APPLICATION REPORT

PHARMACOGENETIC ANALYSIS USING WHOLE EXOME SEQUENCING OF SALIVA-DERIVED DNA COLLECTED FROM SALETTO® DNA SALIVA COLLECTION DEVICE

Assessing Saletto® DNA's Suitability for Next Generation Sequencing Applications

POREX
Life Sciences Institute™

Introduction

This study was conducted to demonstrate suitability of extracted DNA from the Saletto® DNA's Saliva Collection Device for next generation sequencing assays, specifically, Whole Exome Sequencing using the Illumina NovaSeq 6000 systems.

In this study we sequenced DNA extracted from saliva collected from 3 donors using Saletto® DNA to examine specific pharmacogenetic markers involved in drug metabolism of Warfarin, an oral anticoagulant medication. The 3 donors were tested for variants of the CYP2C9 and VKORC1 genes.

To assess the performance of the saliva samples collected using Saletto®DNA, we present quality control data and compare against saliva samples collected with an alternate saliva collection device from the same donors. The alternate saliva collection device is an FDA-cleared Class II device that has demonstrated through validation studies that it is a viable alternative to blood for use in molecular diagnostic applications.

Materials and Methods

Using Saletto® DNA OFCD-325, 1 mL of saliva was collected from each of the 3 donors. The alternate device was used to collect 200 ul of saliva.

DNA was extracted using a Prepito DNA Cyto Pure Kit (PerkinElmer) on a chemagic Prepito instrument (PerkinElmer). DNA quality was checked with spectrophotometer NanoPhotometer® N120 (Implen) and quantified using PicoGreen reagent (Thermo Fisher Scientific) on a Wallac 1420 Victor™ 2D microplate reader (PerkinElmer).

Sequencing was performed on genomic DNA using an Agilent targeted sequence capture method to enrich the exome. Direct sequencing of the amplified captured regions was performed using 2x150bp reads on Illumina NovaSeq 6000 systems. A base is considered to have sufficient coverage at 20x and an exon is considered fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20X or more. Alignment to the human reference genome (GRCh37) was performed and variants were identified in the targeted region. Primary data analysis was performed using Illumina bcl2fastq converter v2.19. Secondary analysis was performed using Illumina DRAGEN Bio-IT Platform v.3.10.8.

Results and Discussion

For all samples, “average alignment coverage” exceeded 100x, indicating high coverage and sequencing depth for all regions, conveying good quality and quantity of DNA used. Average alignment coverage indicates the sequencing read depth at which sequencing data generated covers the targeted exome regions on average. After sequencing, the resulting reads are aligned to the reference genome, and the depth of coverage at each position is calculated. Average align coverage can impact the accuracy of variant calling due to missing regions or low coverage.

Table 1: Coverage and Sequencing Depth Comparison between Saletto DNA and Alternate Saliva Collection Device

	Donor 1		Donor 2		Donor 3	
	Saletto DNA	Alternate Saliva Collection Device	Saletto DNA	Alternate Saliva Collection Device	Saletto DNA	Alternate Saliva Collection Device
Average Align Coverage	>100x	>100x	>100x	>100x	>100x	>100x
%Bases> 20x	97.15%	97.13%	96.90%	97.23%	97.51%	97.51%

Table 1: Coverage and sequencing depth comparison for all regions demonstrated by “average align coverage,” and targeted region demonstrated by “%bases>20x” between saliva from both devices

As table 1 shows, the percentage of bases covered in the target region (important for variant calling) for each sample has been sequenced at a depth of at least 20x, and all samples showed at least 97% coverage.

Concordance between saliva collected from both devices was examined for SNPs and variants. The concordance for SNPs and all variants between saliva collected from both devices was greater than 98% and 95% respectively, as shown in table 2.

Table 2: SNP and Variant Overlap Comparison between Saletto DNA and Alternate Saliva Collection Device

	SNP				All Variants			
	SNPs in Saletto DNA Sample	SNPs in Alternate Saliva Collection Device Sample	Common SNPs	Concordance	All Variants in Saletto DNA Sample	All Variants in Alternate Saliva Collection Device Sample	Common Variants	Concordance
Donor 1	268	202	32,511	98.57%	821	723	34,925	95.77%
Donor 2	272	259	31,137	98.32%	799	804	33,440	95.43%
Donor 3	273	211	32,103	98.51%	749	707	34,608	95.96%

Table 2: Coverage and sequencing depth comparison for all regions demonstrated by "average align coverage," and target region demonstrated by "%bases>20x" between saliva from both devices

Saliva collected from both devices was successfully used to investigate genetic variants using next generation sequencing technologies as demonstrated in table 3 below. The SNPs for each allele matched perfectly between saliva collected from both devices for all donors. For CYP2C9*2, all 3 donors are homozygous for the variant. For CYP2C9*3, donor 1 is heterozygous for the variant, and donors 2 and 3 do not have the variant. For VKORC1, donors 1 and 3 do not have the variant and donor 2 is heterozygous for the variant. Individuals who inherit two copies of the CYP2C9*2 variant (one from each parent) are considered homozygous for the variant and have significantly reduced enzyme activity, whereas those who inherit only one copy of the variant (from one parent) are considered heterozygous and have intermediate enzyme activity.

Table 3: SNP Check Comparison between Saletto DNA and Alternate Saliva Collection Device

		Donor 1		Donor 2		Donor 3	
Allele	rsID	Saletto DNA	Alternate Saliva Collection Device	Saletto DNA	Alternate Saliva Collection Device	Saletto DNA	Alternate Saliva Collection Device
CYP2C9*2	rs1799853	C	C	C	C	C	C
CYP2C9*3	rs1057910	A/C	A/C	A	A	A	A
VKORC1	rs9923231	C	C	C/T	C/T	C	C

Table 3: SNP check comparison for alleles CYP2C9*2, CYP2C9*3 and VKORC1 between saliva from both devices

Conclusion

DNA extracted from the Saletto® DNA Saliva Collection Device is suitable for next generation sequencing techniques such as Whole Exome Sequencing as demonstrated by this study, which allowed us to examine the SNPs of interest involved in drug metabolism. The high coverage and sequencing depth as demonstrated by "average alignment coverage" for all regions and "%bases> 20x" for the target region, allowed us to confidently examine the SNPs of interest. The high concordance of 98% for SNPs and 95% for all variants between saliva samples collected from both devices, and perfect correlation of SNPs read for the alleles involved in drug metabolism of Warfarin, provides further confidence in the effectiveness of Saletto DNA for next generation sequencing studies, proving equivalence to other traditional saliva collection methods.

Saletto® DNA Saliva Collection Device is for Research Use Only.

1. This testing was performed at PerkinElmer Genomics (CLIA ID: 39D0673919; CAP ID: 1286403). This test result is being used for research purposes only.

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