DNA genotek

DNA recovery from samples collected with sponges in the Oragene® disc kit†

This protocol has been created as a suggested method for extracting DNA from saliva samples that have been collected from infants or young children who are unable to spit.

Once collected, Oragene®/saliva samples are stable at ambient temperature for years without processing. Heating the samples as indicated below (step 1) ensures the DNA will be uniformly distributed in the sponges and the free liquid. If 5 sponges are used to collect saliva, about ½ of the liquid will be trapped in the sponges and the other ½ will be free.

To recover DNA from saliva samples collected with sponges, we recommend the following methods:

- If you require DNA for a small number of tests or want a fast and accurate estimate of the total amount of DNA collected, proceed with method A.
- If you wish to recover DNA from the total sample for a battery of tests, proceed with method B.

Alternatively, one can purify DNA from a sample with both method A and B. For instance, one can quickly estimate the total amount of DNA in a sample and run some preliminary tests by purifying a small aliquot (method A), store the sample at room temperature until all the samples have been collected for the study, and then proceed with method B to recover the remainder of the DNA. DNA recovered from the same donor using method A and B can be pooled.

Method A: purification of DNA from a 0.5 mL aliquot

1. Ensure that the cap of the Oragene kit is firmly closed. Mix gently by inversion 5 times. Incubate at 50°C for 1 hour in a water bath or for 2 hours in an air incubator.
2. Carefully open the kit and remove 0.5 mL of the free liquid.
3. Purify DNA according to the prepIT™•L2P *Laboratory protocol for manual purification of DNA from 0.5 mL of sample*[1].

Method B: purification of DNA from the whole sample

1. Ensure that the cap of the Oragene kit is firmly closed. Mix gently by inversion 5 times. Incubate at 50°C for 1 hour in a water bath or for 2 hours in an air incubator.
2. Remove as much of the free liquid as possible and transfer to a 15 mL conical centrifuge tube.
3. Place the barrel of a 5 mL disposable plastic syringe (i.e., without the plunger) into the same 15 mL conical tube.
4. Using fine forceps, transfer the sponges from the blue base into the barrel of the syringe.
5. Centrifuge the syringe barrel containing sponges in the conical tube at 200 × *g* (e.g., 1,000 rpm in a Sorvall RT6000D centrifuge) for 10 minutes at 20°C.
6. Remove and discard the syringe barrel containing the dry sponges.

Syringe barrel with sponges inserted in conical tube for centrifuge.

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

7. DNA can be extracted from the Oragene/saliva liquid in the centrifuge tube, following instructions in the prepIT•L2P *Laboratory protocol for manual purification of DNA from whole sample*[2]. Ensure the volumes of reagents used in this protocol for a 4 mL sample are adjusted for the actual volume of Oragene/saliva liquid recovered from samples collected with saliva sponges.

References

1 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.
2 Laboratory protocol for manual purification of DNA from whole sample. DNA Genotek. PD-PR-015.

Technical support is available Monday to Friday (9h00 to 17h00 EST):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com