Comparison of DNA Extraction Methodologies and Quality of DNA from Buccal Cells Collected in Oragene Saliva Collection Kit and Scope Mouthwash

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INTRODUCTION

Venipuncture, the standard DNA collection method, cannot be used in many situations for medical, logistic, or cultural reasons. In other situations, it is feasible but prohibitively expensive. In a pilot study on Danish nurse cohort conducted by Hansen et al. it was shown that 31% of the requested participants delivered blood sample, whereas 72%, 80%, and 76% delivered a saliva sample, buccal cell sample via mouth swabs, or buccal cell sample on FTA card, respectively (1). Collection of DNA from buccal cells is becoming more common in epidemiological studies. Buccal cells provide convenient source of DNA for genetic testing and to establish DNA banks for large epidemiological studies. Human genomic DNA found in saliva can be used in various applications in diagnostics including the detection of biomarkers to diagnose a disease, follow the disease's progress or monitor the effects of a particular treatment. Isolation of DNA from saliva for diagnostics has become an attractive alternative to isolation from blood or tissue due to the fact that sample collection is non-invasive. Simple methods of collecting DNA samples in large-scale community studies could extend the range of molecular epidemiological studies. In this study, we examined the yield and quality of DNA obtained from Oragene saliva kit and mouthwash for suitability for PCR amplification and analysis. We compared buccal cell collection formats and automated vs. manual extraction methodologies for DNA yields and quality. Each method was tested on adult volunteers. Experiments were conducted to select an efficient extraction method for isolating DNA. The performance of DNA was evaluated in downstream processes such as real-time PCR, human DNA content and SNP analysis.

METHODS

DNA Extractions

Manual DNA extractions from saliva and mouthwash were performed using Oragene manual extraction protocol (DNA Genotek Inc) and Puregene DNA Extraction Kits (Gentra Systems, Minneapolis, MN) as per the manufacturer's protocol. Both kits use the salting out method for DNA isolation. Automated DNA extraction was performed using Gentra System's Autopure LS work station. The DNA was dissolved in 500µl of TE buffer, and the yields were quantitated by OD reading at 260 nm using the SpectraMax Plus Spectrophotometer (Molecular Devices) and Picogreen quantitation was performed using Quant-iT™ PicoGreen® dsDNA Assay Kit From Molecular Probes (Invitrogen).

PCR Assay to Quantitate Human Specific DNA

Real time PCR assay for Quantitation of human specific DNA from saliva and mouthwash was performed on an ABI 7500 Sequence Detector System (Applied Biosystems, Inc., Foster City, CA, USA) using the BRCA 1 primers and probe as described by Haque et al. (2)

Analysis of Extracted DNA for Quality Control

Quality of the DNA is determined by performing agarose gel analysis and PCR amplification on the extracted DNA. The presence of high molecular weight DNA with no smearing on the gel suggests that the DNA is of high quality. PCR amplification was performed on 50ng of purified DNA by using the β-globin primer pair that amplifies a ~536 bp DNA fragment. Successful amplification suggests that the extracted DNA does not contain any amplification inhibitors.

SNP Analysis

DNA extracted from Saliva (various extraction methods) and Mouthwash samples were tested for Single Nucleotide Polymorphisms (SNP's) using ABI's MTHFR_A1298C SNP assay on ABI 7500 Sequence Detector System (Applied Biosystems, Inc., Foster City, CA, USA).

Table 1

DNA Yields from saliva and mouthwash using various extraction methods

Extraction Method	Material Type	Sample Volume (uL)	# of samples	Total DNA (ug)		Human DNA (ug)		% of Human DNA
				Mean	Median	Mean	Median	
Autopure	Saliva	2mL	24	34.83	32.92	26.08	20.97	75
Manual Puregene	Saliva	2mL	20	39.00	32.30	30.39	22.78	78
Manual Oragene	Saliva	2mL	20	57.85	52.05	46.32	37.25	80
Autopure	Mouthwash	10mL	16	20.37	10.20	12.72	8.96	62

Manual oragene protocol resulted in higher DNA yield compared to other extraction methods. Saliva samples (2mL) collected in oragene DNA collection kits gave significantly higher DNA yields compared to 10mLof mouthwash sample.

Figure 2

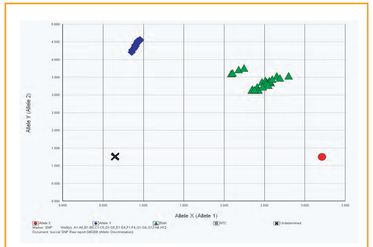

Figure 2: SNP Assay using MTHFR A1298C and ABI 7500 platform

DNA extracted from saliva and mouthwash samples were successfully genotyped using ABI's MTHFR SNP showing that the DNA is of good quality and amplifiable.

Figure 3

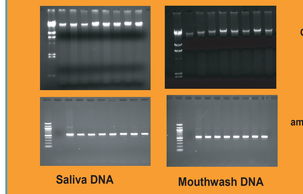

Figure 3: Agarose Gel Photographs of Genomic DNA and Amplified PCR DNA

The DNA extracted from saliva and mouthwash samples was of good quality because the high molecular weight genomic DNA was intact and the B-globin gene was able to be amplified in a PCR reaction.

Table 2 and Figure 1

DNA Yields from 4 mL of saliva using Oragene manual extraction method

	Total DNA OD (ug)	Total DNA Picogreen (ug)	Human DNA TaqMan	% of Human DNA
Mean	139.30	63.65	38.53	61
Median	107.81	54.16	36.05	
Minimum	8.08	4.32	2.64	
Maximum	488.72	215.79	76.09	
Sample Size (N	28	28	28	

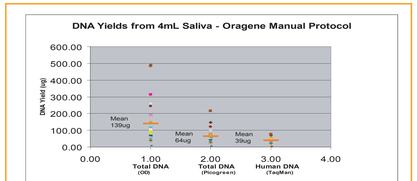

Fig 1: DNA yield - Spectrophotometer, Picogreen and TaqMan assays

Quantitation of total DNA using OD was 2 fold higher as compared to picogreen fluoresence assay. It is highly recommended that the total DNA be quantitated using fluoresence based assay to accurately account for the DNA yield. Based on the human specific TaqMan assay the mean human DNA content is around 65%.

CONCLUSIONS

- Oragene saliva collection kits provide an excellent format for DNA for genetic studies.
- The mean DNA yields from 4mL Oragene Saliva collection kit was 64ug (median 54.16ug) and the mean DNA yields from 10mL mouthwash was 20.37ug (median 10.20ug).
- Manual Oragene extraction method gave more DNA compared to other extraction methods.
- OD260/280 ratio, DNA gels and SNP analysis indicate the extracted DNA was of good quality.
- The use of Oragene kit coupled with manual/automated DNA extraction provide high quality DNA for genetic analyses, and sufficient quantities to be archived for future studies.
- The recommended extraction protocols would be Autopure workstation or Oragene manual method. Taking into consideration the cost for extraction Autopure would be ideal for high throughput extractions.

REFERENCES

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2. Haque et al. (2003) Performance of high-throughput DNA quantification methods. BMC Biotechnol. 28:3(1):20.

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