

# Maximizing DNA yield with Oragene<sup>®</sup>•DNA

## 1. Collect the recommended volume of saliva

The recommended volume of saliva is 2 mL. The volume of the Oragene•DNA solution in the vial is 1.9 mL. Therefore the total volume of 2 mL of saliva plus the Oragene•DNA solution should be 3.9 mL.

## 2. Expect variation in DNA yield between different donors

The median yield of DNA is 110 µg from 4 mL of Oragene•DNA/saliva solution but the yield may range from a minimum of 15 µg to more than 300 µg. Even with the same donor, the yield may change from day to day.

## 3. Finish spitting within 30 minutes

The full saliva sample should be collected within 30 minutes and the Oragene•DNA vial should be capped immediately. Waiting longer than 30 minutes may decrease the yield and quality of the DNA.

## 4. Take an aliquot for DNA processing after incubation at 50°C

Saliva is quite viscous and can trap DNA. The 50°C incubation step releases DNA from the Oragene•DNA/saliva sample. Taking an aliquot taken prior to the incubation step may give a lower DNA yield.

## 5. Add the correct amount of alcohol to precipitate the DNA

It is important to add an equal volume of room-temperature ethanol (95-100%) to the clear supernatant after the centrifugation step. For example, 500 µL of ethanol should be mixed with 500 µL of supernatant. The aqueous phase should be mixed gently and thoroughly with the ethanol to ensure complete precipitation of DNA. A visible clot of DNA will usually be seen as the ethanol mixes with the aqueous phase. Adding more than 1 volume of ethanol and/or using cold ethanol will not improve DNA recovery and may increase the amount of impurities that precipitate.

## 6. Allow a sufficient period of time to rehydrate the DNA

Oragene•DNA-extracted DNA is high molecular weight and may take some time to dissolve completely in buffer. The rehydration solution should be a low salt buffer such as TE. We recommend overnight incubation at room temperature to ensure complete rehydration of the DNA. For best results, the final concentration of DNA should be less than 200 µg/mL (200 ng/µL).

Drying the sample after ethanol precipitation is not recommended because this can significantly increase the rehydration time. The rehydration time may be shortened by incubating at 50°C for 10 minutes. Once the DNA is dissolved, you should briefly vortex or pipet the sample several times prior to taking an aliquot.

## 7. Quantifying the DNA

It is important to remember that Oragene•DNA collects about 2 mL of saliva and mixes it with 1.9 mL of Oragene•DNA. DNA solution. Therefore, the total volume of Oragene•DNA/saliva solution is about 4 mL. The total DNA yield for the 4 mL should be > 20 µg.

DNA quantification by absorbance is accurate enough for PCR and most downstream applications. A more accurate method of quantifying DNA is by fluorimetry using a fluorescent dye such as SYBR<sup>®</sup> Green.

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.