ORAgene®•ANIMAL
For DNA Collection

Laboratory protocol for manual purification of DNA from 250 µL of Oragene®•ANIMAL/saliva

The following step-by-step protocol describes how to purify DNA from a 250 µL aliquot of an animal saliva sample that has been collected and preserved in Oragene®•ANIMAL chemistry with the OA-400 collection kit.

When animal saliva is collected using the collection sponges and mixed with the Oragene•ANIMAL solution, the DNA is immediately stabilized. Oragene•ANIMAL/saliva samples are stable at room temperature for months without processing. If it is your laboratory practice to store frozen samples, Oragene•ANIMAL/saliva samples can be stored indefinitely at -15°C to -20°C, and can undergo multiple freeze-thaw cycles without deterioration of the DNA.

Equipment and reagents

- Microcentrifuge capable of running at 13,000 rpm (15,000 × *g*)
- Air or water incubator at 50°C.
- Ethanol (95% to 100%) at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1mM EDTA, pH 8.0) or similar solution
- (Optional) Glycogen (20 mg/mL) (e.g., Invitrogen Cat. No. 10814-010)
- Ethanol (70%) at room temperature
- 5M NaCl solution

Procedure

Purification steps	Notes
1. Remove the existing cap on the Oragene•ANIMAL tube. Without removing collection sponges, replace with the supplied red coloured cap.	• The red cap is required to ensure successful incubation in step 3.
2. Thoroughly mix the Oragene•ANIMAL/saliva sample in the tube by inversion and vigorous shaking for 30 seconds.	• This is to ensure that viscous saliva samples are properly mixed with the Oragene•ANIMAL solution.
3. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours.	• DNA in Oragene•ANIMAL is stable at room temperature even without the incubation step. • This heat-treatment step is essential to ensure that DNA is adequately released and that nucleases are permanently inactivated. • This incubation step may be performed at any time after saliva is collected from the animal and before it is purified. • Incubation of the entire sample is recommended. • The sample may be incubated at 50°C overnight if it is more convenient. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator.

Purification steps	Notes
4. (Optional) Carefully grasp the handles of the collection sponges and thoroughly remove additional Oragene•ANIMAL/saliva sample by pressing the sponges against the side if the tube. Discard sponges.	• Sponges can be removed one at a time, or simultaneously.
5. Transfer 250 µL of the mixed Oragene•ANIMAL/saliva sample to a 1.5 mL microcentrifuge tube.	• The remainder of the Oragene•ANIMAL/saliva sample can be stored at room temperature or frozen (-15°C to -20°C). Do not store in refrigerator (4°C).
6. For 250 µL of Oragene•ANIMAL/animal saliva, add 10 µL (1/25th volume) of Purifier (OG-L2P, supplied) to the microcentrifuge tube and mix by vortexing for a few seconds.	• The sample will become turbid as impurities and inhibitors are precipitated.
7. Incubate on ice for 10 minutes.	• Room temperature incubation can be substituted but will be slightly less effective in removing impurities.
8. Centrifuge at room temperature for 5 minutes at 13,000 rpm (15,000 × *g*).	• A longer period of centrifugation (up to 15 minutes) may be beneficial in reducing the turbidity (high A_{320}) of the final DNA solution.
9. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. Discard the pellet containing impurities. (Optional) Addition of Glycogen	• The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged. Optional: Addition of Glycogen • Some users may prefer to add 2.5 µL (50 µg) of Glycogen to the supernatant to make the pellet more easily visible.
10. To 250 µL of supernatant, add 15 µL of 5 M NaCl.	• Addition of NaCl is necessary to ensure efficient recovery of DNA.
11. To 250 µL of supernatant, add 300 µL of room temperature 95% to 100% ethanol. Mix gently by inversion 10 times.	• During mixing with ethanol, the DNA will be precipitated. This may appear as a clot of DNA fibers or as a fine precipitate, depending upon the amount of DNA in the sample. • Even if no clot is seen, DNA will be recovered by carefully following the next steps.
12. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	• Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
13. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for 2 minutes at 13,000 rpm (15,000 × *g*).	• For example, place each tube in the microcentrifuge with the hinge portion of the cap pointing away from the centre of the rotor. After centrifugation, the position of the pellet can be located (even if too tiny to be easily visible); it will be at the tip of the tube below the hinge.

Purification steps	Notes
14. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.	• This pellet contains DNA. Loss of the pellet will result in loss of the DNA. • Rotating the tube such that the pellet is on the upper wall will allow you to safely move a pipette tip along the lower wall and remove all of the supernatant. • The supernatant may contain impurities and should be removed as completely as possible. • Excessive drying of the pellet can make the DNA more difficult to dissolve.
15. Ethanol wash step: Carefully add 250 µL of 70% ethanol. Let stand at room temperature for 1 minute. Completely remove the ethanol without disturbing the pellet.	• Take care not to disturb the DNA pellet. • The DNA pellet may be small. Addition of a carrier such as glycogen at step #7 will increase the visibility of the pellet. • Should the pellet detach, centrifuge the sample for 5 minutes at 13,000 rpm (15,000 x *g*). • The 70% ethanol wash helps to remove residual inhibitors.
16. Add 50 µL of TE solution (see page 1) to dissolve the DNA pellet. Vortex for at least 5 seconds.	• Note that large amounts of high molecular weight DNA can be slow to hydrate (dissolve) completely. • Incomplete hydration of the DNA is a cause of inaccuracy in estimating DNA concentration and of failure of downstream applications such as PCR.
17. (Optional) Additional steps to ensure complete hydration of the DNA.	a) Additional vigorous pipetting and vortexing, and/or b) Incubation at 50°C for 1 hour with occasional vortexing, and/or c) Incubation at room temperature for 1-2 days **Note:** for applications such as Southern blotting that require very high molecular weight DNA, (c) is recommended.
18. Storage of the fully rehydrated DNA.	• In TE at 4°C for up to 1-2 months. • Recommended in TE in aliquots at -20°C for long-term storage. **Note:** freezing of purified DNA in TE will cause DNA to precipitate. When thawing a sample of frozen purified DNA, pay careful attention to rehydration, as discussed in step 13.

Quantification of DNA

By fluorescence method

Assays that use fluorescent dyes are more specific than absorbance at 260 nm for quantifying the amount of double-stranded DNA (dsDNA) in a DNA sample. We recommend using fluorescent dyes such as PicoGreen® or SYBR® Green I to quantify dsDNA since there is less interference by contaminating RNA. An inexpensive

protocol using SYBR Green I is described in PD-PR-075, *DNA quantification using SYBR Green I Dye and a micro-plate reader*[1]. Alternatively, commercially available kits such as Invitrogen's Quant-iT™ PicoGreen dsDNA Assay Kit (Cat. No. Q-33130) can be used. For either protocol, we recommend that the purified DNA be diluted 1:50 with TE solution and that 5 µL be used in the quantification assay.

By absorbance method

If you choose to quantify DNA by absorbance, we recommend that you first treat the purified sample with RNase to digest contaminating RNA and then remove the RNA fragments by ethanol precipitation of the DNA. A detailed protocol is described in PD-PR-040, *RNA removal by double-RNase digestion*[2]. Please note that DNA from an oral sample typically contains appreciably more RNA than found in blood samples. Ensure that alcohol-precipitated DNA is fully dissolved before reading the absorbance.

Conversion factor: An absorbance of 1.0 at 260 nm corresponds to a concentration of 50 ng/µL (50 µg/mL) for pure dsDNA.

Ensure that absorbance values are within the linear range of the spectrophotometer. Re-dilute and re-measure samples that fall outside of the linear range. See your instrument documentation for more information.

Method:

1. Dilute a 10 µL aliquot of purified RNase-treated DNA with 90 µL of TE (1/10 dilution). Mix by gently pipetting up and down. Wait for bubbles to clear.
2. Use TE in the reference (blank) cell.
3. Measure absorbance at 320 nm, 280 nm and 260 nm.
4. Calculate corrected A_{280} and A_{260} values by subtracting the absorbance at 320 nm (A_{320}) from the A_{280} and A_{260} values.
5. DNA concentration in ng/µL = corrected A_{260} × 10 (dilution factor) × 50 (conversion factor).
6. A_{260}/A_{280} ratio: Divide corrected A_{260} by corrected A_{280}.

Example

1. Assume the measured A_{320}= 0.025, A_{280}= 0.175 and A_{260}= 0.295
2. The DNA concentration of the undiluted sample will be:
   $(A_{260} - A_{320}) \times 10$ [dilution factor] × 50 [conversion factor]
   $= (0.295 - 0.025) \times 10 \times 50$
   $= 0.270 \times 10 \times 50$
   = 135 ng/µL or 135 µg/mL
3. The corrected A_{260}/A_{280} ratio will be:
   $(A_{260} - A_{320}) \div (A_{280} - A_{320})$
   $= (0.296 - 0.025) \div (0.175 - 0.025)$
   $= 0.270 \div 0.150$
   = 1.80

References

1 DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.
2 RNA removal by double-RNase digestion. DNA Genotek. PD-PR-040.