DNA genotek

DNA yield: collection from infants and young children with sponges (CS-1, CS-2) and the Oragene® disc collection kit† (OG-250, OGR-250)

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DNA Genotek has developed an easy, non-invasive DNA collection procedure for infants and young children. Saliva sponges, used in conjunction with the Oragene® self-collection kit in disc format, yield high quality DNA from saliva for genetic research.

Introduction

The Oragene self-collection kit is designed for completely non-invasive collection of DNA from saliva samples. Traditional DNA collection methods require a painful blood draw or uncomfortable and unreliable cheek scrapes. With the Oragene disc kit, a donor simply provides a 2 mL saliva sample into the Oragene disc. Once the kit is closed, the saliva mixes with a liquid that stabilizes the DNA at ambient temperature until it is extracted.

Some donors, such as infants, young children and individuals with limitations may not be able to spit the required 2 mL of saliva. To address this donor group, DNA Genotek has developed an easy and non-invasive collection procedure that uses saliva sponges together with the Oragene kit to obtain a sample. With this method, one or more sponges (up to 5 sponges per kit) are used to collect saliva which is resident in the cheek pouches of the donor. The sponge tips are then cut with scissors (provided) into an Oragene kit to preserve the DNA at ambient temperature and to prevent bacterial growth.

The saliva collection accessory kit for young children is provided with a detailed protocol[1] to purify DNA from either a portion of the sample (0.5 mL) or the entire sample. The amount of DNA recovered from the oral cavity can vary based on the effectiveness of the collection process (e.g., cooperativeness of the donor/saturation of the sponges with saliva) and the number of sponges used per donor. Our experience with this method to collect DNA from 32 infants and young children is presented. All samples were collected by parents who used the recommended collection protocol and who had no previous experience conducting this type of activity. It is expected that trained or experienced technicians/clinicians would obtain higher DNA yields.

Materials and methods

Saliva collection and DNA purification

Parents were provided with saliva collection kits for young children and asked to collect saliva samples from their children at home using 5 sponges per child. In total, 32 children, ranging in age from newborn to 7 years, provided samples. Details of the collection and purification procedure are described in protocols supplied with the saliva collection kit for young children[1, 2, 3, 4].

DNA analysis

The amount of DNA (µg) recovered from 32 children's samples was measured using a fluorescent dye (SYBR® Green I[5], Figures 1 to 4). Agarose gel (0.8%) electrophoresis with ethidium bromide staining was used to assess the molecular weight of DNA purified from samples collected with 5 sponges. DNA samples were analyzed by polymerase chain reaction (PCR) and agarose gel (1%) electrophoresis for a 560 bp fragment of the human thymidylate synthetase (TS) gene.

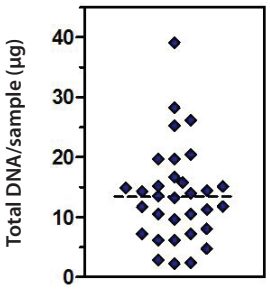

***Figure 1**: Scattergram of total DNA recovered using sponge/saliva samples from 32 infants and young children. The dashed line represents the median value of 13.4 µg.*

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

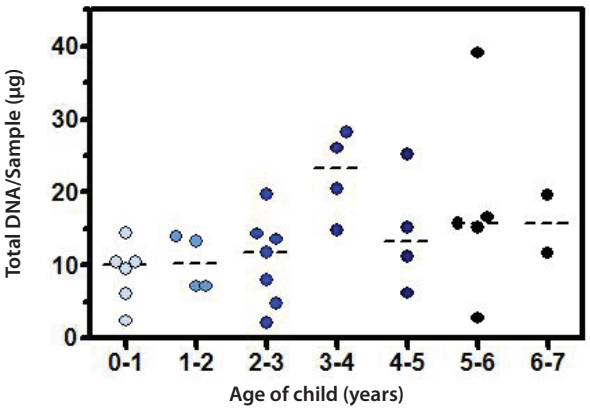

***Figure 2**: Scattergram of total DNA recovered from sponge/saliva samples as a function of age. The dashed line represents the median value collected per age group.*

Age group (years)	25%	Median	75%	Mean
0–1	4.3	10.0	12.5	8.9
1–2	7.2	10.2	13.6	10.4
2–3	4.7	11.8	14.3	10.6
3–4	17.7	23.3	27.2	22.4
4–5	8.7	13.2	20.2	14.5
5–6	9.0	15.8	27.9	17.9
6–7	N/A	15.7	N/A	15.7

***Table 1**: Comparison of DNA (µg) recovered with 5 sponges from children of each age group.*

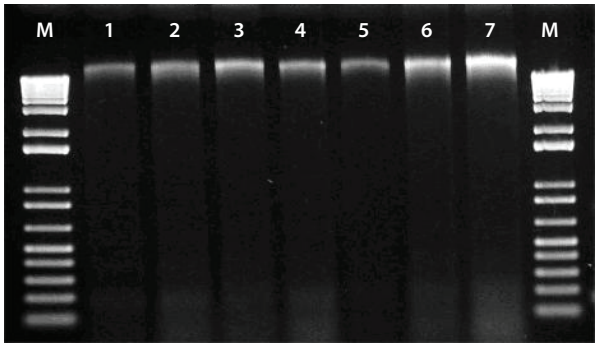

***Figure 3**: Integrity of DNA following storage of sponge samples collected with sponges in the Oragene kit at ambient temperature for 3 months. Lane 1–7, shows DNA purified from samples of 7 young children (age 14 months to 5 years). 1 kb plus DNA ladder was used as the marker (M).*

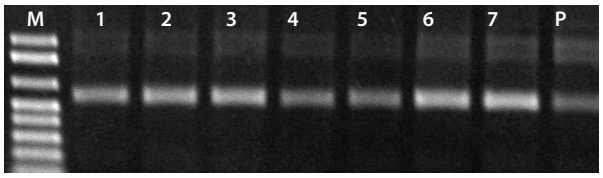

***Figure 4**: Agarose gel electrophoresis of PCR products. Lane 1–7, the 560 bp fragment of human TS gene was successfully amplified by PCR using DNA purified from samples of the same 7 children, as shown in Figure 3. 1 Kb plus DNA ladder was used as the marker (M). Positive control (P), a reaction with control DNA (50 ng, human white blood cell DNA).*

Results

The amount of DNA obtained from 32 children using saliva sponges is shown in Figure 1. The median amount of DNA recovered was 13.4 µg (25th percentile, 7.6 µg; 75th percentile, 16.2 µg).

Discussion and conclusions

The saliva collection accessory kit for young children successfully combines the proven Oragene solution for DNA stabilization and purification, with sponges that are ideal for collecting saliva samples from infants and young children. Agarose gel electrophoresis analysis of purified sponge samples showed that the integrity and quality of the DNA was excellent. The median DNA recovered from 32 young children using 5 sponges is 13.4 µg. Amplification of a 560 bp fragment of the human TS gene from samples stored at ambient temperature for several months, indicates the DNA is stable and suitable for PCR.

References

1. DNA recovery from samples collected with sponges in the Oragene disc kit. DNA Genotek. PD-PR-017.
2. CS-1 or CS-2 with the Oragene disc kits (OG-250 and OGR-250): using saliva sponges to collect DNA samples from infants and young children. DNA Genotek. PD-PR-018.
3. Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.
4. Laboratory protocol for manual purification of DNA from whole sample. DNA Genotek. PD-PR-015.
5. DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced with DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.