DNA genotek

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

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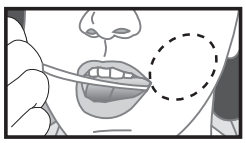
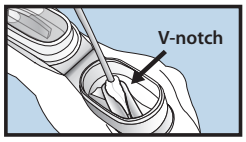
Abstract

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 the Oragene® kit† as the source of genomic DNA for downstream applications. Human genomic DNA from saliva can be used for 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 cannot spit) we describe a new method of using sponges to transfer saliva from a donor's mouth into an Oragene kit. Unlike buccal swabs, this method transfers saliva which contains high quality and quantity DNA into the Oragene kit which preserves the DNA and prevents bacterial growth. We report that this method allows for the collection of a median yield of 17.3 µg of genomic DNA with median A_{260}/A_{280} ratio of 1.8 and a molecular weight > 23 kb. The collected samples are stable at ambient temperature for years and can be used in a multiple downstream applications.

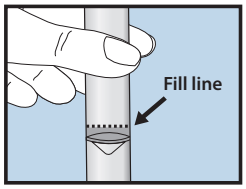
Materials and methods



1 Place one sponge in cheek pouch. Gently move the sponge along the gums and inner cheeks for 30 seconds to soak up as much saliva as possible.

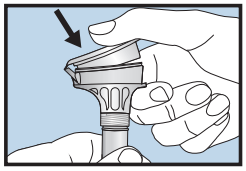


2 Once the sponge is saturated with saliva, insert sponge in V-notch of funnel. Wring saliva out of sponge using a twisting and pushing motion against the inner wall of the V-notch. Saliva will flow into tube.

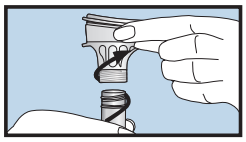


3 Repeat these steps (1 to 2) USING THE SAME SPONGE until the liquid saliva (not bubbles) reaches the fill line. Check sponge for damage each time before inserting into donor's mouth. Use second sponge if first sponge shows any signs of wear or tear.

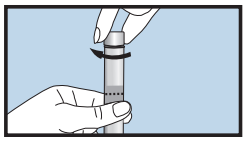
Tap tube bottom against hard surface to reduce number of bubbles.



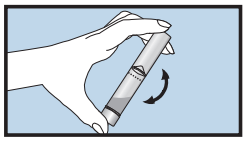
4 Hold the tube upright with one hand. Close the lid with the other hand (as shown) by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the saliva. Make sure the lid is closed tightly.



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



6 Use the small cap to close the tube tightly.



7 Shake the capped tube for 5 seconds. Discard or recycle the funnel. Discard sponges.

***Figure 1**: Assisted collection of saliva using a sponge with the Oragene kit.*

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

Results

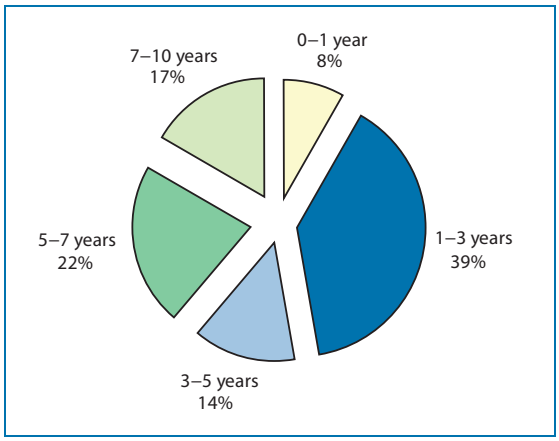


***Figure 2**: Distribution of donor ages*

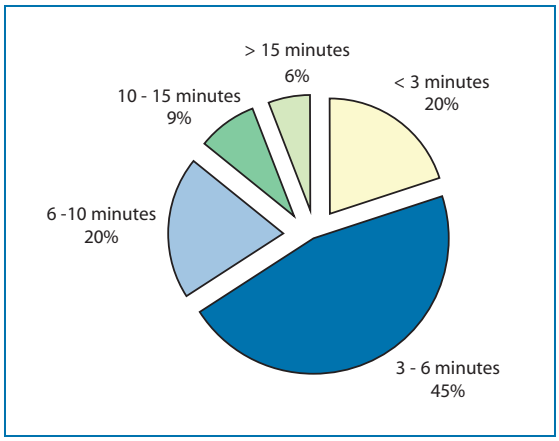


***Figure 3**: Time required to collect saliva to the fill-to-line*

- 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.

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

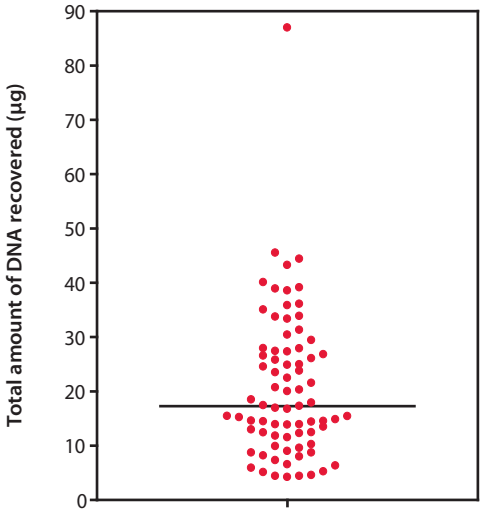


***Figure 4**: Total amount of DNA recovered.*

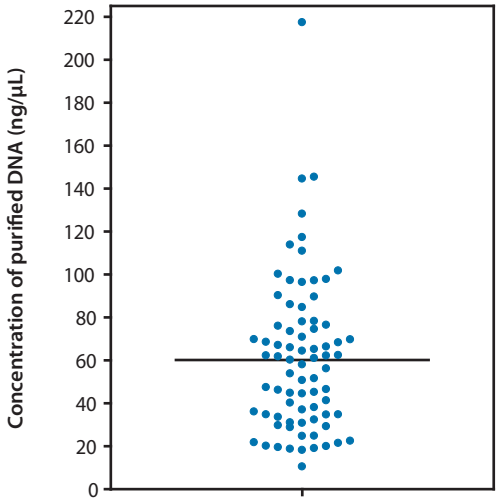


***Figure 5**: Concentration of DNA purified from collected saliva.*

- Collected samples were purified according to the prepIT™•L2P (DNA Genotek) purification protocol[1].
- Purified DNA was quantified by fluorescence using SYBR® Green I dye[2].
- 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.

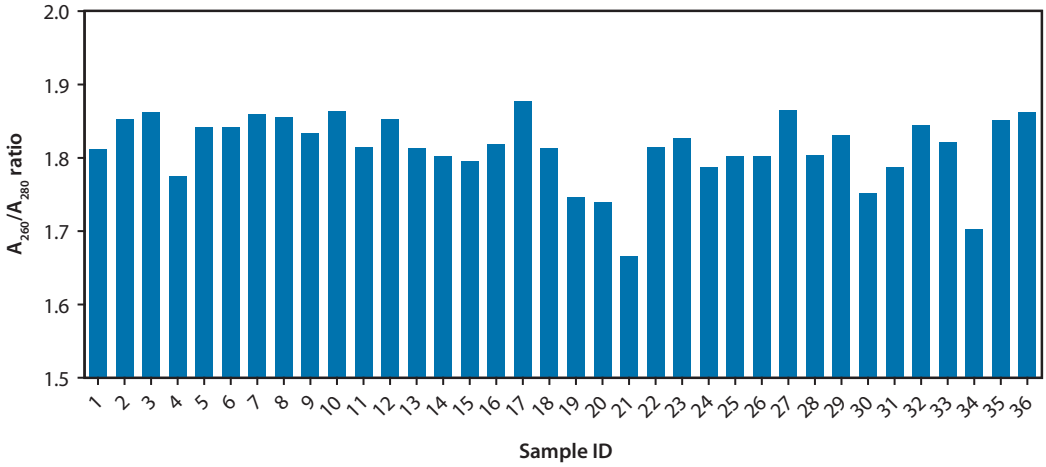


***Figure 6**: Corrected A_{260}/A_{280} ratio of purified DNA.*

- The corrected A_{260}/A_{280} ratio was calculated by subtracting the A_{320} value (which represents scattered light due to insoluble material) from both the A_{260} and A_{280} values.
- The purified DNA had a median A_{320} corrected A_{260}/A_{280} ratio of 1.82.

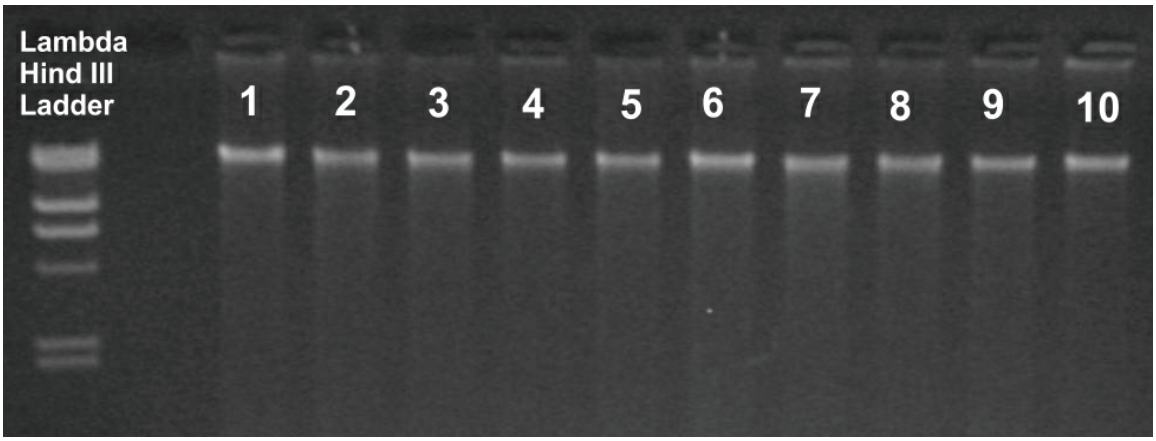


***Figure 7**: Representative agarose gel showing high molecular DNA.*

- 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-Hind III ladder.

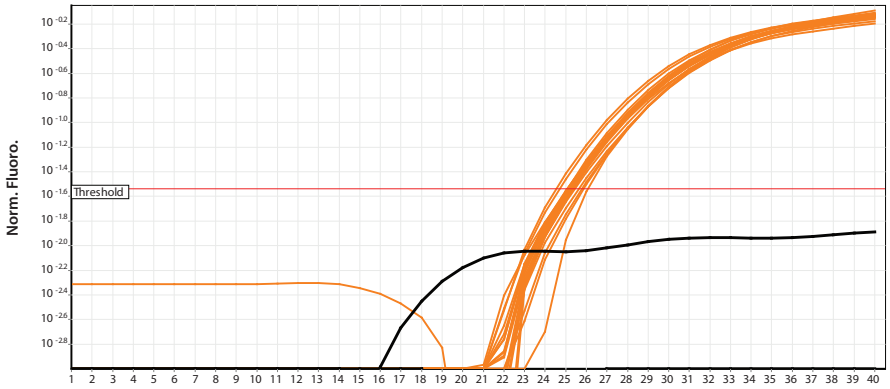


***Figure 8**: Real-time PCR results of human thymidylate synthetase gene.*

- 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 donors. Using a sponge, saliva was successfully collected from all 77 participants.
- Following sample purification using the prepIT purification protocol[1], 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.

Age of donor	3.5 years
Total DNA yield	17.3 µg
Purified DNA concentration	60.2 ng/µL
A_{260}/A_{280} ratio	1.82

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

References

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