Non-invasive, assisted collection of high quantity and quality genomic DNA from saliva of young children

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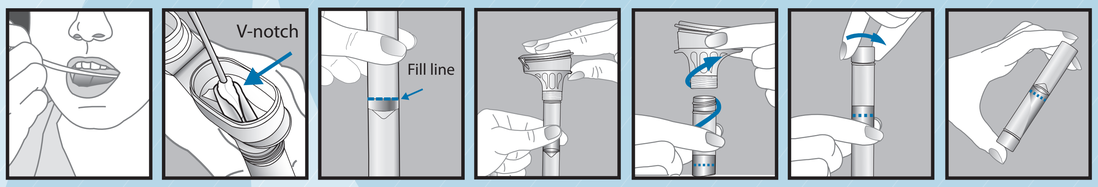
Abstract

Large population-based studies, involving thousands of subjects, are increasingly being used to investigate the genetic determinants of complex diseases. Saliva is a convenient source of genomic DNA because it can be collected in a painless and non-invasive manner. Non-invasive methods and techniques that permit self-collection are preferred because they increase compliance rates and reduce costs. For this reason, many large-scale studies now use DNA from saliva collected using Oragene®•DNA as the source of genomic DNA for downstream applications. Human genomic DNA from saliva can be used in various applications, some of which include genotyping, sequencing and micro-array analysis. In order to facilitate the non-invasive collection of genomic DNA from a population of all ages (including those individuals who can not spit) we describe a new method of using sponges to transfer saliva from a donor's mouth into an Oragene•DNA device. Unlike buccal swabs, this method transfers saliva which contains high quality and quantity of DNA into the Oragene•DNA device which preserves the DNA and prevents bacterial growth. This approach allows for easy collection of high quality DNA for complex downstream applications from children who are too young to spit. We report that using this new method allows for the collection of on average 17.3 µg of genomic DNA with an average A260/A280 ratio of 1.8 and a molecular weight > 23 kb. The collected samples are stable at room temperature for > 5 years and can be used in a multitude of downstream applications.

Materials and Methods

Figure 1: Assisted collection of saliva using a sponge into Oragene•DNA



1. Place one sponge in cheek pouch. Gently move the sponge along the gums and inner cheeks to soak up saliva.

2. Wring saliva out of sponge using a twisting and pushing motion against the inner wall of the V-notch. Saliva will flow into tube.

3. Repeat steps 1-2 USING SAME SPONGE until the saliva (not bubbles) reaches the fill line. Tap tube bottom against hard surface to reduce number of bubbles.

4. Close the lid by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the saliva.

5. Hold the tube upright. Unscrew the funnel from the tube and discard the funnel.

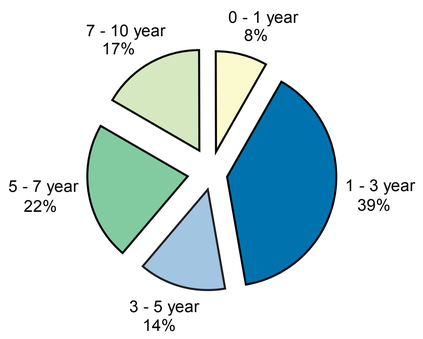
6. Use the small cap in the collection kit to close the tube tightly.

7. Shake the capped tube for 5 seconds. Discard sponges.

- Saliva was collected according to DNA Genotek protocol PD-PR-122.

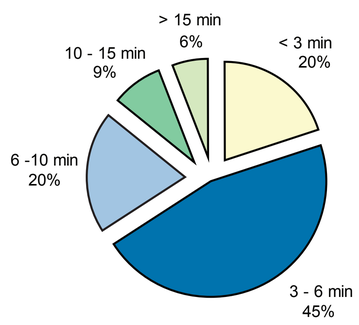
Results

Figure 2: Distribution of donor ages



- Children aged 9 months to 10 years old were recruited for this study
- Median age of children was 3.5 years
- Samples from a total of 77 children were analyzed

Figure 3: Time required to collect saliva to the fill to line.



- 65% of all donors irrespective of age were able to complete the collection of saliva in under 6 minutes

Figure 4: Total amount of DNA recovered

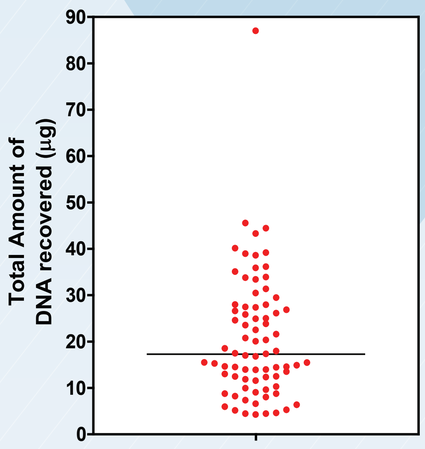
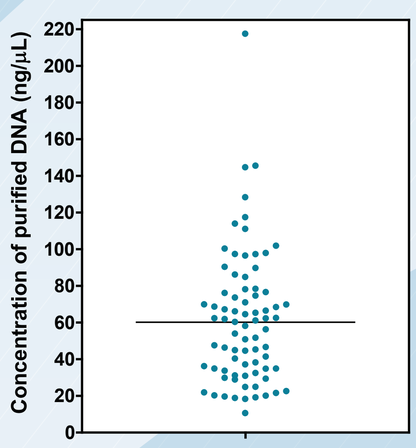
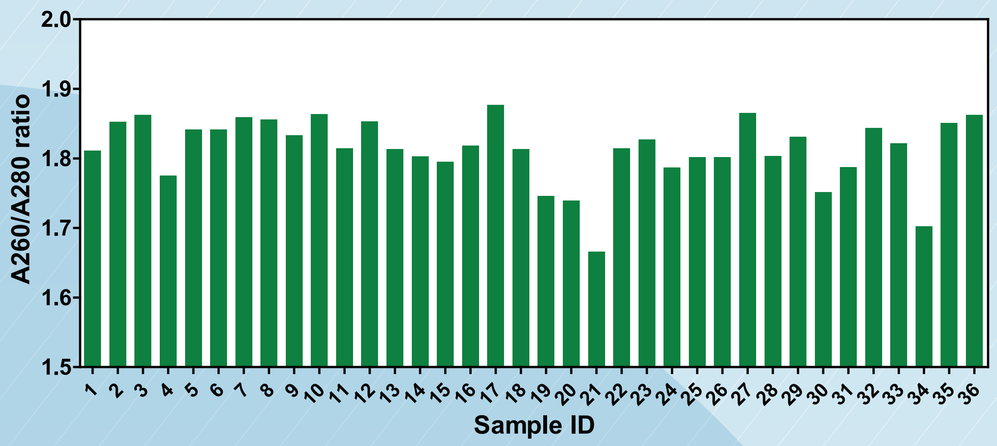


Figure 5: Concentration of DNA purified from collected saliva



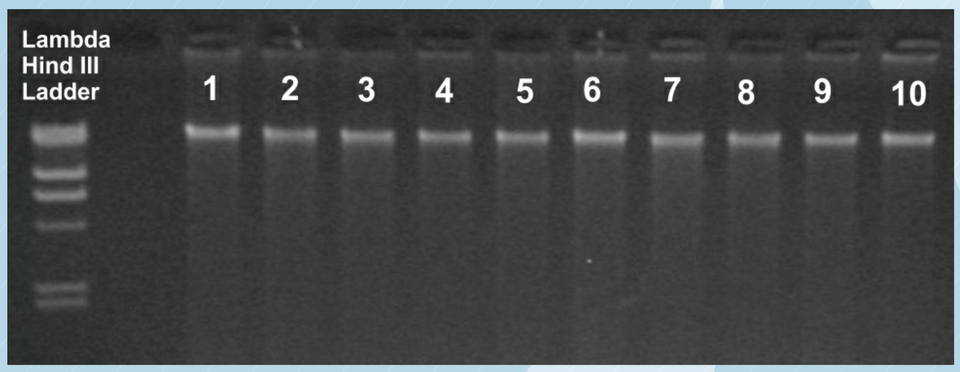
- Collected samples were purified according to DNA Genotek protocol PD-PR-121.
- Purified DNA was quantified by fluorescence using Sybr Green I dye
- The total amount of DNA collected from each child is reported in Figure 4. The median amount of DNA recovered was 17.3 µg.
- The resulting purified DNA had a median concentration of 60.2 ng/µL.

Figure 6: Corrected A260/A280 ratio of purified DNA



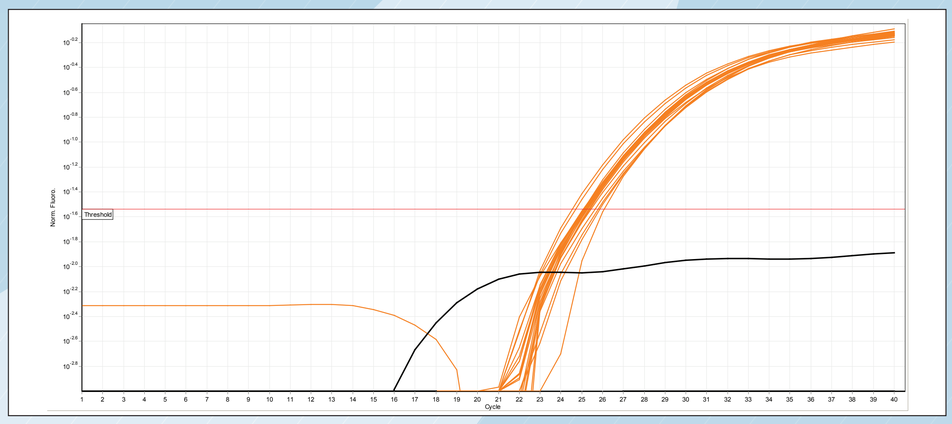
- The corrected A260/A280 ratio was calculated by subtracting the A320 value (which represents scattered light due to insoluble material) from both the A260 and A280 values.
- The purified DNA had a median A320 corrected A260/A280 ratio of 1.82

Figure 7: Representative agarose gel showing high molecular DNA



- The molecular weight of purified DNA was assessed by running a 0.8% agarose gel. The purified DNA consistently had a molecular weight > 23 kb as compared with the Lamda/HindIII ladder.

Figure 8: Real-time PCR results of human thymidylate synthetase gene



- Purified DNA was suitable for use in real-time PCR. Purified DNA was diluted to 4 ng/µL and 20 ng was used in the PCR reaction.
- Orange lines represent the 20 samples tested. Each sample performed equally well and no inhibition was observed. Black line represents the no template control.

Conclusions

- This study enrolled 77 children ranging in age from 9 months to 10 years. Following the instructions provided an adult assisted with the collection of saliva samples from each of the 77 donors. Using a sponge, saliva was successfully collected from all 77 participants.
- Following sample purification using a simple alcohol precipitation protocol the quality of the sample was assessed. The analytical specifications of all samples are summarized in Table 1.
- This method provides a simple non-invasive technique for collecting large amounts of high quality genomic DNA suitable for downstream applications.

Table 1: Summary of study results; median values reported.

Age of Donor	3.5 years
Total DNA Yield	17.3 µg
Purified DNA Concentration	60.2 ng/µL
A260/A280 Ratio	1.82

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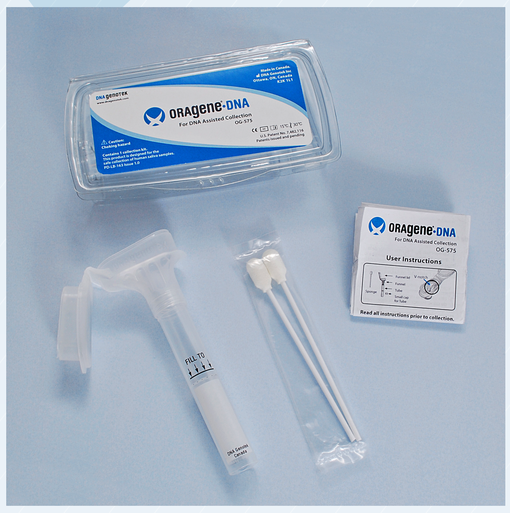
Community Design No. 001095186-0002
Community Design No. 001095186-0003
Patents pending



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