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Genomic DNA from saliva samples stored >5 years in Oragene® chemistry at room temperature is equivalent to DNA from freshly collected samples as shown on the Illumina® HumanExome v1.1 array

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Introduction

Non-invasive sample collection can greatly improve the success of a research study by ensuring donor compliance. Use of saliva collection for gDNA has been demonstrated to increase compliance rates. However, large epidemiological studies may require significant time to collect 1000s of samples. To benefit from economies of scale in processing and analysis, batching of samples is required. Oragene®•DNA stabilized saliva samples offer long-term room temperature stability to ensure the nucleic acids do not change from time of collection to time of processing. In this study we compare exome array data from >5 year old Oragene•DNA/saliva samples stored at room temperature to those freshly collected prior to processing, demonstrating that the Oragene•DNA stabilizing solution provides an effective biological "snap shot".

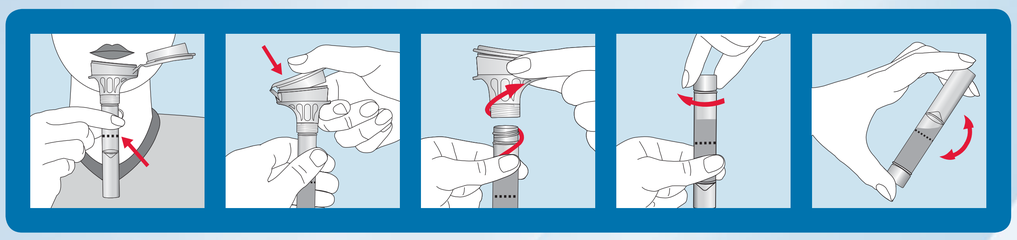
Methods

Oragene•DNA/saliva samples collected and stored at room temperature >5 years were compared against freshly collected samples from the same donors.

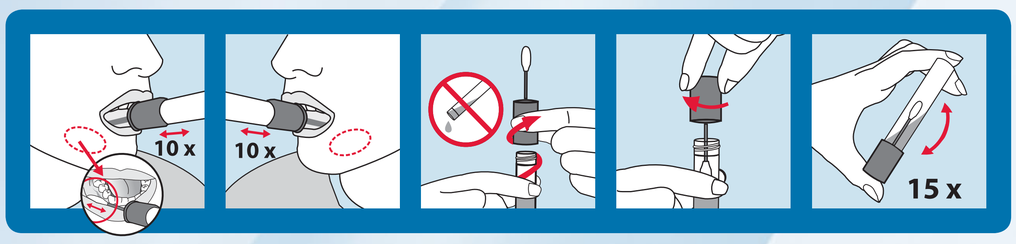
Collection

Under IRB consent, saliva samples were collected from 38 donors between 2004 and 2006 and 10 donors in 2012. Eight (8) of the donors participated in both collections. In the early collections, 2 mL of saliva was collected using the Oragene•DNA self-collection kit. In 2012, 10 donors were asked to donate two samples each: one using the Oragene•DNA self-collection kit (OG-500) and one using the ORAcollect•DNA (OCR-100) collection kit. All samples were collected according to the instructions provided with the kit.

OG-500



OCR-100



Storage

Oragene•DNA/saliva samples collected in 2004 were stored in the collection tube at ambient temperature (~23°C).

Purification

All samples were purified using the prepIT®•L2P DNA extraction kit from DNA Genotek. The kit uses a proprietary solution to remove inhibitors followed by alcohol precipitation of DNA. For all Oragene•DNA collected samples an aliquot of 500 µL was purified and eluted in 50 or 100 µL TE buffer. For all ORAcollect•DNA samples an aliquot of 650 µL was purified and eluted in 50 µL TE buffer.

Quality control

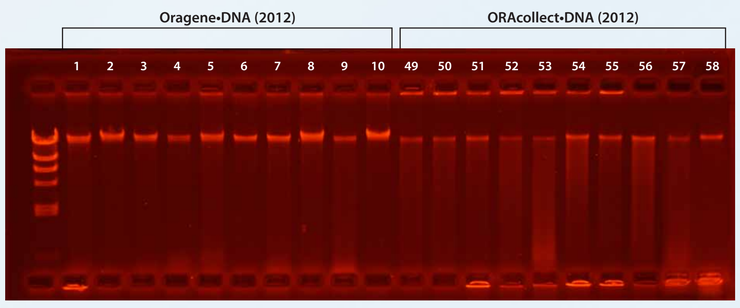
Purified DNA was assessed using 4 different methods. First, the sample was quantified using PicoGreen® to accurately quantify the amount of DNA present. Next, the A_{260}/A_{280} ratio was measured using a NanoDrop® spectrophotometer and the integrity of the DNA was assessed using agarose gel electrophoresis. 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 on the Illumina HumanExome v1.1 arrays. Correlation between aged and fresh samples as well as correlation between collection methods was assessed.

Results

Sample ID	Collection date	Collection method	Donor ID	Yield (µg)	Concentration (ng/µL)	A_{260}/A_{280}	% Bact	Exome array call rates
1	2012	Oragene•DNA	OGP1	52.7	131.8	1.8	N/A	99.96%
2	2012	Oragene•DNA	OGP2	50.2	125.6	1.8	6.53%	99.97%
3	2012	Oragene•DNA	OGP3	101.8	254.4	1.9	18.21%	99.95%
4	2012	Oragene•DNA	OGP4	102.4	256.1	2.0	35.63%	99.93%
5	2012	Oragene•DNA	OGP5	136.3	340.7	1.8	6.51%	99.96%
6	2012	Oragene•DNA	OGP6	28.3	70.7	1.8	9.62%	99.88%
7	2012	Oragene•DNA	OGP7	21.2	53.0	1.9	27.56%	99.90%
8	2012	Oragene•DNA	OGP8	60.3	150.7	1.8	10.28%	99.92%
9	2012	Oragene•DNA	OGP9	85.1	212.7	1.8	14.91%	99.94%
10	2012	Oragene•DNA	OGP10	49.3	123.1	1.8	7.21%	99.91%
11	2004	Oragene•DNA	2	18.8	23.5	1.9	8.9%	ND
12	2004	Oragene•DNA	13	8.8	10.9	1.9	20.0%	ND
13	2004	Oragene•DNA	17	15.3	19.1	1.9	28.8%	ND
14	2004	Oragene•DNA	18	17.5	21.9	1.9	41.7%	ND
15	2004	Oragene•DNA	20	13.2	16.5	1.8	14.1%	ND
16	2004	Oragene•DNA	24	34.2	42.7	1.9	35.1%	ND
17	2004	Oragene•DNA	36	65.5	81.9	1.9	43.4%	ND
18	2004	Oragene•DNA	45	11.6	14.5	1.9	26.9%	ND
19	2004	Oragene•DNA	46	16.9	21.1	1.8	13.2%	ND
20	2004	Oragene•DNA	51	46.0	57.4	1.9	25.4%	ND
21	2004	Oragene•DNA	58	13.2	16.6	1.9	32.1%	ND
22	2004	Oragene•DNA	64	22.7	28.4	1.8	11.4%	ND
23	2004	Oragene•DNA	68	54.5	68.2	1.8	7.5%	ND
24	2004	Oragene•DNA	72	68.2	85.2	1.8	8.0%	ND
25	2004	Oragene•DNA	73	24.3	30.4	1.9	14.3%	ND
26	2004	Oragene•DNA	87	52.2	65.3	1.8	31.8%	ND
27	2004	Oragene•DNA	92	18.1	22.6	1.8	35.4%	ND
28	2004	Oragene•DNA	99	21.5	26.9	1.8	3.0%	ND
29	2004	Oragene•DNA	101	62.0	77.6	1.8	3.7%	ND
30	2004	Oragene•DNA	109	15.1	18.8	1.8	32.6%	ND
31	2004	Oragene•DNA	111	36.8	46.0	1.9	30.9%	ND
32	2004	Oragene•DNA	122	42.1	52.6	1.8	18.3%	ND
33	2004	Oragene•DNA	148	31.7	39.6	1.9	34.5%	ND
34	2004	Oragene•DNA	154	77.5	96.8	1.9	40.6%	ND
35	2004	Oragene•DNA	186	61.9	77.4	1.8	3.9%	ND
36	2004	Oragene•DNA	201	390.0	487.5	2.0	34.2%	ND
37	2004	Oragene•DNA	202	18.5	23.2	1.8	7.1%	ND
38	2004	Oragene•DNA	203	88.6	110.7	1.9	7.8%	ND
39	2004	Oragene•DNA	204	120.7	150.8	2.0	33.2%	ND
40	2004	Oragene•DNA	205	37.2	46.5	1.8	32.1%	ND
41	2006	Oragene•DNA	AGP1	94.5	236.2	1.9	17.4%	99.94%
42	2006	Oragene•DNA	AGP2	28.6	71.5	1.9	29.9%	99.88%
43	2006	Oragene•DNA	AGP3	52.3	130.7	1.9	18.3%	99.90%
44	2006	Oragene•DNA	AGP4	52.3	130.8	1.9	14.0%	99.91%
45	2006	Oragene•DNA	AGP5	61.7	154.3	1.9	37.2%	99.94%
46	2006	Oragene•DNA	AGP6	192.1	480.2	2.0	44.8%	99.90%
47	2006	Oragene•DNA	AGP7	70.3	175.6	1.8	10.1%	99.81%
48	2006	Oragene•DNA	AGP8	66.0	164.9	1.9	35.7%	99.89%
49	2012	Oracollect•DNA	OCP1	5.4	70.0	1.7	48.45%	99.90%
50	2012	Oracollect•DNA	OCP2	5.5	71.8	1.7	29.30%	99.91%
51	2012	Oracollect•DNA	OCP3	5.8	75.7	1.6	31.26%	99.95%
52	2012	Oracollect•DNA	OCP4	3.3	43.7	1.7	12.59%	99.97%
53	2012	Oracollect•DNA	OCP5	2.9	37.5	1.7	5.87%	99.97%
54	2012	Oracollect•DNA	OCP6	3.9	51.6	1.7	1.55%	99.92%
55	2012	Oracollect•DNA	OCP7	7.7	101.3	1.6	2.72%	99.91%
56	2012	Oracollect•DNA	OCP8	2.1	27.0	1.6	3.15%	99.90%
57	2012	Oracollect•DNA	OCP9	3.4	45.1	1.7	22.27%	99.94%
58	2012	Oracollect•DNA	OCP10	1.2	15.2	1.8	19.10%	99.92%



SNP genotyping concordance measured against freshly collected Oragene•DNA/saliva samples

Donor ID	Aged Oragene•DNA sample	ORAcollect sample
1	0.99997	0.99998
2	1.00000	0.99998
3	0.99997	0.99998
4	0.99997	0.99997
5	0.99997	0.99999
6	0.99995	0.99999
7	0.99997	0.99999
8	0.99999	0.99998
9	ND	0.99998
10	ND	0.99999

Conclusions

- Samples purified after ambient temperature storage in Oragene•DNA for up to 9 years exhibit high yields of high molecular weight human genomic DNA
- Aged Oragene•DNA samples stored for extended period of time perform equally to freshly collected saliva as demonstrated by 100% concordance across >230,000 SNPs
- Oral samples collected using either Oragene•DNA or ORAcollect•DNA are an excellent source of gDNA for use with Exome arrays as demonstrated with call rates >99.8%
- Samples collected using either Oragene•DNA or ORAcollect•DNA perform equally as assessed by 100% concordance using the Illumina HumanExome v1.1 arrays

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