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Project: Extraction and Genotyping of DNA Collected with Mawi Collection Kits and Comparison to a Sample From the Same Individual Collected Using a DNA Genotek OraCollect Kit

Project Commencement Date: 6/15/2016
Report Date: 8/25/2016

Project overview:
Samples were collected from 40 individuals. Each participant provided a sample collected using the Mawi ISWAB-ID collection kit (ISWAB-ID ,Mawi, Hayward, CA) and a sample collected using an OraCollect collection device (OC-100, DNA Genotek, Ottawa, Canada). For the purposes of this document Mawi and ISWAB-ID are interchangeable as are OraCollect and OC-100. Samples were extracted using two different methods. Samples from 20 individuals had both the Mawi and OC-100 collected samples extracted manually using Qiagen PureGene reagents and a validated laboratory protocol. Additionally, samples from another 20 individuals had both the Mawi and OC-100 collected samples extracted using an automated system, Chemagen, MSMI instrument (Perkin Elmer, Waltham, MA) and a manufacturer's protocol. Following extraction, samples quantity and quality were estimated by UV spectrophotometry (NanoDrop, Thermo Scientific, Wilmington, DE). DNA from both the Mawi and the OC-100 manual extractions for 10 individuals was then genotyped on a panel of 118 SNPs and 1 copy number variant (CNV). Life Technologies' Taqman® Open Array was used for the SNPs and a Life Technologies real-time PCR assay was used for the CNV. The SNP and CNV assays were executed using a laboratory-developed protocol.

Comparison of the two collection kit types included average yield, 260/280 ratios, 260/230 ratios, genotyping success and genotyping concordance.

Summary of activities:
Extraction:

- Manual extraction-20 individuals. Both Mawi and OC-100 kits collected from the same individual were extracted for a total of 40 samples.
- Automated extraction-20 individuals. Both Mawi and OC-100 kits collected from the same individual were extracted for a total of 40 samples.
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Genotyping

- 118 single nucleotide polymorphisms-10 individuals. Both Mawi and OC-100 kits collected from the same individual were genotyped for a total of 20 samples.

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- Copy Number Variant-10 individuals. Both Mawi and OC-100 kits collected from the same individual were genotyped for a total of 20 samples.

Project metrics:
80/80 samples were extracted. Average yield of the manually extracted Mawi samples was 8.4 ug (1.8-20.4 ug) (Table 1.). Average yield of the manually extracted OC-100 samples was 9.1 ug (0.88-30.5 ug) (Table 2). Average yield of the automated extracted Mawi samples was 2.3 ug (0.45-10.4 ug) (Table 3). Average yield of the automated extracted OC-100 samples was 3.1 ug (0.47-10.63 ug) (Table 4). The samples for all kit types and extraction methods produced acceptable and comparable 260/280 ratios.

Table 1. Mawi manually extracted samples

Sample ID	ng/ul	260/280	260/230	Type	Yield (ug)
1000038392	159.0	1.88	1.42	MAWI	15.9
1000038394	59.5	2.10	1.51	MAWI	6.0
1000038396	80.2	1.98	1.58	MAWI	8.0
1000038398	121.6	1.93	1.37	MAWI	12.2
1000038400	123.5	2.00	1.64	MAWI	12.3
1000038402	34.6	1.90	0.61	MAWI	3.5
1000038404	49.3	2.08	1.75	MAWI	4.9
1000038406	20.9	2.38	1.09	MAWI	2.1
1000038408	51.3	2.08	1.76	MAWI	5.1
1000038410	63.9	1.94	1.38	MAWI	6.4
1000038412	125.2	1.98	1.74	MAWI	12.5
1000038414	77.4	2.04	1.62	MAWI	7.7
1000038416	46.3	2.13	1.72	MAWI	4.6
1000038418	73.6	2.13	1.65	MAWI	7.4
1000038420	80.9	2.01	1.72	MAWI	8.1
1000038422	203.8	1.92	1.46	MAWI	20.4
1000038424	116.8	1.97	1.52	MAWI	11.7
1000038426	17.7	2.70	0.53	MAWI	1.8
1000038428	88.3	2.01	1.50	MAWI	8.8
1000038430	82.9	1.92	1.61	MAWI	8.3

Averages		2.054	1.459		8.38275
Range		1.88-2.70	0.53-1.76		1.8-20.4

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Table 2. OC-100 manually extracted samples

Sample ID	ng/ul	260/280	260/230	Type	Yield (ug)
1000038391	66.2	1.91	1.73	OC100	6.6
1000038393	27.0	2.17	1.43	OC100	2.7
1000038395	10.5	2.03	1.22	OC100	1.0
1000038397	67.2	1.93	1.46	OC100	6.7
1000038399	137.6	2.03	1.88	OC100	13.8
1000038401	49.9	1.77	0.90	OC100	5.0
1000038403	55.2	2.06	1.63	OC100	5.5
1000038405	241.4	1.95	1.57	OC100	24.1
1000038407	51.1	2.04	1.52	OC100	5.1
1000038409	48.2	1.81	0.98	OC100	4.8
1000038411	109.0	1.88	1.62	OC100	10.9
1000038413	8.8	1.90	0.78	OC100	0.9
1000038415	12.6	1.52	0.80	OC100	1.3
1000038417	78.6	2.00	1.56	OC100	7.9
1000038419	208.8	2.00	1.75	OC100	20.9
1000038421	187.7	1.93	1.73	OC100	18.8
1000038423	304.8	1.78	1.25	OC100	30.5
1000038425	15.9	2.01	1.22	OC100	1.6
1000038427	39.8	1.95	1.69	OC100	4.0
1000038429	95.4	2.00	1.87	OC100	9.5

Averages		1.9335	1.4295		9.07755
Range		1.52-2.17	0.78-1.88		0.88-30.5

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Table 3. Mawi automated extraction samples

Sample ID	ng/ul	260/280	260/230	Type	Yield (ug)
1000038432	32.1	2.10	1.69	MAWI	2.4
1000038434	26.9	2.03	1.58	MAWI	2.0
1000038436	78.0	1.93	1.65	MAWI	5.8
1000038438	29.2	2.07	1.64	MAWI	2.2
1000038440	28.8	2.08	1.48	MAWI	2.2
1000038442	32.6	2.07	1.69	MAWI	2.4
1000038444	26.0	2.12	1.78	MAWI	2.0
1000038446	6.0	2.65	0.87	MAWI	0.4
1000038448	35.2	1.95	1.64	MAWI	2.6
1000038450	138.3	2.05	1.98	MAWI	10.4
1000038452	10.4	1.95	1.09	MAWI	0.8
1000038454	25.8	2.22	1.68	MAWI	1.9
1000038456	9.8	2.49	1.25	MAWI	0.7
1000038458	24.9	2.08	1.40	MAWI	1.9
1000038460	18.7	2.26	1.38	MAWI	1.4
1000038462	6.4	2.67	1.08	MAWI	0.5
1000038464	29.2	1.95	1.66	MAWI	2.2
1000038466	22.6	1.99	1.60	MAWI	1.7
1000038468	27.3	2.06	1.72	MAWI	2.1
1000038470	8.9	2.84	1.26	MAWI	0.669

Averages		2.178	1.506		2.315025
Range		1.93-2.84	0.87-1.98		0.45-10.4

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Table 4. OC-100 automated extraction samples

Sample ID	ng/ul	260/280	260/230	Type	Yield (ug)
1000038431	62.2	1.92	1.30	OC100	4.7
1000038433	20.8	1.88	1.13	OC100	1.6
1000038435	93.7	1.80	1.17	OC100	7.0
1000038437	32.8	2.05	1.55	OC100	2.5
1000038439	49.0	1.93	1.01	OC100	3.7
1000038441	55.4	1.92	1.40	OC100	4.2
1000038443	24.0	2.02	1.42	OC100	1.8
1000038445	10.5	2.07	0.94	OC100	0.8
1000038447	26.2	2.12	1.54	OC100	2.0
1000038449	141.8	1.89	1.23	OC100	10.6
1000038451	46.0	1.87	1.17	OC100	3.5
1000038453	38.6	1.98	1.29	OC100	2.9
1000038455	6.3	3.73	0.85	OC100	0.5
1000038457	83.6	1.88	1.22	OC100	6.3
1000038459	17.7	2.14	1.37	OC100	1.3
1000038461	21.6	2.50	1.62	OC100	1.6
1000038463	30.5	2.12	1.59	OC100	2.3
1000038465	27.4	2.05	1.25	OC100	2.1
1000038467	14.3	2.24	1.04	OC100	1.1
1000038469	24.2	2.01	1.27	OC100	1.8

Averages		2.106	1.268		3.0977625
Range		1.8-3.73	0.85-1.62		0.47-10.63

An aliquot of 10 sample pairs, paired samples from the same individual, of the Mawi and OC-100 manually extracted samples, was normalized to approximately 3 ng/ul for genotyping using Taqman® chemistry. All samples were analyzed for 118 SNPs and 1 CNV. All genotypes from the Taqman® Open Array were concordant between the two extraction kits. Data from the CNV was concordant between the two extraction kits. Samples from both kits genotyped a rate of 99.5%. 11/12 undetermined genotypes amplified, but could not be definitively placed in a genotype cluster. One undetermined genotype did not amplify. The undetermined genotypes were equally distributed between the 2 collection kits, Mawi 7 undetermined genotypes and OC-100 5 undetermined genotypes. There were no failures in the CNV assay and all CNV results were concordant.

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Conclusions:
DNA extracted from both collection kits had similar yields and quality. The automated extraction as is typical gave lower average yields, but there was no significant difference between extraction kits. DNA extracted from both collection kits performed equally well in Taqman genotype and copy number variant analysis with a high genotyping rate.

Materials and methods:

DNA Extraction:
Manual extraction was completed using Qiagen PureGene reagents and a validated extraction protocol. Automated extraction was completed using a Perkin Elmer MSMi instrument and Chemagen reagents. Concentrations of the samples were determined by UV spectrophotometry measurements. An estimate of purity was determined with UV spectrophotometry by measuring the A260/A280 absorbance ratio.

Taqman® Open Array Genotyping:
Genotyping for 118 single nucleotide polymorphisms (SNPs) was accomplished using a TaqMan® Open Array genotyping assay. The Taqman® assay is an allele discrimination assay using PCR amplification and a pair of fluorescent dye detectors that target the SNP. One fluorescent dye is attached to the detector that is a perfect match to the first allele (e.g. an "A" nucleotide) and a different fluorescent dye is attached to the detector that is a perfect match to the second allele (e.g. a "C" nucleotide). During PCR, the polymerase will release the fluorescent probe into solution where it is detected using real-time PCR in a Life Technologies QuantStudio thermocycler. Samples and Taqman® assays are loaded onto the Open Array slide using a Life Technologies 12K Flex Accufil instrument. The open array was designed by and obtained from Life Technologies design and manufacturing. Genotypes were determined using Life Technologies' Taqman® Genotyper v1.0.1 software. Genotyper software is based on a clustering algorithm where the three expected genotypes are separated into clusters. The 178 SNPs detected using this methodology had adequate separation in the clusters to ensure a high accuracy of genotyping.

Copy Number Variant Taqman® Genotyping

Taqman® Copy Number assays are design to detect and measure copy number variation within the human genome. Taqman® Copy Number assays are run simultaneously with a Taqman® Copy Number reference assay (RNase P) in a real-

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time polymerase chain reaction assay. This copy number assay detects the target of a known copy number variant, which is associated with certain pharmacological responses. The reference assay detects a sequence known to exist as two copies in the diploid human genome. One fluorescent dye (FAMM) is attached to the CNV assay, and a different fluorescent dye (VIC) is attached to the reference assay. During PCR, the polymerase will release the fluorescent probe into solution where it is detected using real-time analysis in a Life Technologies, Inc. (Foster City, CA) 7900HT Real-Time instrument. Copy number was determined by comparison of the CNV assay as compared to the diploid reference assay, based on the mean Ct value, using Life Technologies' Copy Caller v2.0 software. All samples were repeated in quadruplicate for both the CNV and reference assays to ensure accurate copy number determination. Each sample was analyzed in triplicate for telomere length. Samples with 3 Ct values that differ by ≤ 1 Ct were analyzed.