Verification of buccal swab and saliva sample types for PharmacoScan Solution

Abstract

The Applied Biosystems™ PharmacoScan™ Solution enables comprehensive and accurate genotyping using a DNA microarray of ~4,600 markers in over 1,000 genes involved in drug-metabolizing enzymes and transporters.

Because personalized medicine is increasingly important in patient care, this study set out to expand the application of the PharmacoScan platform to various DNA sample types and extraction methodologies. To this end, the characterization and verification of genotyping performance of saliva and buccal cell sample types were carried out with the Applied Biosystems™ PharmacoScan™ Assay Kit, 96-Format, and PharmacoScan™ Assay Kit, 24-Format, array plates.

This study verified the use of both magnetic bead–based high-throughput DNA isolation and precipitation-based manual DNA extraction methods. Both extraction methods provided high-quality genomic DNA (gDNA) that was compatible with the specifications detailed in the PharmacoScan user guide. Additionally, similar genotyping performance was observed between DNA extracted from oral samples and whole blood.

All sample types analyzed passed the PharmacoScan assay in-process quality control (QC) checks and met the genotyping and copy number performance metrics. Results showed that DNA derived from saliva and buccal cells performed similarly to whole blood in arrays with both the PharmacoScan Assay Kit, 96-Format, using the Beckman™ Biomek™ FXP automated target preparation workflow, and the PharmacoScan Assay Kit, 24-Format, using manually prepared DNA. This study supports the use of gDNA derived from oral samples, such as saliva and buccal cells, in the PharmacoScan assay.

Introduction

Variation in drug absorption, distribution, metabolism, and excretion (ADME) genes forms the basis of each individual's clinical response to xenobiotics. The PharmacoScan Solution enables a comprehensive analysis of ADME genes. Performance of the PharmacoScan assay has been extensively characterized with gDNA from peripheral whole blood, while the performance of orally derived DNA has not been systematically evaluated. Increasingly, clinicians collect oral samples for human gDNA because they can be obtained noninvasively, in contrast to whole blood samples. Therefore, in this study gDNA from saliva and buccal cells was tested as an alternative to DNA from blood.

Matched samples from over 100 healthy donors were collected using the collection kits specified in Table 1, following the manufacturers' recommendations. DNA was extracted using two different methods: a manual, precipitation-based approach using Qiagen™ Gentra™ Puregene™ kits, and an automated, bead-based approach using the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit. DNA samples were quantified, quality-checked via gel electrophoresis for degradation, and then evaluated by the PharmacoScan assay to assess genotyping and copy number performance. Here the verification of oral sample types with the PharmacoScan Assay Kits, 96-Format and 24-Format, is described.

Materials and methods

Sample collection

Whole blood was collected in EDTA tubes (Table 1), and the tubes were slowly inverted, manually, at least 10 times. The filled tubes should not be shaken, since vigorous mixing can cause foaming or hemolysis that can result in DNA degradation. On the other hand, inadequate mixing may result in platelet clumping, clotting, or lower-quality DNA [1].

For oral sample collection, it is critical to avoid food for at least 30 minutes prior to sample collection to prevent inclusion of substances that may interfere with downstream analyses (Table 1). Saliva sample collection using the Oragene™ Discover collection kit (DNA Genotek OGR-500) followed the manufacturer's instructions to mix the collection tube contents effectively to help prevent DNA degradation over time (Table 1) [2]. For optimal DNA yield and quality of buccal sample DNA, each donor's cheek was rubbed at least 10 times, and then the swab was stored at the temperature specified in Table 1 [3].

DNA extraction

DNA was extracted using two different methods, including a manual precipitation-based approach [4] (Gentra Puregene kit) and an automated magnetic bead–based approach using the MagMAX kit [5] on the Thermo Scientific™ KingFisher™ platform (Table 1). Extraction was carried out within 8 weeks after initial sample collection, and samples were stored as indicated in Table 1. DNA purity was checked by measuring absorbance at 260 nm, 280 nm, and 230 nm on a Thermo Scientific™ NanoDrop™ instrument. DNA size integrity was evaluated using agarose gel electrophoresis as indicated in the PharmacoScan user guide [6,7].

Table 1. Sample collection methods, relevant factors that help prevent negative effects on DNA yield and quality, and DNA extraction kits.

Sample type	Collection kit	Critical step that may impact DNA quality	Sample storage before aliquoting (temp; time)	Long-term sample storage (temp; time)	DNA extraction kit (Cat. No.)
Blood	BD EDTA tube (Cat. No. 367863) [1]	Mixing step	4°C; no more than 24 hr	–80°C; NA	MagMAX DNA Multi-Sample Ultra 2.0 Kit; Cat. No. A36570 and Gentra Puregene kits; Cat. No. 158489 (Bl, Sa)/ Cat. No. 158845 (Bu)
Saliva	Oragene•DISCOVER collection kit (DNA Genotek OGR-500) [2]	Avoid food 30 min prior to collection; mixing step	RT; no more 24 hr	RT; over 1 yr	
Buccal swab	Copan 4N6FLOQSwabs™ buccal swabs (Cat. No. 4520CS01) [3]	Avoid food 30 min prior to collection; rub donor's cheek 10 times	RT; no more than 30 days	–20°C; NA	

Bl = blood; Sa = saliva; Bu = buccal cells; RT = room temperature; NA = not available

Analysis of oral sample types in the PharmacoScan assay

DNA input for the PharmacoScan assay was based on quantitation using Invitrogen™ Qubit™ products for double-stranded DNA (dsDNA) quantitation. All samples tested met the minimum DNA concentration requirement of 5 ng/µL (Table 2). The PharmacoScan assay required a total of 150 ng of input DNA. The Applied Biosystems™ Axiom™ amplification reaction used 100 ng (20 µL of 5 ng/µL) and the multiplex PCR used 50 ng (10 µL of 5 ng/µL).

Arrays were hybridized, washed, and imaged on an Applied Biosystems™ GeneTitan™ Multi-Channel Instrument controlled by GeneChip™ Command Console Software version 4.3.

Data analysis was performed using software tools available with Applied Biosystem™ Axiom™ Analysis Suite version 3.1. Genotype calls were made using the BRLMM-P algorithm. BRLMM-P is a batch genotyping method wherein prior information on genotype cluster positions is combined with information from supplied data to determine posterior cluster positions and probabilities for making final genotype call assignments. The copy number calls were made by the small fixed regions (SAFER) algorithm. The SAFER algorithm is a single-sample analysis method where the range of $\log_2$ signal ratios associated with each copy number state is predefined. Release 6 analysis library packages were used for both PharmacoScan plate formats. Default analysis settings were used. Samples were grouped into multiple analysis batches by the same plate format, sample type, and extraction method. Kit control samples from relevant plates were included in each analysis batch.

Table 2. DNA QC summary for samples extracted with the MagMAX DNA Multi-Sample Ultra 2.0 Kit and Gentra Puregene kit.

Sample type (N = 109)	Collection kit	Starting amount of collected samples	A_{260}/A_{280}*	A_{260}/A_{230}*	Concentration (Qubit method) (ng/µL)**	Yield (Qubit method) (µg)**	Yield range (µg)
Gentra Puregene kit							
Blood	BD EDTA tube	4 mL	1.9	2.6	110.0	32.3	3.0–99.5
Saliva	Oragene Discover collection kit	2 mL of stabilized saliva	1.8	1.2	183.0	13.7	0.1–90.0
Buccal swab	4N6FLOQSwabs buccal swabs	1 swab	1.8	2.6	39.6	1.4	0.1–4.2
MagMAX DNA Multi-Sample Ultra 2.0 Kit							
Blood	BD EDTA tube	400 µL	1.9	2.3	25.6	2.6	0.8–12.2
Saliva	Oragene Discover collection kit	450 µL of stabilized saliva	1.9	1.8	32.6	2.6	0.1–14.1
Buccal swab	4N6FLOQSwabs buccal swabs	1 swab	1.9	2.4	18.2	0.8	0.1–3.5

* Mean

** Median

Results

Oral sample collection kits simplified sample collection and provided adequate DNA yield of high molecular weight gDNA, as shown in Table 2. Overall, gDNA extracted from saliva and buccal cells showed good DNA integrity, comparable with that of whole blood, as shown in a representative agarose gel in Figure 1. Some saliva and buccal samples showed a faint smear on the gel that may indicate a minor percentage of degraded DNA.

The gDNA extracted using two different methods had A_{260}/A_{280} and A_{260}/A_{230} ratios that met the guidelines recommended in the user guides (A_{260}/A_{280} = 1.8–2.0, A_{260}/A_{230} >1.5) (Table 2). The exception was the A_{260}/A_{230} ratio for the DNA extracted from saliva with the Gentra Puregene kit (Table 2). However, the assay concordance for the Gentra Puregene kit–extracted saliva samples was similar to that observed for the MagMAX 2.0 kit–extracted saliva samples (Table 3).

The majority of samples, based on dsDNA measurements, had concentrations above 5 ng/µL, along with highly comparable DNA integrity (Table 2). Donor variability in DNA yield was observed across the three sample types. Nevertheless, DNA yields from the two different kits were equivalent when considering the starting volume of blood and saliva samples (Table 2).

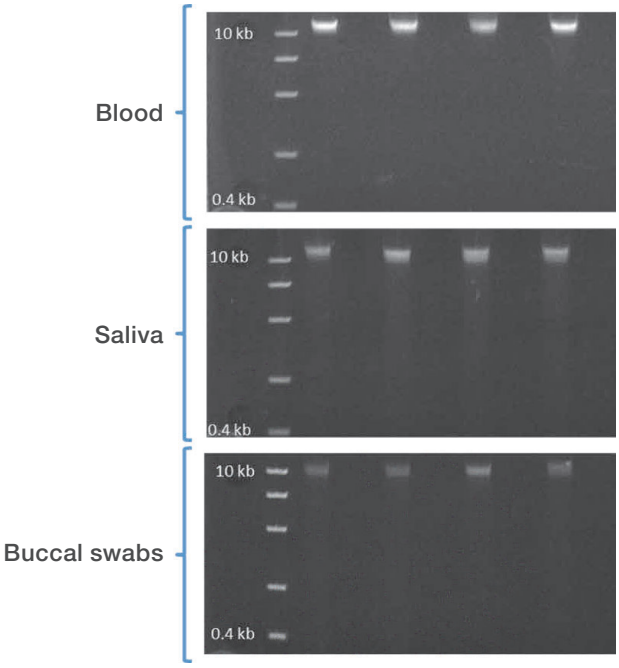


Figure 1. DNA samples derived from blood, saliva, and buccal swabs were analyzed for integrity on Invitrogen™ E-Gel™ 1% agarose gels.

Table 3. Genotype concordance of buccal and saliva sample data to whole blood data. Overall concordance considers all genotype calls of the 4,330 ADME markers among the markers genotyped by default in the PharmacoScan release 6 library package. Heterozygous concordance is the concordance to the subset of ADME marker data called heterozygous in whole blood.

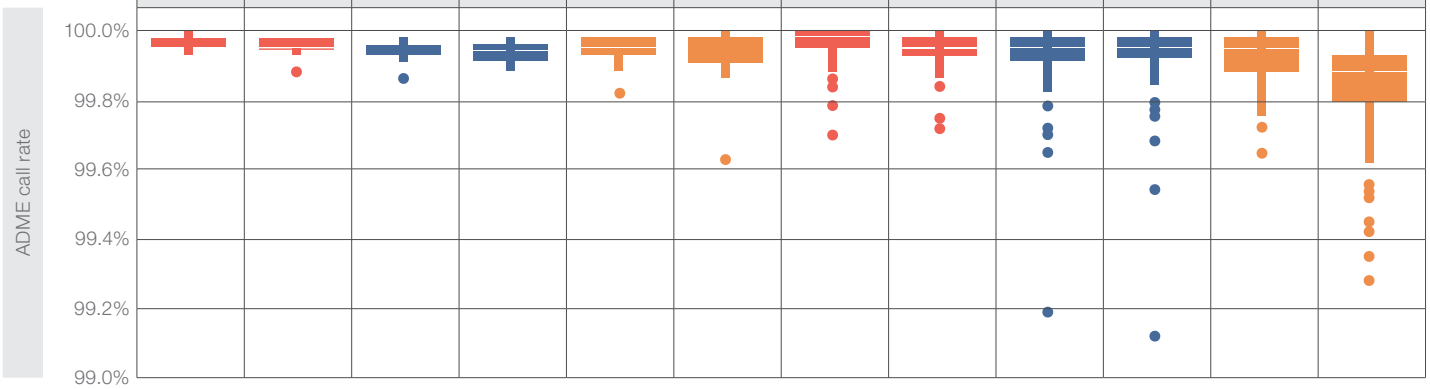
Plate format	Sample type	Extraction method	Sample count	Overall concordance (%)	Heterozygous concordance (%)
24-well	Buccal	MagMAX kit	12	99.92	99.92
24-well	Buccal	Puregene kit	12	99.95	99.99
24-well	Saliva	MagMAX kit	11	99.90	99.98
24-well	Saliva	Puregene kit	12	99.78	99.94
96-well	Buccal	MagMAX kit	104	99.95	99.97
96-well	Buccal	Puregene kit	102	99.96	99.98
96-well	Saliva	MagMAX kit	103	99.96	99.97
96-well	Saliva	Puregene kit	103	99.84	99.89

Multiple sample types can be assayed on the same PharmacoScan plate. However, for batch genotype calling, samples should be grouped by known factors, like sample type and extraction method, when there are at least 20 samples that belong to the same group. Grouping by known factors will minimize batch effects that may impact genotyping accuracy. In this study, samples were grouped for genotype calling by sample type and extraction method. Each analysis group also includes DNA control samples; however, results from the controls are not reported here.

Sample pass rates were high in this study for all tested sample types and extraction methods. Of the 105 unique samples tested in the PharmacoScan 96-array format, at least 104 samples passed sample QC checks among each of the three sample types and two extraction methods. Among the 12 unique samples tested in the PharmacoScan 24-format array across the same combination of sample types and extraction methods, one sample failed minimum sample QC criteria in one of the conditions (data not shown).

As shown in Figure 2, the median ADME genotype call rates for all tested sample types and extraction methods exceeded 99.9%. The ADME call rate is computed for the 4,330 markers genotyped by default that are annotated in the PharmacoScan release 6 library package as being associated with drug adsorption, distribution, metabolism, and excretion. Buccal and saliva samples showed somewhat more variability in sample call rates than did whole blood samples, which may be partly attributable to buccal and saliva sample quality being a function of the inherent oral microbial load as well as the collection technique.

As shown in Table 3, the concordance of ADME genotype calls of buccal and saliva samples was also high when compared to genotype calls from whole blood samples. Buccal samples reported at least 99.9% overall concordance to blood. Buccal sample calls were also at least 99.9% concordant to the heterozygous variant calls reported on blood samples. Saliva samples in this study performed almost the same as buccal samples. While saliva samples with MagMAX extraction also had at least 99.9% overall and heterozygous concordance to blood samples, saliva samples with Gentra Puregene extraction showed a slightly lower concordance—around 99.8%.



	PharmacoScan Assay Kit, 24-Format						PharmacoScan Assay Kit, 96-Format					
	Whole blood		Buccal		Saliva		Whole blood		Buccal		Saliva	
	MagMAX kit	Puregene kit	MagMAX kit	Puregene kit	MagMAX kit	Puregene kit	MagMAX kit	Puregene kit	MagMAX kit	Puregene kit	MagMAX kit	Puregene kit
Count	16	16	12	12	15	16	105	104	104	103	107	108
Median	99.98%	99.95%	99.94%	99.94%	99.95%	99.94%	99.98%	99.95%	99.95%	99.95%	99.95%	99.88%
Avg	99.97%	99.95%	99.94%	99.94%	99.94%	99.92%	99.96%	99.94%	99.89%	99.90%	99.90%	99.82%
Min	99.93%	99.88%	99.86%	99.88%	99.82%	99.63%	99.70%	98.48%	97.07%	97.97%	98.52%	98.45%

Figure 2. Sample call rate for ADME markers by plate format, sample type, and extraction method.

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The PharmacoScan assay also reports the total copy number state in nine regions spanning five genes: *CYP2A6*, *CYP2D6*, *GSTM1*, *GSTT1*, and *UGT2B17*. All tested conditions for a sample were used to determine the consensus copy number state. The most common reported copy number state for each sample's region was used as the consensus call. Ties were resolved to the larger copy number state as consensus. High copy number concordance was observed for all tested sample types and extraction methods (Table 4). The small number of copy number discordances were most often called as 2 where the consensus copy number was 3. The number of comparisons varies across conditions within plate format because of occasional sample QC failure and because some of the conditions included technical replicates.

Table 4. Copy number concordance by plate format, sample type, and extraction method.

Plate format	Sample type	Extraction method	Number of comparisons	Copy number concordance (%)
24-well	Buccal	Puregene kit	99	100.0
24-well	Buccal	MagMAX kit	99	100.0
24-well	Saliva	Puregene kit	135	100.0
24-well	Saliva	MagMAX kit	135	98.5
24-well	Whole blood	Puregene kit	135	100.0
24-well	Whole blood	MagMAX kit	135	100.0
96-well	Buccal	Puregene kit	918	99.9
96-well	Buccal	MagMAX kit	918	99.8
96-well	Saliva	Puregene kit	954	99.8
96-well	Saliva	MagMAX kit	954	99.6
96-well	Whole blood	Puregene kit	954	99.7
96-well	Whole blood	MagMAX kit	963	98.7

Conclusion

The PharmacoScan assay performs well in genotyping and copy number analysis with DNA from both buccal cells and saliva. Genotyping call rates and concordance were high for all samples tested, in spite of different sample types and different extraction methods, on both 96-format and 24-format array plates. The PharmacoScan assay also showed compatibility with DNA samples prepared by both precipitation-based and magnetic bead–based DNA extraction methods.

We have successfully developed a robust, high-throughput workflow that enables processing of clinical research sample types—whole blood, buccal swabs, and saliva—to genotype them for DNA biomarkers of pharmacogenomic interest, using Applied Biosystems™ Axiom™ chemistry for microarray detection.

References

1. Manufacturer's instructions: EDTA plasma. Cat. No. 367863: 13 x 100 mm x 6.0 mL BD Vacutainer™ Plus plastic whole blood tube, lavender Hemogard™ closure.
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3. How to guide—How to collect buccal cells. Available at **copanusa.com**. Cat. No. 4520CS01 available at **copanusa.com**; Cat. No. 4473979 available at **thermofisher.com**.
4. Gentra Puregene Handbook (2014, Pub. No. 1090287). Available at **qiagen.com**.
5. MagMAX™ DNA Multi-Sample Ultra 2.0 User Guide (2018, Pub. No. MAN0017324, saliva, Rev. C.0; 2017, Pub. No. MAN0017205, buccal swabs, Rev. B.0; 2017, Pub. No. MAN0017325, whole blood, Rev. B.0). Available at **thermofisher.com**.
6. PharmacoScan™ Assay 96-Array Format Automated Workflow User Guide for Beckman™ Biomek FX^P (Windows™ 7) Rev. 3 (2018, Pub. No. 703472). Available at **thermofisher.com**.
7. PharmacoScan™ Assay 24-Array Format Manual Workflow User Guide Rev. 4 (2018, Pub. No. 703286). Available at **thermofisher.com**.

All donor samples used in this study were procured For Research Use Only.

Ordering information

Product	Cat. No.
PharmacoScan Assay Kit, 96-Format	903026
PharmacoScan Assay Kit, 24-Format	903010TS
PharmacoScan Training Kit, 96-Format	913027
PharmacoScan Training Kit, 24-Format	903011TS
KingFisher Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630

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