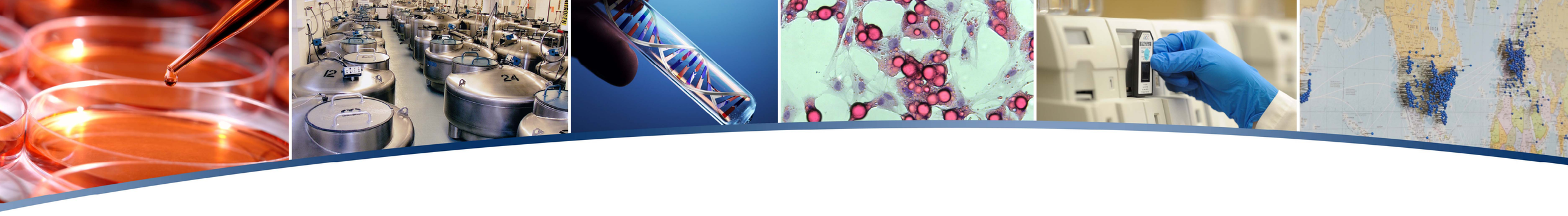

Performance analysis of saliva generated genomic DNA used for genotyping on the Affymetrix DMET Plus array as part of the Coriell Personalized Medicine Collaborative

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ABSTRACT

The use of saliva as a source of genomic DNA for research and clinical studies has grown in popularity due to the ease of collection and participant compliance. The Coriell Personalized Medicine Collaborative (CPMC) has been using Oragene collection kits for the past six years as the source of genomic DNA, initially for genotyping on the Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 array, and for the past four years, also for genotyping on the Affymetrix DMET Plus array. Although multiple studies on the SNP 6.0 array have utilized saliva as the source of DNA, the CPMC is one of the first large scale studies to also use that DNA on the DMET Plus array. To date, the study has successfully processed more than 5000 samples on the DMET Plus array with an average call rate of 99.59%. This was similar to the 99.4% call rate achieved on the SNP 6.0 array. The genotyping results from saliva generated genomic DNA have also proven to be highly reproducible on DMET Plus. Independent extractions from individual Oragene kits as well as across multiple kits for 4 samples used as processing controls have resulted in average call rates between 99.5% and 99.8% across thirty or more replicates of each control. Furthermore, in the control replicates, the concordance rates in a set of 166 variants of interest to the CPMC study ranged between 99.7% and 99.9 %. Finally, because the genomic DNA samples were run on both Affymetrix arrays, it was also possible to examine the performance of 212 SNPs that are present on both platforms. For a set of 1920 samples, the average concordance was 98.2%. When excluding SNPs where either or both arrays had a No Call, the average concordance increased to 99.5%. The use of saliva generated genomic DNA in the Affymetrix DMET Plus assay has proven to be very successful and has allowed the CPMC to expand its genotyping options while maintaining a single DNA source.

DMET PLUS VALIDATION

Prior to processing clinical samples on the DMET PLUS array for the CPMC study, the assay needed to be validated in the Coriell Genotyping and Microarray Center. The Validation was carried out as 2 separate experiments as outlined below:

Experiment 1	**Experiment 2**
40 HapMap Samples	40 Saliva Samples

In each sample set, the samples were processed as duplicates in 3 separate plates for a total of 240 possible replicates

Processing of the saliva samples included independent extraction of DNA for each replicate. DNA was extracted using an Agencourt DNAdvance kit and a 600 µL aliquot of saliva . Minimum required extraction yield ≥ 50ng/µL.

16 saliva replicates failed extraction

↓

Each plate was analyzed separately in Affymetrix DMET Console using the Dynamic Genotyping algorithm

Call Rate threshold for inclusion in downstream marker analysis ≥ 97%

239 HapMap and 219 Saliva Samples passed the call rate threshold

↓

Independent analysis of the HapMap/Saliva data sets for discordancies and no calls to determine markers with data quality acceptable for clinical reporting

Discordancy threshold for exclusion from reporting > 5 % in either set
No Call threshold for exclusion from reporting > 10 % in either set

↓

Total number of markers excluded: 12/1931 (0.62%)

Table 1: Markers excluded from reporting due Discordancy and/or No Call rate.

	HapMap		Saliva	
Probeset	% No Call	% Discordant	% No Call	% Discordant
AM_11474	2.09	0.43	8.68	6.50
AM_11702	0.00	2.51	0.00	5.02
AM_11203	5.02	3.52	2.74	6.10
AM_11913	0.00	0.84	3.65	5.21
AM_11611	0.00	0.00	14.61	10.70
AM_12057	19.25	16.58	0.91	4.15
AM_13024	7.53	2.26	11.87	7.25
AM_10056	4.18	0.00	5.02	14.42
AM_11547	0.00	0.42	5.02	5.29
AM_11089	0.00	4.18	3.20	8.02
AM_12278	17.57	0.00	1.83	0.47
AM_10090	3.35	0.00	9.59	15.15

Discordancy rates were calculated as # calls different from consensus/(total # replicates – # replicates with No Call)

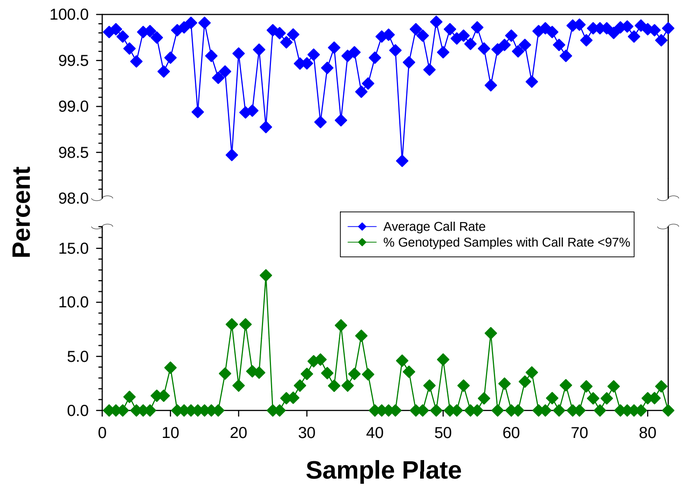
CPMC SAMPLE COLLECTION & PROCESSING

Participants attend and informed consent session and provide a saliva sample in a DNA Genotek OG-500 kit.

Participants activate their online account and complete a medical, family, lifestyle questionnaire.

DNA is extracted from the participants saliva sample and genotyped on the Affymetrix SNP 6.0 and DMET Plus arrays.

DMET PLUS ASSAY QUALITY METRICS



Average Call Rate Across All Plates
99.59%

Total Number of Genotyped Samples with a Call Rate <97%

120/5164 (1.74%)

Figure 1. DMET Plus assay performance using DNA from saliva as the starting material. Within the CPMC study, the laboratory uses a per sample call rate of 97% as the threshold for data release.

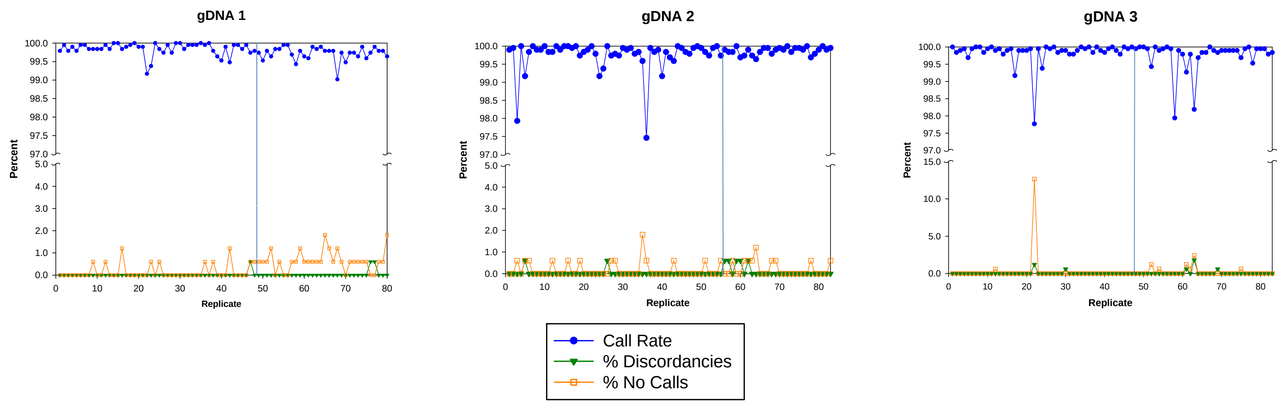


Figure 2. DMET Plus assay performance of gDNA controls provided in the reagent kit. The vertical line in each plot indicates a change in the sample provided with the DMET Plus kit. The replicates represent sample processing during the period of October, 2009 – September, 2013.

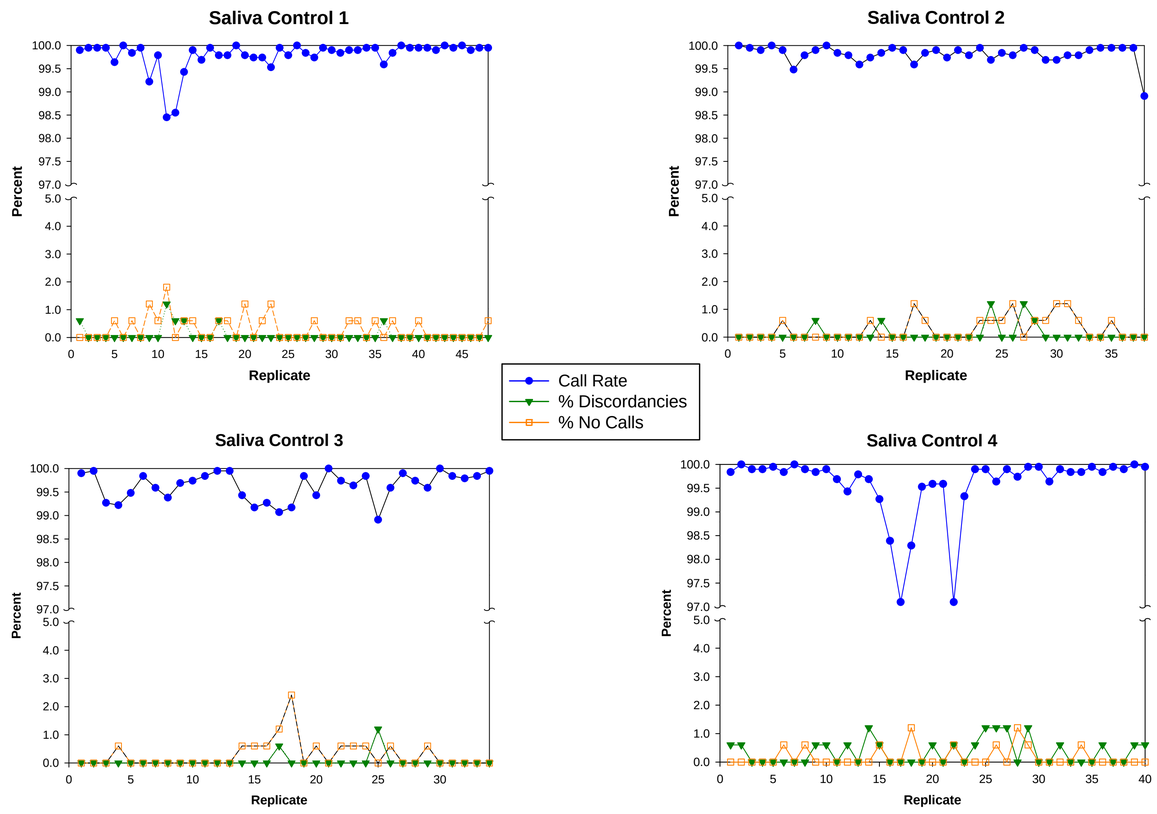


Figure 3. DMET Plus assay performance of saliva controls. Two of the four controls are randomly selected by the LIMS to be run in each plate of samples. The replicates represent sample processing during the period of October, 2009 – September, 2013.

Table 2: Average Call and Concordance Rates for Controls Used in DMET Sample Plates

	Average Call Rate	Average Concordance	Averge No Call Rate
gDNA 1	99.80	99.98	0.35
gDNA 2	99.79	99.95	0.18
gDNA 3	99.81	99.94	0.24
Saliva Control 1	99.80	99.91	0.30
Saliva Control 2	99.82	99.79	0.29
Saliva Control 3	99.63	99.95	0.28
Saliva Control 4	99.53	99.66	0.21

The Average Call Rates were calculated using the call rates generated by Affymetrix DMET Console for the entire array. The Average Concordance and No Call Rates were calculated using a subset of 166 markers that are candidates for reporting within the CPMC study.

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