

ORAgene®•DNA purification using the Puregene® DNA Purification Kit

DNA Purification Protocol for 4 ml Saliva Samples

Cell Lysis

1. Incubate ORAgene•DNA/Saliva samples at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours.
2. Transfer 4 ml lysate sample (2 ml saliva plus 2 ml ORAgene•DNA-preserving solution) to a 15 or 50 ml centrifuge tube.
3. Add 1 ml Cell Lysis Solution and 25 µl Gentra RNase A Solution (4 mg/ml). Vortex on high speed for 10 seconds to mix sample and incubate 10 minutes at room temperature.

Protein Precipitation

4. Add 1.67 ml Protein Precipitation Solution to the cell lysate.
5. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.
6. Centrifuge at 2,000 x g for 5 minutes. The precipitated proteins will form a tight dark brown pellet. If the protein pellet is not tight, repeat Step 4 followed by incubation on ice for 5 minutes and then repeat Step 5.

DNA Precipitation

7. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 15 or 50 ml tube containing 5 ml 100% Isopropanol (2-propanol) and 40 µl of Gentra Glycogen Solution (20 mg/ml).
8. Mix the sample by inverting gently 50 times.
9. Centrifuge at 2,000 x g for 3 minutes; the DNA will be visible as a small white pellet.
10. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 5 ml 70% Ethanol and invert tube several times to wash the DNA pellet.
11. Centrifuge at 2,000 x g for 1 minute. Carefully pour off the ethanol. Pellet may be loose so pour slowly and watch pellet.
12. Invert and drain the tube on clean absorbent paper and allow sample to air dry 5-10 minutes.

DNA Hydration

13. Add 400 µl DNA Hydration Solution (400 µl will give a concentration of 200 µg/ml if the total yield is 80 µg DNA).
14. Rehydrate DNA by incubating at 65°C for 1 hour and overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.
15. For storage, sample may be centrifuged briefly and then transferred to a 1.5 ml tube. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.