Evaluation of methodologies for the analysis of human exomes using DNA extracted from saliva

R. Iwasiow and M. Tayeb
DNA Genotek Inc., Ottawa, Canada

Introduction

Non-invasive sample collection has been demonstrated to dramatically increase donor compliance[1]. Saliva collected with Oragene® provides a non-invasive alternative to blood samples for collecting large amounts of high quality genomic DNA that is suitable for array based GWAS and Next Generation Sequencing studies. Oragene offers a non-invasive collection method and also contains a stabilizing reagent that ensures the sample is of high quality and allows long-term storage at ambient temperature. For these reasons, DNA isolated from saliva collected using the Oragene self-collection kit has been used in many large scale epidemiological array based GWAS studies[2, 3, 4]. In recent years it has become more practical and economical to analyze samples using Next Generation Sequencing technologies, in particular Whole Exome Sequencing.

In this study we extracted DNA from 7 year old Oragene/saliva samples stored at room temperature (~23°C) and evaluated the data from both the Illumina® HumanExome v1.1 array and Whole Exome Sequencing on the Illumina HiSeq 2000 after enrichment using the Agilent SureSelect Human All Exon v4+UTRs 71Mb Kit.

Methods

Collection and storage

Saliva samples were collected from 8 consented donors in 2006. Two millilitres (2 mL) of saliva was collected using the Oragene self-collection kit. After collection, the samples were heated for 1 hour at (50°C) and then stored at room temperature (~23°C) in the original collection tube for 7 years, until full purification in 2013.

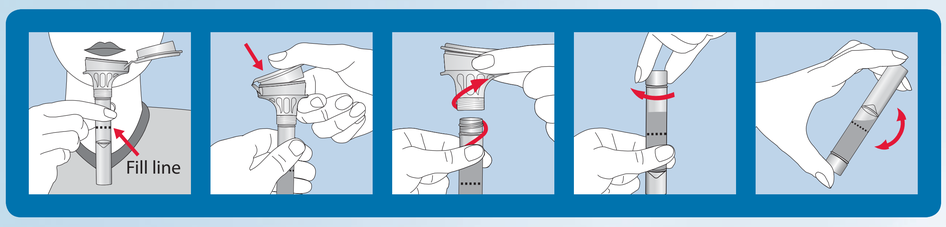


Figure 1: Oragene collection instructions

Purification

All samples were purified using the prepIT®•L2P DNA extraction kit from DNA Genotek (protocol PD-PR-006). The kit uses a proprietary solution to remove inhibitors followed by alcohol precipitation of DNA. For all Oragene collected samples an aliquot of 500 µL was purified and eluted in 50 mL TE buffer.

Quality control

Purified DNA was assessed using 4 different methods. First, the sample was quantified using PicoGreen® to accurately quantify the amount of DNA present. Next, the A_{260}/A_{280} ratio was measured using a NanoDrop® spectrophotometer and the integrity of the DNA was assessed using agarose gel electrophoresis. Approximately 100 ng of DNA as determined by PicoGreen was loaded per sample on the 0.8% agarose gel. Finally, bacterial DNA content was assessed using an in-house developed qPCR method (protocol PD-PR-065).

Exome arrays

Samples were processed by Affiliated Genetics Inc. on the Illumina HumanExome v1.1 arrays in accordance to Illumina protocols.

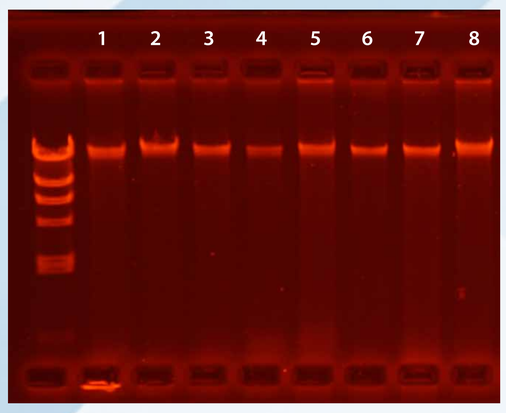
Exome sequencing

Library preparation and sequencing was performed at Expression Analysis. The DNA was enriched for the exome using the Agilent SureSelect Human All Exon v4+UTRs 71Mb Kit. The enriched library was sequenced on an Illumina HiSeq 2000 to a mean depth of 119×.

Results

After 7 years room temperature storage (~23°C) the Oragene/saliva samples were purified. The purified DNA was of high yield, quality and molecular weight.

Sample ID	Collection date	Total yield (µg)	Concentration (ng/µL)	A_{260}/A_{280}	% Bact
1	2006	94.5	236.2	1.86	17%
2	2006	28.6	71.5	1.86	30%
3	2006	52.3	130.7	1.85	18%
4	2006	52.3	130.8	1.86	14%
5	2006	61.7	154.3	1.92	37%
6	2006	192.1	480.2	1.95	45%
7	2006	70.3	175.6	1.84	10%
8	2006	66.0	164.9	1.87	36%



Each sample was barcoded and 4 samples were run per lane on the Illumina HiSeq 2000. The average sequencing yield per sample was 12.5 Gb, with 98% of sequences prior to clipping aligned to the human hg19 reference.

Sample	Sequencing yield (Mb)	% Align genome	Insert mean	Mean quality	% Duplication	Mean depth
1	11610	97.70	223	36.5	0.41	111
2	11145	97.57	246	36.4	0.43	104
3	11936	97.94	220	36.6	0.37	114
4	13989	97.86	234	36.6	0.32	131
5	13399	97.60	265	36.3	0.28	124
6	13428	96.89	256	36.3	0.34	125
7	12271	98.07	213	36.5	0.35	119
8	12718	97.69	246	36.6	0.30	121

The call rates on the Illumina HumanExome v1.1 array ranged between 99.81% and 99.94%. Similarly, based on sequencing results we observed between 99.71% and 99.84% coverage of the Agilent SureSelect Human All Exon v4+UTRs 71Mb Kit. We observed, on average, 76% of sequenced bases within the captured exon regions.

Sample	Coverage (%)	# Variants (in target)	Het SNPs in target	Hom SNPs target	Indels in target	kbases not in Exon	kbases in Exon	Proportion in Exon (%)	Array call rates (%)
1	99.80	67500	42294	20899	4307	2,338,203	7,896,136	77.2	99.94%
2	99.71	64602	40651	19869	4082	2,440,522	7,422,073	75.3	99.88%
3	99.73	68642	43090	21083	4469	2,311,256	8,130,362	77.9	99.90%
4	99.75	69488	43574	21499	4415	2,863,802	9,338,541	76.5	99.91%
5	99.84	69410	43388	21493	4529	3,228,553	8,836,106	73.2	99.94%
6	99.83	69497	43565	21462	4470	3,135,419	8,939,596	74.0	99.90%
7	99.82	67223	41809	21158	4256	2,324,367	8,465,341	78.5	99.81%
8	99.81	69332	43596	21289	4447	2,925,642	8,657,029	74.7	99.89%

The Illumina HumanExome v1.1 array contains 242,901 markers of which 201,756 overlap with content located on the Agilent SureSelect Human All Exon v4+UTRs 71Mb Kit. After filtering the sequencing data for Q>20 we observed a concordance >99.2% between the two technologies across all samples. Filtering for quality > Q30 had no significant impact on concordance. As the data was further filtered to consider depth of coverage we observed increased concordance, >99.7%. We did not observe any increase in concordance when filtering at higher depths of coverage, 100×.

Sample	Concordance no filtering	Depth > 20	Depth > 30	Depth > 50	Depth > 100	Qual > 20	Qual > 30
1	99.29%	99.47%	99.64%	99.77%	99.76%	99.29%	99.30%
2	99.24%	99.42%	99.62%	99.78%	99.76%	99.24%	99.24%
3	99.34%	99.50%	99.68%	99.79%	99.78%	99.34%	99.34%
4	99.44%	99.57%	99.69%	99.80%	99.79%	99.44%	99.45%
5	99.40%	99.54%	99.69%	99.78%	99.77%	99.40%	99.40%
6	99.31%	99.46%	99.62%	99.73%	99.71%	99.31%	99.31%
7	99.22%	99.37%	99.54%	99.67%	99.66%	99.22%	99.22%
8	99.35%	99.50%	99.67%	99.75%	99.78%	99.35%	99.35%

Highlights

- Saliva samples stored in Oragene for 7 years exhibit high yields of high molecular weight human genomic DNA.
- Saliva samples collected using Oragene are an excellent source of gDNA for array-based and Whole Exome Sequencing studies.
- The >99.7% concordance between array and exome sequencing results indicates that Oragene/saliva samples are a reliable source of gDNA which can be safely stored for years at room temperature with no impact on genotyping results.

References

1 *Cancer Epidemiol Biomarkers Prev*; 15(9) Sep 2006. Quality and quantity of saliva DNA obtained from the self administrated oragene method—a pilot study on the cohort of Swedish men. Rylander-Rudqvist et al.
2 *Cancer Epidemiol Biomarkers Prev*; 19(3) March 2010. Saliva-Derived DNA Performs Well in Large-Scale, High-Density Single-Nucleotide Polymorphism Microarray Studies. Bahlo et al.
3 *Nature Genetics*; 44(6) May 2012. Genome-wide association analyses identify 13 new susceptibility loci for generalized vitiligo. Jin et al.
4 *Genomics*; 98(2) Aug 2011. Next generation genome-wide association tool: design and coverage of a high-throughput European-optimized SNP array. Hoffman et al.

DNA genotek

Superior samples
Proven performance

MK-00230 Issue 1/2013-05

www.dnagenotek.com • support@dnagenotek.com