



# ORAgene™ DNA Purification Protocol

## DNA yield and stability in ORAgene™

ORAgene yields a large amount of DNA from saliva. The median yield from 4 mL of ORAgene/saliva solution is 110 µg, with a 25<sup>th</sup> percentile yield of 62 µg and a 75<sup>th</sup> percentile of 158 µg.

When saliva is mixed with ORAgene, the DNA is immediately stabilized. ORAgene/saliva samples are stable at room temperature for years without any processing. Alternatively, the samples may be stored at -20°C if this is more convenient. ORAgene/saliva samples may undergo multiple freeze-thaw cycles without any degradation.

## Equipment and reagents to be supplied by user

- Microcentrifuge capable of running at 15,000 × g (13,000 rpm)
- Water bath or air incubator, heated to 50°C
- Ethanol (95 to 100%) at room temperature
- TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0) or other standard buffer

## DNA purification steps

DNA may be purified from (A) a 500 µL aliquot, or (B) the total 4 mL sample. Volumes other than 500 µL or 4 mL may also be purified provided that the ORAgene Purifier and ethanol are used in proportional amounts.

### (A) Purification from a 500 µL aliquot

1. Incubate the ORAgene/saliva sample in the ORAgene vial at 50°C in a water bath or air incubator for a minimum of 1 hour. The sample may be incubated overnight if this is more convenient. This incubation step only needs to be done once.
2. Transfer 500 µL of the ORAgene/saliva sample to a 1.5 mL microcentrifuge tube. The rest of the ORAgene/saliva sample can be stored at room temperature until ready for further use.
3. Add 20 µL (1/25<sup>th</sup> volume) of ORAgene Purifier (supplied with kit) and mix gently by inversion. The sample will become turbid as impurities are precipitated.
4. Incubate on ice for 10 minutes.
5. Centrifuge for 3 minutes at 15,000 × g (13,000 rpm) at room temperature. Carefully pipet the clear supernatant into a fresh microcentrifuge tube without disturbing the pellet. Discard the pellet.
6. Add 500 µL (equal volume) of room-temperature 95% ethanol to the supernatant and mix gently by inversion. Invert at least 5 times. A clot of DNA may be visible.
7. Let the solution stand for 10 minutes at room temperature so that the DNA is fully precipitated. Do not incubate at -20°C because impurities will co-precipitate with the DNA.
8. Centrifuge for 1 minute at 15,000 × g (13,000 rpm) at room temperature. Discard the supernatant without disturbing the DNA pellet (may or may not be visible).
9. If necessary, centrifuge again for 10 seconds and remove excess ethanol.
10. Once all of the ethanol has been removed, dissolve the DNA pellet in 100 µL of TE buffer or other standard buffer. The expected concentration of the rehydrated DNA is 10 to 100 ng/µL.
11. To fully dissolve the DNA, we recommend vigorous vortexing followed by incubation for a minimum of 1 hour at room temperature, preferably overnight. Alternatively, incubation for 10 minutes at 50°C is also effective.

### (B) Purification of the total 4 mL sample

1. Incubate the ORAgene/saliva sample in the ORAgene vial at 50°C in a water bath or air incubator for a minimum of 1 hour. The sample may be incubated overnight if this is more convenient. This incubation step only needs to be done once.
2. Divide the total 4 mL ORAgene/saliva sample into four 1.5 mL microcentrifuge tubes, each containing approximately 1 mL of sample.
3. Add 40 µL (1/25<sup>th</sup> volume) of ORAgene Purifier (supplied with kit) to each tube and mix gently by inversion. The sample will become turbid as impurities are precipitated.

4. Incubate the four tubes on ice for 10 minutes.
5. Centrifuge the four tubes for 3 minutes at  $15,000 \times g$  (13,000 rpm) at room temperature. Carefully pipet the clear supernatant from each tube and combine them all into one 15 mL centrifuge tube without disturbing the pellets. Discard the pellets.
6. Add 4 mL (equal volume) of room-temperature 95% ethanol to the supernatant and mix gently by inversion. Invert at least 5 times. A clot of DNA may be visible.
7. Let the solution stand for 10 minutes at room temperature so that the DNA is fully precipitated. Do not incubate at  $-20^{\circ}\text{C}$  because impurities may co-precipitate with the DNA.
8. Centrifuge for 10 minutes at  $1,100 \times g$  (3,500 rpm) at room temperature.
9. Discard the supernatant without disturbing the DNA pellet (may or may not be visible). Remove ethanol as thoroughly as possible.
10. Once all of the ethanol has been removed, dissolve the DNA pellet in 500  $\mu\text{L}$  of TE or other standard buffer. The expected concentration of the rehydrated DNA is 20 to 200  $\text{ng}/\mu\text{L}$ .
11. To fully dissolve the DNA, we recommend vigorous vortexing followed by incubation for a minimum of 1 hour at room temperature, preferably overnight. Alternatively, incubation for 10 minutes at  $50^{\circ}\text{C}$  is also effective.

### Quantification of DNA

Quantification by absorbance is accurate enough for PCR and most downstream applications, but quantification by fluorescence is preferred. To ensure accuracy, absorbance readings at 260 nm should fall between 0.1 and 1.0. The sample dilution should be adjusted accordingly. Absorbance at 320 nm ( $A_{320}$ ) measures light scattering and gives an estimate of the background turbidity. A high  $A_{320}$  reading results in artificially high estimates of yield ( $A_{260}$ ) but also reduces estimated DNA purity ( $A_{260}/A_{280}$ ). Many spectrophotometers will automatically subtract the  $A_{320}$  reading from the  $A_{260}$  and  $A_{280}$  values. DNA from Oragene should have an  $A_{260}/A_{280}$  ratio  $> 1.6$ .

#### (A) Quantification

1. Take an aliquot of the dissolved DNA and prepare a 1:20 dilution in water.
2. Measure the absorbance at 260 nm ( $A_{260}$ ) and 320 nm ( $A_{320}$ ).
3. DNA concentration in  $\text{ng}/\mu\text{L}$  =  $(A_{260} - A_{320}) \times 20$  (dilution factor)  $\times 50$   $\text{ng}/\mu\text{L}$  (conversion factor)  
E.g. if the  $(A_{260} - A_{320})$  value = 0.250, then the concentration of the original DNA sample is 250  $\text{ng}/\mu\text{L}$  (250  $\mu\text{g}/\text{mL}$ ).

#### (B) $A_{260}/A_{280}$ ratio

1. Take an aliquot of the dissolved DNA and prepare a 1:20 dilution in water.
2. Measure the absorbance at 260 nm, 280 nm and 320 nm.
3.  $A_{260}/A_{280}$  ratio =  $(A_{260} - A_{320}) / (A_{280} - A_{320})$

Technical support is available Monday to Friday (8h30 to 16h30 EST) by phone at +1(613) 723-5757 ext. 235 or by email at support@DNAgenotek.com.