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.

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