QIAGEN Supplementary Protocol:

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

Please refer to the *QIAamp® DNA Mini Kit and QIAamp DNA Blood Mini Kit Handbook* 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 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 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. **Place a QIAamp Spin Column in a 2 ml collection tube (provided). Carefully apply the mixture from step 6 to the QIAamp Spin Column without moistening the rim, close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.**

   Do not wet the rim of the QIAamp Spin Column, and seal each spin column in order to avoid aerosol formation during centrifugation.

8. **Carefully open the QIAamp Spin Column and add 500 µl of Buffer AW1. Centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.**

9. **Carefully open the QIAamp Spin Column and add 500 µl of Buffer AW2. Centrifuge at full speed for 3 min.**

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

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

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

11. **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 AE 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_{260} ratios of 1.7–1.9.