Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.*
Repeat this step twice.

8. Carefully open the QIAamp Mini spin column and add 700 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min.
Repeat this step twice.

9. Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.
This step helps to eliminate the chance of possible Buffer AW2 carryover.

10. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 100 µl Buffer AE. Incubate at room temperature for 3 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.
Reload the elution to the column and incubate for 3 min and spin 6000 x g for 1 min.

Check the OD and 260/280.
If 260/280 is out of range (1.7-2.0), try to re-precipitate with 0.3M NaAc and glycogen.