Development and analytical verification of a Pharmacogenomics Research Ion AmpliSeq sequencing assay covering 139 variants and CYP2D6 CNV

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ABSTRACT

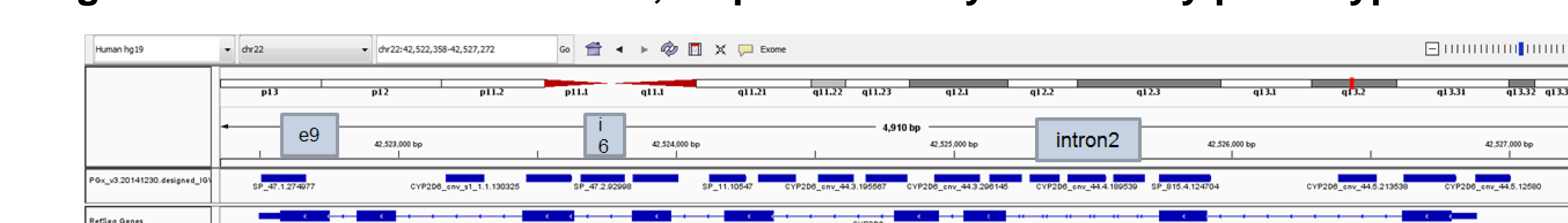
Cytochrome P450 enzymes metabolize about 75% of drugs, including oncology drugs, with UGT enzymes metabolizing about another 15%. Variations in gene sequence or in copy number may result in an inactive, defective, unstable, mis-spliced, low expressed, or absent enzyme, an increase in enzyme activity, or an altered affinity for substrates. Pharmacogenomics genes can predict whether an individual is a poor or rapid metabolizer, facilitating dose optimization. Failure to adjust dosage of drugs metabolized by the relevant enzyme can lead to adverse drug reaction, or conversely to too rapid drug metabolism and no drug response.

Here, we present a pharmacogenomics (PGx) Research panel to detect 139 SNV/Indel targets in 42 genes (Figure 1) and CYP2D6 copy number variation (CNV, Figure 2). This panel covers the commonly known targets in genes encoding drug metabolism enzymes and associated transport proteins. The panel design is particularly challenging due to high levels of sequence homology between the cytochrome P450 genes. This assay uses Ion AmpliSeq™ technology and contains 146 amplicons in an ultrahigh-multiplex PCR in a single pool, followed by Ion Torrent™ semiconductor sequencing. The assay requires as little as 10 ng of input DNA. This customizable panel allows target addition or removal, and will be the first NGS PGx Research panel on the market.

Figure 1. List of 42 genes covered in the Pharmacogenomics Ion AmpliSeq panel

ABCB1	CYP1A2	CYP3A4	F2	HTR2C	OPRM1	UGT2B15
ABCG2	CYP2B6	CYP3A5	F5	ITGB3	SLC6A3	UGT2B7
ADRA2A	CYP2C19	DBH	GABRA6	KIF6	SLC6A4	VKORC1
ANKK1	CYP2C8	DPYD	GABRP	MTHFR	SLCO1B1	HLA-A*3101
APOE	CYP2C9	DRD1	GRIK4	OPRD1	TPMT	HLA-B*5701
COMT	CYP2D6	DRD4	HTR2A	OPRK1	UGT1A1	HLA-B*1502

Figure 2. Common CYP2D6 CNV, TaqMan™ assays and likely phenotypes



	Exon 9	Intron 6	Intron 2	Likely phenotype
Taqman Assay	Hs00010001	Hs04502391	Hs04083572	-
CNV Gain (CN=3)	3	3	3	Ultrarapid metabolizer
CNV Normal (CN=2)	2	2	2	Extensive metabolizer
CNV Loss (CN=1)	1	1	1	Intermediate metabolizer
Exon 9 conversion (*36)	2	3	3	Intermediate metabolizer
NULL (CN=0)	0	0	0	Poor metabolizer

CYP2D6 CNV: gene-level (CN=0~3) and *36 at exon-level (Eon 9: CN=2). Predicted metabolizer phenotype is used to determine drug selection and dosages.

E.g., with respect to the metabolism of codeine to morphine by CYP2D6:

- **Ultrarapid metabolizers** avoid codeine use due to potentially toxic morphine levels.
- **Poor metabolizers** avoid codeine use due to lack of efficacy.
- **Extensive & Intermediate metabolizers** use age- & weight-specific dosing
  - Intermediate metabolizers may not respond as well as extensive metabolizers

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MATERIALS AND METHODS

Coriell cell lines and buccal swab samples: 91 well characterized Coriell cell lines and archived buccal swab samples from various sources were used to establish the analytical verification of the panel. The genotypes and copy numbers detected by the panel were compared to the gold standard TaqMan Assays.

Sequencing: Libraries were generated with the Ion AmpliSeq™ protocol and the Ion AmpliSeq™ Library Kit 2.0 according to the product manual. Libraries were quantified by qPCR and templating was performed on the Ion OneTouch™ 2 instrument. Enriched Ion Sphere™ Particles were loaded onto a Ion 318™ Chip v2 and sequenced using the Ion PGM™ Sequencing 200 Kit v2.

Genotyping and Copy Number Analysis: Signal processing, base-calling and alignment were performed with the Ion Torrent Suite™ Software. An Ion Pharmacogenomics Analysis Plugin is implemented and consisted of three modules: 1) a genotyping module, parsing results of Torrent Suite variant caller plugin with specific variant hotspots and optimized json parameters; 2) a copy number analysis module, calculating normalized coverage of amplicons and performing Clustering with Gaussian Mixture Model to infer copy number status (proprietary method, see RESULTS section for illustration), and 3) an export module, providing outputs with various format (vcf, Open Array, PCR assay) downstream analysis.

Annotation: Variant interpretation and haplotype calling will be handled by downstream software, either by AlleleTyper™, Translational Software, or customer in-house solution.

RESULTS

Genotyping Research analysis: 91 well characterized Coriell samples and 5 buccal swab samples were first used to access the genotyping performance on the PGx Research panel (Figure 3). Concordance with TaqMan was > 99.9%, reproducibility was > 99.7%, and the no-call rate was < 0.5.

Figure 3. Genotyping performance on Coriell cell lines and swab samples

Coriell samples (Run1-3: 96-plex, Run4 and Run5: 48-plex)

Index	Samples /Targets	Mean Depth	NOCALL Per Target	Targets with OA validation	Concordance with validation	TPs	FNs	NOCALLs OA Target
Run1	91 / 139	418.97	0.3%	121	99.97%	10666	3	10(0.09%)
Run2	91 / 139	344.04	0.29%	121	99.98%	10666	2	11(0.1%)
Run3	91 / 139	204.18	0.427%	121	99.98%	10644	2	33(0.3%)
Run4	26 / 139	899.6	0.388%	121	99.97%	3017	1	10(0.32%)
Run5	26 / 139	609.6	0..0554%	121	99.97%	3025	1	2(0.06%)

5 swab samples with CYP2D6 CN=0 (in 96-plex)

Index	Samples /Targets	Mean Depth	NOCALL Per Target	Targets with OA validation	Concordance with validation	TPs	FNs	NOCALLs OA Target
Run1	5 / 139	320.26	12.7%	34	100%	169	0	1(0.6%)
Run2	5 / 139	266.16	12.3%	34	100%	170	0	0(0%)
Run3	5 / 139	154.08	13.1%	34	100%	169	0	1(0.6%)

- Two False Negatives are consistent across Coriell runs due to rare SNPs on the design primer. Refinement of primer design and Sanger verification are in progress.
- High no-call rate on swab samples is due to deletion of CYP2D6 gene.

Copy Number Analysis: A mixture of Coriell samples and swab samples was first used to verify the Clustering algorithm (Figure 4). With the assumption that a majority of samples have CN=2 in a run, the priors of CN=1,2,3 can be established to handle potential missing clusters. Expectation–maximization algorithm is used for model fitting (Exon-level: one-dimensional Gaussian; Gene-level: mixtures of Gaussian), and posteriors are used to infer copy number status. CYP2D6 null samples can be also easily Identified by a threshold value on the normalized coverage (Figure 5). Figure 6 demonstrated that the CNV analysis is robust on several cell line and swab datasets.

Figure 4. Copy number analysis on Coriell and swab samples

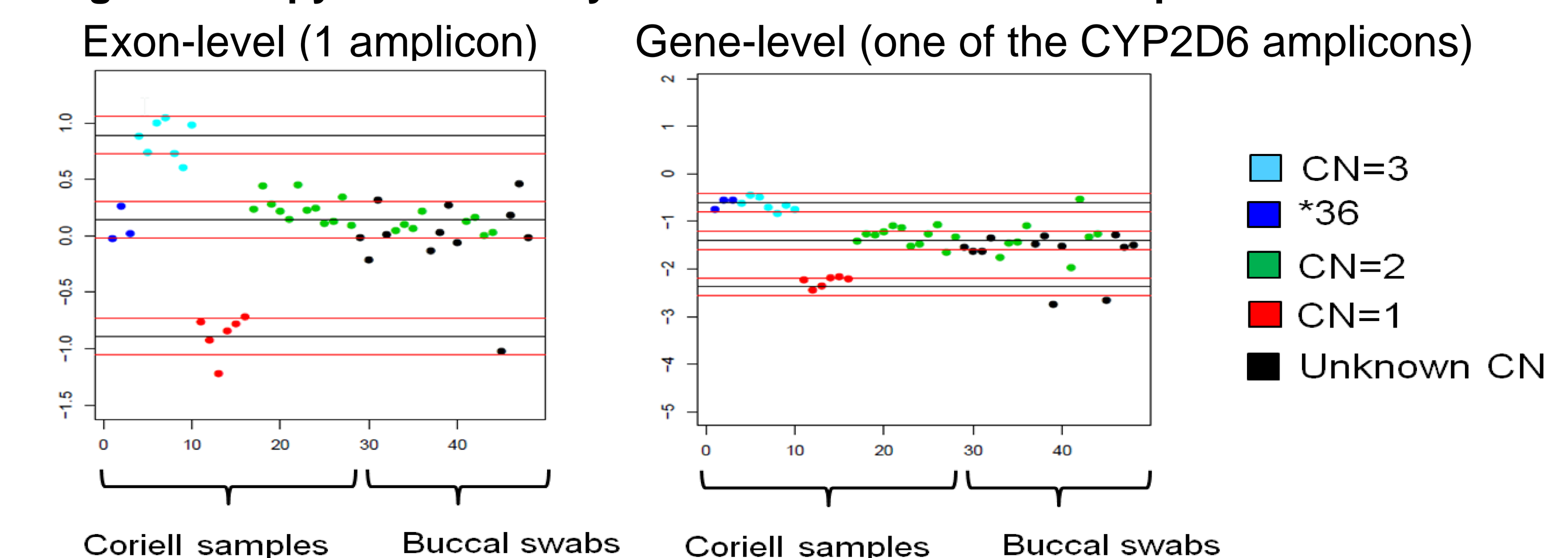

Figure 5. Copy number analysis on CN null samples

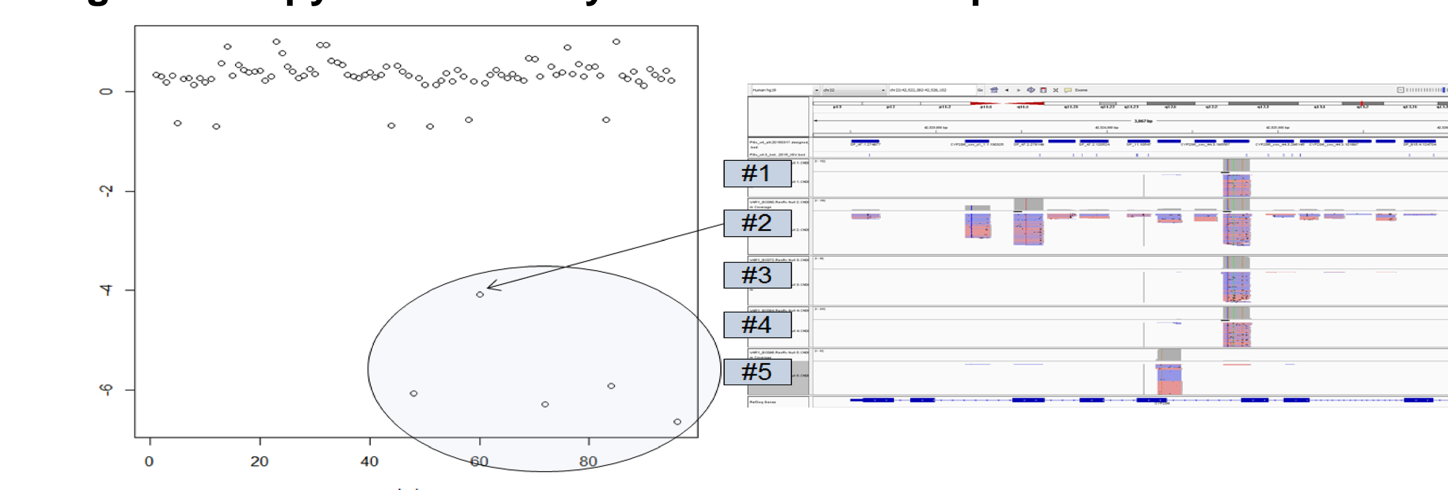

Figure 6. Copy number performance on various datasets

96-plex Coriell	Mean Depth	CN=2	CN=1	CN=3	*36	NOCALL	Concordance
Run1	413.8	75 [70/70]	6 [6/6]	7 [7/7]	3 [3/3]	5.5%(5/91)	100%(86/86)
Run2	339.9	75 [65/65]	6 [6/6]	7 [7/7]	3 [3/3]	11%(10/96)	100%(81/81)
Run3	175.7	75 [72/72]	6 [6/6]	7 [6/6]	3 [3/3]	4.4%(4/91)	100%(87/87)

26 Coriell + 22 swabs	Mean Depth	CN=2	CN=1	CN=3	*36	NOCALL	Concordance
Run4	812.7	19 [17/17]	6 [6/6]	7 [6/6]	4 [4/4]	8.3%(4/48)	100%(33/33)
Run5	895.2	19 [16/16]	6 [6/6]	7 [5/5]	4 [4/4]	17%(8/48)	100%(31/31)

48-plex swabs	Mean Depth	CN=2	CN=1	CN=3	*36	NOCALL	Concordance
Run6	812.7	35 [34/34]	5 [5/5]	4 [3/3]	4 [4/4]	4.2%(2/48)	100%(46/46)
Run7	895.2	35 [32/32]	5 [5/5]	4 [4/4]	4 [4/4]	6.2%(3/48)	100%(45/45)

CONCLUSIONS

The research panel measures PGx gene genotypes and copy numbers with high accuracy. These results demonstrate an assay which can be used to explore potential pharmacogenomic relationships, including the relationship between copy number and genotype of metabolism enzyme targets that may be used to assess drug tolerability and clinical outcomes in the future.

REFERENCES

For more information, please visit www.lifetechnologies.com.
For more information on Ion AmpliSeq™ technology, please visit ampliseq.com.