QIAGEN Supplementary Protocol:

Isolation of genomic DNA from dried blood spots using the QIAamp® 96 DNA Blood Kit

This protocol is designed for the isolation of genomic DNA from dried blood spots using the QIAamp® 96 DNA Blood Kit. Blood, both untreated and treated with anticoagulants, which has been spotted and dried onto filter paper (e.g., S&S 903™, Schleicher and Schuell) is suitable for use with this procedure.

Please be sure to read the *QIAamp 96 DNA Blood Kit Handbook* carefully before beginning this procedure.

Important notes before starting

- Buffer ATL and Proteinase K are not included in the QIAamp 96 DNA Blood Kit, but can be purchased separately (200 ml Buffer ATL, cat. no. 19076; 10 ml QIAGEN Proteinase K, cat. no. 19133).
- Equilibrate Buffer AE to room temperature, for elution in step 18.
- Prepare a 90°C incubator oven for use in step 2, and a 56°C incubator oven for use in steps 4 and 6.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to instructions.
- If a precipitate has formed in Buffer AL or Buffer ATL, dissolve by incubating at 70°C.
- Use of a multichannel pipet is recommended.
- All centrifugation steps are carried out at room temperature.

Procedure

1. **Place 3 punched-out circles from a dried blood spot to the bottom of one well of a Round-Well Block (provided), and add 180 µl of Buffer ATL. Use the register card provided to identify the locations of the samples.**

   Cut 3 mm (1/8 inch) diameter punches from a dried blood spot with a single-hole paper puncher (paper punchers may be purchased from Schleicher & Schuell, order no. 10495010).

   It is a good idea to mark the Round-Well Blocks at this stage so that they can be easily identified throughout the protocol.

2. **Seal the wells thoroughly using the caps (for round-well blocks) provided. Incubate at 90°C for 15 min. Centrifuge briefly at 3000 rpm to collect any solution from the caps.**

   Allow centrifuge to reach 3000 rpm, then stop the centrifuge.

3. **Add 20 µl Proteinase K solution. Seal the wells thoroughly using the caps provided. Mix by vigorously shaking the Round-Well Block for 15 s.**

   Note: To avoid cross-contamination when sealing the wells with caps, do not touch the rim of the wells with the pipet tips.

   Note: The addition of Proteinase K is essential.

4. **Centrifuge briefly at 3000 rpm to collect any solution from the caps and incubate at 56°C for 1 h in an incubator oven.**

   Allow centrifuge to reach 3000 rpm, then stop the centrifuge.

   Note: Placing a weight on top of the round-well block will prevent the lids from popping off occasionally during incubation.

5. **Centrifuge briefly at 3000 rpm to collect any solution from the caps. Add 200 µl Buffer AL to each of the samples, taking care not to wet the rims of the wells. Seal the wells thoroughly using the caps provided.**

   Note: Use only the caps provided, since using AirPore™ tape at this stage of the procedure will lead to cross-contamination. Ensure that the wells are sealed thoroughly to avoid spurting during shaking.

6. **Mix thoroughly by shaking vigorously for 15 s and incubate at 56°C for 10 min.**

   In order to ensure efficient lysis, it is essential that the samples and Buffer AL are mixed immediately and thoroughly to yield a homogeneous solution. Hold the Round-Well Block with both hands and shake up and down vigorously.

7. **Centrifuge briefly at 3000 rpm to collect any solution from the caps.**

   Allow centrifuge speed to reach 3000 rpm, then stop the centrifuge.

8. **Remove the caps and add 200 µl ethanol (96-100%) to each of the samples, taking care not to wet the rims of the wells.**

9. **Seal the wells thoroughly using new caps. Shake vigorously for 15 s. Centrifuge briefly at 3000 rpm to collect any solution from the caps.**

   Allow centrifuge speed to reach 3000 rpm, then stop the centrifuge.

10. **Place the QIAamp 96 Plate on top of an S-Block. Mark the QIAamp 96 Plate for later identification.**

11. **Carefully apply the mixture from step 9 (600 µl per well) to the QIAamp 96 Plate.**

    Take care not to wet the rims of the wells to avoid aerosol formation during centrifugation.

    Ensure that blood card punches are not transferred or do not block pipet tips.

    Note: Due to sample volume, lowering pipet tips to the bottoms of the wells may cause overflow if extended, narrow pipet tips (such as Matrix cat. no. 8255) are not used. It is best to remove one strip of caps at a time and begin drawing up samples as soon as pipet tips contact the sample. Repeat until all the samples have been applied to the QIAamp 96 Plate.

12. **Seal the QIAamp 96 Plate with an AirPore tape sheet. Load the S-Block and QIAamp 96 Plate onto the carrier, then place it in the rotor bucket. Centrifuge at 6000 rpm for 4 min.**

13. **Remove the AirPore tape. Carefully add 500 µl Buffer AW1 to each well.**

14. **Seal the QIAamp 96 Plate with a new AirPore tape sheet. Centrifuge at 6000 rpm for 2 min.**

15. **Remove the AirPore tape. Carefully add 500 µl Buffer AW2 to each well.**

16. **Centrifuge at 6000 rpm for 15 min.**

    The heat generated during centrifugation allows for evaporation of any residual ethanol in the sample (from Buffer AW2) that may otherwise inhibit PCR and other downstream reactions.

    Note: In order to ensure efficient ethanol evaporation, do not seal the plate with AirPore tape during this centrifugation step.

17. **Place the QIAamp 96 Plate on top of a rack of elution microtubes (provided).**

18. **To elute the DNA, add 150 µl Buffer AE or distilled water, equilibrated to room temperature, to each well using a multichannel pipet, and seal the QIAamp 96 Plate with a new AirPore tape sheet. Incubate for 1 min at room temperature. Centrifuge at 6000 rpm for 4 min. Use the caps provided to seal the wells of the microtubes for storage.**

    Note: Do not elute the DNA with volumes of less than 100 µl.

    Three punched-out circles (3 mm diameter) typically yield 150 ng and 75 ng of DNA from anticoagulant-treated and untreated blood, respectively. If the yield from untreated blood is not sufficient, use 6 circles per prep instead of 3.

    The volume of the DNA eluate used in a PCR assay should not exceed 10% — for a 50 µl PCR, add no more than 5 µl of eluate.