QIAGEN Supplementary Protocol:

Isolation of genomic DNA from saliva and mouthwash using the QIAamp® DNA Blood Mini Kit; vacuum procedure

This protocol is designed for purification of DNA using QIAamp® Spin Columns with VacConnectors and a QIAvac 6S, QIAvac 24, or other vacuum manifold with luer connectors.

Please be sure to read the *QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook* and the detailed Blood and Body Fluid Vacuum Protocol carefully before beginning this procedure.

Important notes before starting

- Ensure that Buffer AL, Buffer AW1, Buffer AW2, and QIAGEN® Protease have been prepared according to the *QIAamp DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook*.
- If a precipitate has formed in Buffer AL, dissolve it by incubating at 70°C.
- All centrifugations are carried out at room temperature.

Procedure

1. **Collect 1 ml saliva by spitting in a 50 ml Falcon® tube. Or collect mouthwash in a 50 ml Falcon tube.**

   Note: Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection.

2. **Add 4 ml PBS (not provided) to the sample and centrifuge at 1800 x *g* for 5 min.**

3. **Carefully decant the supernatant. Resuspend the pellet in 180 µl PBS.**

   QIAamp Spin Columns copurify RNA and DNA in parallel when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not the PCR itself. If RNA-free genomic DNA is required, 20 µl of an RNase A stock solution (20 mg/ml) should be added to the sample prior to the addition of QIAGEN® Protease and Buffer AL.

4. **Add 20 µl QIAGEN Protease stock solution and 200 µl Buffer AL to the sample. Mix immediately by vortexing for 15 s.**

   In order to ensure efficient lysis, it is essential that the sample and Buffer AL are mixed immediately and thoroughly.

   Note: Do not add QIAGEN Protease directly to Buffer AL.

5. **Incubate at 56°C for 10 min.**

6. **Add 200 µl ethanol (96–100%) to the sample, and mix again by vortexing.**

7. **Insert a VacConnector into a Luer Adapter on the QIAvac 6S or QIAvac 24.**

   VacConnectors are designed to prevent cross-contamination of the QIAamp Spin Columns. VacConnectors should be removed from Luer Adapter immediately after each preparation and disposed of properly.

8. **Insert the QIAamp Spin Column into the VacConnector. Seal unused Luer Adapters with Luer plugs.**

9. **Apply the mixture from step 6 to the QIAamp Spin Column. Switch on the vacuum pump to draw the lysate through the QIAamp Spin Column. After the lysate has passed through the QIAamp Spin Column, switch off the vacuum pump.**

10. **Add 750 µl Buffer AW1. Switch on the vacuum pump to draw Buffer AW1 through the QIAamp Spin Column. Switch off the vacuum pump.**

11. **Transfer the QIAamp Spin Column to a collection tube and discard VacConnectors. Add 750 µl Buffer AW2 to the QIAamp Spin Column. Centrifuge 3 min at full speed (14,000 rpm, 20,000 x *g*).**

    The full-speed spin removes all traces of Buffer AW2 from the QIAamp Spin Column before elution.

    Immediately after this step, the used VacConnectors should be disposed of properly and the waste tray of the QIAvac Manifold should be emptied.

    Note: Residual ethanol in the eluate may inhibit PCR and can cause false-negative results.

12. **Place the QIAamp Spin Column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate.**

13. **Carefully open the QIAamp Spin Column. Elute the DNA with 150 µl of Buffer AE or distilled water. Incubate at room temperature for 1 min then centrifuge at 6000 x *g* (8000 rpm) for 1 min.**

    For higher final DNA concentrations (e.g., for RFLP applications), first elute the DNA with 100 µl buffer and then use this 100 µl eluate for a second elution step.

    For long term storage of DNA, eluting in Buffer AE and placing at –20°C is recommended.

    This procedure typically yields samples of 5–15 µg DNA with A_{260}/A_{280} ratios of 1.7–1.9.