Complete High Throughput Pharmacogenomics Workflow: From Sample to Results

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Introduction

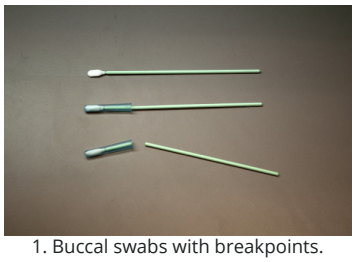
Rapid, high throughput, cost-effective and accurate screening of human DNA for the identification of single nucleotide polymorphisms (SNPs) is a useful tool for the development of therapeutics and diagnostics. Here, we demonstrate these metrics with an automated pharmacogenomics workflow consisting of a non-invasive buccal swab collection system and DNA extraction chemistry (Omega Bio-tek) on 2 open automated liquid handling platforms (Hamilton's Microlab® STAR™ and Qiagen's BioSprint® 96), followed by specific SNP identification via a high throughput genotyping instrument (Douglas Scientific). For demonstration purposes, this system was used to determine the SNP status of warfarin anticoagulant sensitivity mutations within the human VKORC1 and CYP2C9 genes (Ref 1). Genomic controls (Introl HD Multiplex WT, HET, and MUT) were provided by Maine Molecular Quality Controls, Inc.

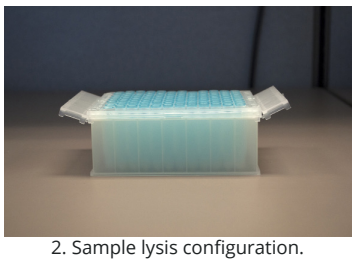
Table 1. Extracted DNA quantification. Values are the average of duplicate extractions.

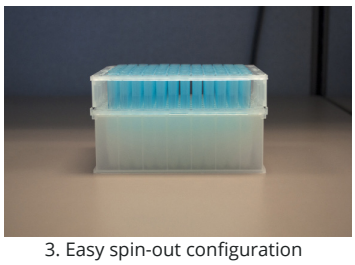
Sample	Qiagen BioSprint® 96		Hamilton Microlab® STAR™	
	Conc. (ng/µL)	Total Yield (ng)	Conc. (ng/µL)	Total Yield (ng)
1	6.6	658	15.6	1559
2	17.0	1700	18.1	1808
3	7.0	697	9.8	982
4	8.2	825	17.4	1744
5	6.8	680	21.7	2166
6	7.9	791	5.5	548
7	13.6	1360	64.9	6487
8	7.0	702	13.2	1317
9	9.9	986	8.0	797
10	9.6	959	7.3	733
11	5.0	502	16.3	1634
12	6.5	646	30.0	3003
13	13.9	4388	33.7	3367
14	25.7	2575	26.1	2611
15	10.3	1031	15.3	1530
16	8.7	869	4.0	404
17	17.1	1711	9.9	987
Average	**10.6**	**1063**	**20.8**	**2079**

Workflow & Methods

Omega Bio-tek's E-Z 96® Spin-Out buccal swab collection device allows for the rapid arraying of sample swabs (1) into 96-well plates for lysis configuration (2), followed by the raised configuration (3) for easy eluate epin-out and start of automated workflow.


1. Buccal swabs with breakpoints.


2. Sample lysis configuration.


3. Easy spin-out configuration

Omega Bio-tek's Mag-BIND® Blood & Tissue DNA HDQ 96 Kit is designed for the rapid and reliable isolation of quality genomic DNA from swabs and other sample types. Mag-BIND® Particles HDQ provide quick magnetic response time. Utilizing paramagnetic particles allows use on multiple open-ended liquid handling platforms and provides high quality DNA that is suitable for direct use in most downstream applications.

The IntelliQube® by Douglas Scientific combines liquid handling with real-time quantitative PCR (qPCR) analysis in miniaturized reaction volumes. The system utilizes Array Tape® in a unique and innovative 768-well format. Array Tape® is a thin and flexible polypropylene consumable that, in combination with miniaturized reaction volumes (1.6 µL) enables both outstanding PCR performance and profound reagent savings.

Results

The purified DNA from 17 replicate samples was quantified for DNA concentration and total DNA yields via PicoGreen® (Figure 1). DNA from these samples was then analyzed via BHQplus® probe-based qPCR for wild-type, mutant, or heterozygous status for warfarin sensitivity at 3 known SNPs (Figures 2 & 3).

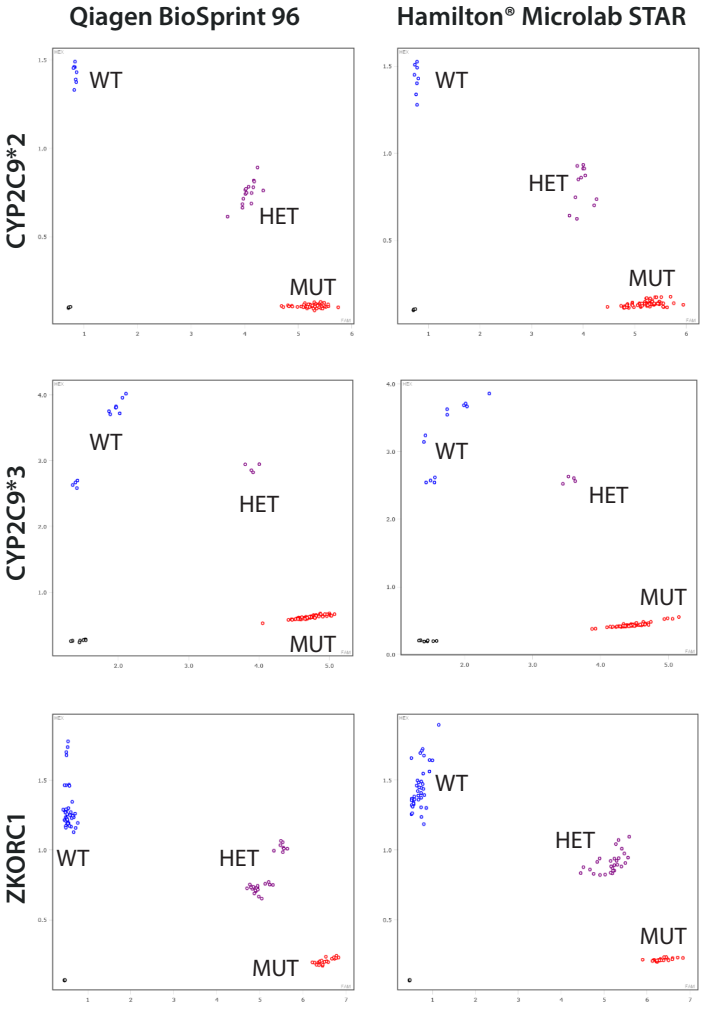


Figure 2. Genotype scatter plots from the IntelliQube®. WT = wild-type, MUT = mutant, HET = heterozygous.

Sample	CYPC2C9*2		CYP2C9*3		VKORC1	
	BS 96	STAR	BS 96	STAR	BS 96	STAR
1	WT	WT	WT	WT	HET	HET
2	WT	WT	WT	WT	HET	HET
3	WT	WT	WT	WT	MUT	MUT
4	WT	WT	WT	WT	WT	WT
5	WT	WT	WT	WT	WT	WT
6	WT	WT	WT	WT	HET	HET
7	WT	WT	WT	WT	WT	WT
8	HET	HET	WT	WT	WT	WT
9	WT	WT	WT	WT	HET	HET
10	WT	WT	WT	WT	WT	WT
11	WT	WT	WT	WT	WT	WT
12	WT	WT	WT	WT	HET	HET
13	WT	WT	WT	WT	WT	WT
14	WT	WT	WT	WT	MUT	MUT
15	WT	WT	WT	WT	MUT	MUT
16	WT	WT	WT	WT	WT	WT
17	WT	WT	WT	WT	MUT	MUT
HET*	HET	HET	HET	HET	HET	HET
MUT*	MUT	MUT	MUT	MUT	MUT	MUT
WT*	WT	WT	WT	WT	WT	WT

Figure 3. Warfarin sensitivity SNP determination for all samples.

Workflow Reagents and Consumables	Cost per Sample
Omega Bio-tek E-Z 96® Spin-Out Collection System	$0.24
Omega Bio-tek Mag-Bind® Blood & Tissue DNA HDQ 96 Kit	$1.85
Disposables (Hamilton tips, etc.)	$0.42
Quanta Biosciences Accustart Genotyping Toughmix	$0.05
Biosearch BHQplus® SNP Assay (Primers and probes)	$0.01
Control DNA	$0.24
Total cost per sample:	**$2.81**

Conclusions

These sample collection, extraction and analysis technologies offer a rapid, high throughput, low cost, and accurate pharmacogenomics workflow solution for human DNA genotyping.

The Douglas Scientific IntelliQube®, Omega Bio-tek's Mag-BIND® Blood & Tissue DNA HDQ 96 Kit and Maine Molecular Quality Controls, Inc.'s products are for research only.

References

1. Takeuchi, F., *et al.* (2009). PLoS Genet 5: e1000433. doi: 10.1371.

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