SNP Genotyping of Saliva DNA using Affymetrix® GeneChip® Targeted Genotyping System

A comparison of the performance of paired blood and saliva DNA samples in the Affymetrix GeneChip Targeted Genotyping assay

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ABSTRACT

With the completion of the HapMap project, researchers are performing SNP genotyping studies in ever increasing numbers. Genomic DNA derived from blood is a common source for these studies, however blood collection has several drawbacks (including costs, infection danger, and sample degradation). The result is interest in developing new collection, transportation and isolation technologies for other sources of genomic DNA. In this poster we evaluate the use of genomic and whole genome amplified (WGA) DNA from both saliva (collected with a new technology - the Oragene™ DNA Self Collection Kits manufactured DNA Genotek) and blood as template in the Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System. The saliva and blood DNA genotyping results (genotyping concordance, trio accuracy and call rates) are compared and differences in performance are determined for the DNAs isolated from the different sources. The results of the Targeted Genotyping assay demonstrate that saliva DNA (in both genomic and WGA form) that is collected with DNA Genotek's Oragene technology performs equivalent to or better than blood extracted DNA. We also demonstrate that the Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System will accept input DNA from different sources, and as both genomic and whole genome amplified DNA.

METHODS

After obtaining informed consent, paired blood and whole saliva samples were collected from 44 healthy human subjects. The sample set contained 5 trios (father, mother, child) among the 44 donors. Saliva samples were collected using Oragene DNA Self Collection Kits (DNA Genotek) according to the manufacturer's instructions, and processed using the PUREGENE® DNA Purification Kit according to Protocol #400244 (Gentra Systems). Blood samples were collected in lavender-top EDTA Vacutainer® tubes (BD Diagnostics), and processed using QIAamp® DNA Blood Mini Kit (Qiagen). Purified DNA was quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) according to the manufacturer's protocol.

Two micrograms of the resulting DNA of each sample was used as template in the Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System and genotyped using the Affymetrix 3K Training Assay Panel, a panel of 2,918 SNPs from Human Ch12 (developed during the HapMap project).

We also investigated the genotyping assay performance of genomic DNA against WGA DNA. 20 to 50 ng of genomic saliva or blood extracted DNA was used as template in the Qiagen Repli-g Whole Genome Amplification assay. The DNA was amplified using a modified Repli-g protocol (developed at Affymetrix) and quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) according to the manufacturer's protocol. One microgram of the resulting amplified DNA was genotyped using the Affymetrix 3K Training Assay Panel.

The genomic blood DNA and genomic saliva DNA results were compared to one another and to the respective WGA blood and saliva DNA results. We compared overall genotyping performance (repeatability, accuracy, completeness, and conversion rate) as well as QC metrics that indicate general sample performance (QC Call rate and Signal/Background Ratio).

RESULTS AND CONCLUSIONS

The results of this study demonstrate that:

1. Genomic DNA from saliva performs as well as genomic DNA from blood as measured using both QC metrics (Table A) and genotypin performance metrics (Graph 1), and that the genotypes derived from both sets of DNA are >99.5% concordant (Graph 2).

2. WGA samples derived from blood and saliva extracted DNAs show some degradation in their QC performance metrics (Tables B & C), and genotyping performance metrics (Graph 1) compared to their genomic counterparts. However concordance to their respective genomic counterparts is still >99% in both cases (Graph 2).

In conclusion, the performance of Affymetrix Targeted Genotyping assay demonstrates that saliva DNA (in both genomic and WGA form) that is collected with DNA Genotek's Oragene technology performs equivalent to or better than blood extracted DNA. This is important as the Oragene technology represents a less invasive DNA isolation protocol than blood extraction, allowing for the use of this technology in situations where blood extraction is not viable. Saliva collected with the Oragene technology can also be stored and shipped at RT, which represents an additional advantage over blood as a source of genomic DNA.

We have also demonstrated that the Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System will accept input DNAs (dervied from different sources) in both genomic and WGA DNA form.

Table A - Genomic Blood DNA vs. Genomic Saliva DNA QC Metrics Comparison

	Blood Genomic DNA (Reference) n = 47		Saliva Genomic DNA n = 47		T-Test % confidence that differences are significant
Condition	Average	St Dev	Average	St Dev	
QC Call Rate %	96.86	0.19	97.02	0.33	99.07%
Sig/Bknd	139.87	14.79	144.45	24.26	72.08%

Table A: Comparison of genotyping QC metrics for 47 blood derived genomic DNA samples vs 47 matched saliva derived genomic DNA samples. The DNA from saliva performs equivalently to the DNA from blood when compared using 3 QC metrics (QC call rate, and Signal to Background Ration (Sig/Bknd))

Table B - Genomic Blood DNA vs. WGA Blood DNA QC Metrics Comparison

	Genomic Blood DNA (Reference) n = 47		WGA Blood DNA n = 47		T-Test % confidence that differences are significant
Condition	Average	St Dev	Average	St Dev	
QC Call Rate %	96.86	0.19	95.10	0.97	100%
Sig/Bknd	139.87	14.79	109.40	17.01	100%

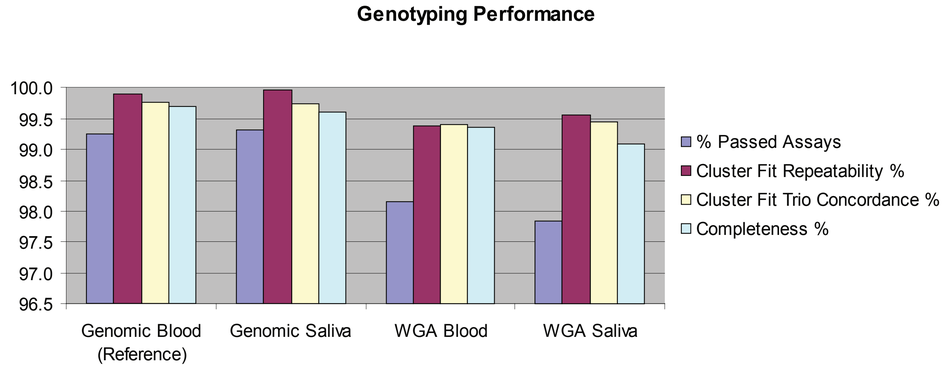
Table B: Comparison of genotyping QC metrics for 47 blood derived genomic DNA samples vs 47 WGA blood derived DNA samples. The QC metrics (QC call rate, and Signal to Background Ration (Sig/Bknd)) for WGA blood derived DNA shows some degradation in performance when compared to genomic blood derived DNA.

Table C - Genomic Saliva vs. WGA Saliva QC Metrics Comparison

	Genomic Saliva DNA (Reference) n = 47		WGA Saliva DNA n = 47		T-Test % confidence that differences are significant
Condition	Average	St Dev	Average	St Dev	
QC Call Rate %	97.02	0.33	94.57	1.16	100%
Sig/Bknd	144.45	24.26	103.87	13.02	100%

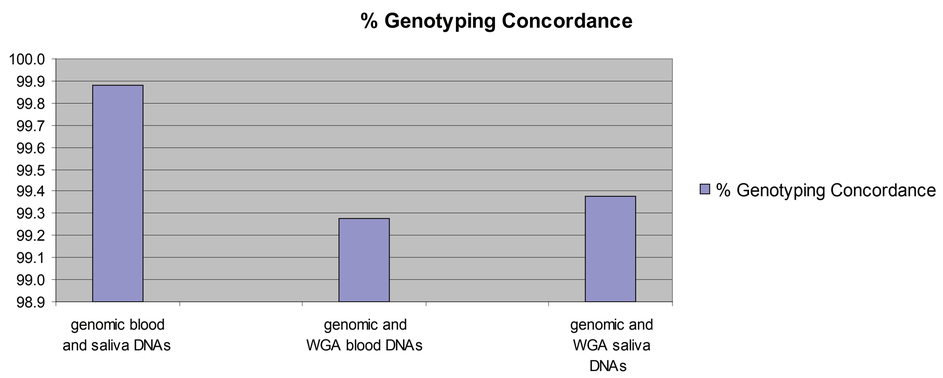
Table C: Comparison of genotyping QC metrics for 47 saliva derived genomic DNA samples vs 47 WGA saliva derived genomic DNA samples. The QC metrics (QC call rate, and Signal to Background Ration (Sig/Bknd)) for WGA saliva derived DNA shows some degradation in performance when compared to genomic saliva derived DNA.

Graph 1 - Comparison of Genotyping Performance Metrics Across Different Targeted Genotyping Assay Templates



Graph 1 - Comparison of Genotyping Performance Metrics Across Different Targeted Genotyping Assay Templates. The results show that for four metrics (% Call Rate, % repeatability, % trio concordance, and % completeness) there is no significant performance difference between genomic blood DNA and genomic saliva DNA. The WGA template DNAs do show lower performance metrics compared to the respective genomic DNA in each case.

Graph 2 - Comparison of % Genotyping Concordance between Saliva and Blood DNAs in both genomic and WGA format



Graph 2 - Comparison of genotyping concordance between genomic saliva and blood DNAs, and WGA DNAs to their respective genomic counterpart. While the WGA DNAs show lower overall genotyping performance (as measured in Tables B, C and Graph 1), the level of concordance between the genomic and WGA genotypes remains >99.2% in both cases.