

Laboratory Protocol for Manual Purification of DNA from 0.5 mL of Oragene®•DNA/saliva

DNA yield and stability in Oragene•DNA

Oragene•DNA is designed to capture the large amount of DNA present in saliva. The median yield of DNA from 2 mL of saliva when captured in 2 mL of Oragene•DNA is 110 µg. Oragene•DNA is robust and can accept up to 4 mL of saliva, which can further increase the DNA yield.

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

Manual purification according to the following protocol will produce a high yield of DNA. Alternatively, Oragene•DNA is also compatible with many high throughput DNA purification systems.

The following step-by-step protocol describes how to purify DNA from a 500 µL aliquot of an Oragene•DNA/saliva sample. Volumes of less than 500 µL may be purified by adjusting the volumes of reagents accordingly.

Equipment and reagents to be supplied by user

- 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 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)
- (Optional) Ethanol (70%) at room temperature

Procedure

Purification Steps	Notes
1. Mix the Oragene•DNA/saliva sample in the Oragene•DNA vial by inversion and gentle shaking for a few seconds.	<ul style="list-style-type: none"> • This is to ensure that viscous saliva samples are properly mixed with the Oragene•DNA solution.
2. 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.	<ul style="list-style-type: none"> • DNA in Oragene•DNA 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 and before it is purified. • Incubation of the entire sample is recommended to ensure that this step has been completed. However, if it is more convenient, a 500 µL aliquot can be heat-treated. • The sample can be incubated either in the original container or after transfer to another tube. • 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.
3. Transfer 500 µL of the mixed Oragene•DNA/saliva sample to a 1.5 mL microcentrifuge tube.	<ul style="list-style-type: none"> • The remainder of the Oragene•DNA/saliva sample can be stored at room temperature or frozen (-15°C to -20°C).

Purification Steps	Notes
4. For 500 μ L of Oragene•DNA/saliva, add 20 μ L (1/25th volume) of Oragene•DNA Purifier (OG-L2P, supplied) to the microcentrifuge tube and mix by vortexing for a few seconds.	<ul style="list-style-type: none"> The sample will become turbid as impurities and inhibitors are precipitated.
5. Incubate on ice for 10 minutes.	<ul style="list-style-type: none"> Room temperature incubation can be substituted but will be slightly less effective in removing impurities.
6. Centrifuge at room temperature for 5 minutes at 13,000 rpm (15,000 \times g).	<ul style="list-style-type: none"> A longer period of centrifugation (up to 15 min) may be beneficial in reducing the turbidity (high A_{320}) of the final DNA solution.
7. Carefully transfer the clear supernatant with a pipet tip into a fresh microcentrifuge tube. Discard the pellet containing impurities. <i>Optional: Addition of Glycogen</i>	<ul style="list-style-type: none"> The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged. <p><i>Optional: Addition of Glycogen</i></p> <ul style="list-style-type: none"> Some users may prefer to add 5 μL (100 μg) of Glycogen to the supernatant to make the pellet more easily visible.
8. To 500 μ L of supernatant, add 500 μ L (i.e., an equal volume) of room-temperature 95-100% ethanol. Mix gently by inversion 10 times.	<ul style="list-style-type: none"> 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.
9. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	<ul style="list-style-type: none"> Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
10. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for 2 minutes at 13,000 rpm (15,000 \times g).	<ul style="list-style-type: none"> 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
<p>11. Carefully remove the supernatant with a pipet tip and discard it. Take care to avoid disturbing the DNA pellet.</p> <p><i>Optional: Ethanol wash step</i></p> <p>Carefully add 250 μL of 70% ethanol. Let stand at room temperature for 1 minute. Completely remove the ethanol without disturbing the pellet.</p>	<ul style="list-style-type: none"> • 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 pipet 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. <p><i>Optional: Ethanol wash step</i></p> <ul style="list-style-type: none"> • 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.
<p>12. Add 100 μL of DNA buffer (see Reagents) to dissolve the DNA pellet. Vortex for at least 5 seconds.</p>	<ul style="list-style-type: none"> • The expected concentration of the fully hydrated DNA is 20 to 200 ng/μL. • 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.
<p>13. (Optional) Additional steps to ensure complete hydration of the DNA.</p>	<p>a) Additional vigorous pipetting and vortexing, and/or</p> <p>b) Incubation at 50°C for 1 hour with occasional vortexing, and/or</p> <p>c) Incubation at room temperature for 1-2 days</p> <ul style="list-style-type: none"> • For applications such as Southern blotting that require very high molecular weight DNA, (c) is recommended.
<p>14. Storage of the fully rehydrated DNA.</p>	<ul style="list-style-type: none"> • In TE at 4°C for up to 1-2 months. • Recommended in TE in aliquots at -20°C for long-term storage. • Note that 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 Absorbance

<p>Quantification by absorbance is relatively insensitive but sufficiently accurate for most PCR and downstream applications. Quantification by fluorescence is preferred where greater sensitivity and accuracy is required. Absorbance readings at 260 nm should be in the range 0.1 and 1.5. Very concentrated samples will need to be diluted accordingly. Absorbance at 320 nm (A_{320}) is a measure of residual turbidity in the sample. Turbidity will also affect the absorbance values at 260 nm and 280 nm. The presence of turbid material will lower the apparent A_{260}/A_{280} ratio. Many spectrophotometers correct for turbidity automatically by subtracting the A_{320} reading from the A_{260} and A_{280} values. We recommend doing this manually if your spectrophotometer does not do this automatically. Purified DNA from Oragene•DNA/saliva should have a corrected A_{260}/A_{280} ratio >1.6.</p>	<p>Notes on turbidity, low A_{260}/A_{280} ratios and RNA fragments. Turbidity results from the presence of very fine particles that are not removed by high speed centrifugation. Centrifuging the final hydrated DNA for up to 15 minutes will lower the turbidity (see DNA Genotek document “A_{260}/A_{280} ratios”). The particles may be residual foodstuffs (such as coffee) that are not removed prior to delivering saliva as recommended by the donor instructions. Glycogen (if added) may contribute to turbidity. Fortunately, small amounts of turbid material are unlikely to interfere with downstream applications such as PCR. Residual RNA fragments that may co-precipitate with DNA do not affect the A_{260}/A_{280} ratio but may lead to an overestimate of the DNA yield.</p>
--	--

Using Absorbance to Calculate DNA Concentration and 260/280 ratio

Notes

Conversion Factor: an absorbance of 1.0 at 260 nm corresponds to a concentration of 50 ng/ μ L (50 μ g/mL) for pure DNA.

- A spectrophotometer capable of reading 100 μ L or less should be used for greater accuracy.
- Absorbance values should be between 0.1 and 1.5. For higher values, samples should be diluted and re-read. For lower values, the undiluted samples can be used, but care must be taken to ensure that the spectrophotometer cells are very clean or that disposable cells are used.

Method

1. Dilute a 10 μ L aliquot of purified DNA with 90 μ L 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/ μ L = corrected $A_{260} \times 10$ (dilution factor) $\times 50$ (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
$$\begin{aligned} & (A_{260} - A_{320}) \times 10 \text{ [dilution factor]} \times 50 \text{ [conversion factor]} \\ & = (0.295 - 0.025) \times 10 \times 50 \\ & = 0.270 \times 10 \times 50 \\ & = 135 \text{ ng}/\mu\text{L or } 135 \mu\text{g/mL.} \end{aligned}$$
3. The corrected A_{260}/A_{280} ratio will be
$$\begin{aligned} & (A_{260} - A_{320}) \div (A_{280} - A_{320}) \\ & = (0.295 - 0.025) \div (0.175 - 0.025) \\ & = 0.270 \div 0.150 \\ & = 1.80 \end{aligned}$$