Enhancing a Blended Genome-Exome Assay for Integrated Pharmacogenomic Analysis

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Background

The blended genome-exome (BGE) assay

One PCR-free library is split: an aliquot is exome-captured, recombined with the rest and sequenced together. A single run yields a 1-2x low-pass genome and a clinical-grade exome at about 90x. The genome supports polygenic risk scores by imputation; the exome supports monogenic diagnosis. BGE is a lower-cost alternative to 30x WGS, built to match WGS across the exome.

Broad Clinical Labs (BCL) already reports clinically validated PGx from WGS: 24 genes, 100% allele concordance with GeT-RM (ref. 6; poster 2032F). Our goal was the same PGx results from BGE.

The pharmacogenomics gap

Two gaps kept comprehensive PGx off standard BGE:

1. **Variants outside the exome.** Six actionable defining variants sit in promoters and deep introns (CYP2C19*17, VKORC1 -1639G>A, UGT1A1*28/*80, DPYD HapB3, CFTR 3849+10kbC>T). Low-pass coverage cannot genotype them.
2. **Structural variation in homologous genes.** Deletions, duplications and hybrids in CYP2D6 and CYP2B6 need even, deep coverage across gene and pseudogene, which exome baits do not give.

Table 1. The 24 pharmacogenes. Same gene set and output files as BCL's clinical WGS PGx deliverable.

Star allele caller (small variants)						Targeted caller (CNV/SV)
ABCG2	BCHE	CACNA1S	CFTR	CYP2C9	CYP2C19	CYP2D6
CYP3A4	CYP3A5	CYP4F2	DPYD	F5	G6PD	CYP2B6
IFNL3	MT-RNR1	NAT2	NUDT15	RYR1	SLCO1B1	
TPMT	UGT1A1	UGT2B17	VKORC1			

Methods

1. Enhanced assay

- Twist Alliance Clinical Research Exome plus PGx content (design PGx_3), manufactured into one bait for research and clinical BGE; no change to the lab workflow.
- Whole-gene tiling of CYP2D6/CYP2D7 and CYP2B6/CYP2B7, plus six single-site probes: about 77 kb, 0.2% of the exome target.
- Standard cBGE library preparation, sequencing (NovaSeq X Plus) and QC specifications.

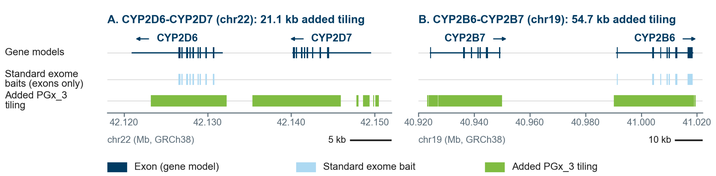


Figure 1. Enhanced bait design. Added tiling across CYP2D6-CYP2D7 and CYP2B6-CYP2B7, compared with standard exome baits (exons only).

2. Calling

- 22 genes: DRAGEN v4.3.6 star allele caller, the same version as the cWGS comparator, run without a target BED.
- CYP2D6 and CYP2B6: BGE-specific DRAGEN targeted callers developed with Illumina, planned for a future DRAGEN release and run here on a pre-release build. Copy number is normalized against an in-run panel of normals; the 32 samples of one NovaSeq X run served as their own panel.

3. Evaluation

- 32 enhanced BGE libraries from 26 reference cell lines (6 in duplicate), each with matched 30x WGS from BCL's validated cWGS PGx pipeline.
- Where WGS and the GeT-RM consensus disagreed, the verified WGS call was used as truth.
- Pre-specified target: >98% diplotype concordance with WGS.
- Star alleles informed by copy number changes in genes other than CYP2D6 and CYP2B6 were not assessed in this initial analysis.
- Planned clinical validation: 25 Coriell controls and 29 clinical samples across five specimen types, compared with cWGS.

Results

CYP2D6 vs 30x WGS (32 libraries, 26 individuals)

- 32/32 diplotypes concordant, with no no-calls (Figure 2).
- In 6 libraries where WGS and GeT-RM disagreed, BGE matched the verified WGS call every time.
- Read-depth copy number matched WGS for the deletion, the duplication and the hybrid (Figure 3). Median CYP2D6 depth was 256x on BGE and 43x on WGS.

NA18873 *17/*5	HG00524 *10+*36/*2	NA16688 *10+*36/*2	NA16688 *10+*36/*2	NA21781 *2x2/*4+*68	NA21781 *2x2/*4+*68	NA23246 *10x2/*10+*36	NA23246 *10x2/*10+*36
NA12878 *3/*4+*68	NA24008 *1/*4+*68	NA24008 *1/*4+*68	HG00436 *2x2/*71	HG00436 *2x2/*71	NA19207 *10/*2x2	NA19207 *10/*2x2	NA20362 *2/*4x2
NA20901 *1x2/*7	HG01083 *1/*2	HG02772 *1/*2	HG02861 *1/*4	HG03716 *1/*1	NA06991 *1/*4	NA12248 *1/*35	NA12753 *2/*3
NA17129 *1/*4	NA17254 *4/*41	NA17296 *1/*9	NA17438 *2/*4	NA18519 *106/*29	NA19213 *1/*1	NA20289 *11/*6	NA20296 *1/*2

Whole-gene deletion (*5) **1** CYP2D6/CYP2D7 hybrid **10** Duplication (non-hybrid) **6** Small variants only **15**

Same individual, two libraries *16/16 duplications includes the 10 tandem hybrids; all 32 concordant with 30x WGS*

Figure 2. CYP2D6 diplotypes called on enhanced BGE, colored by structural class.

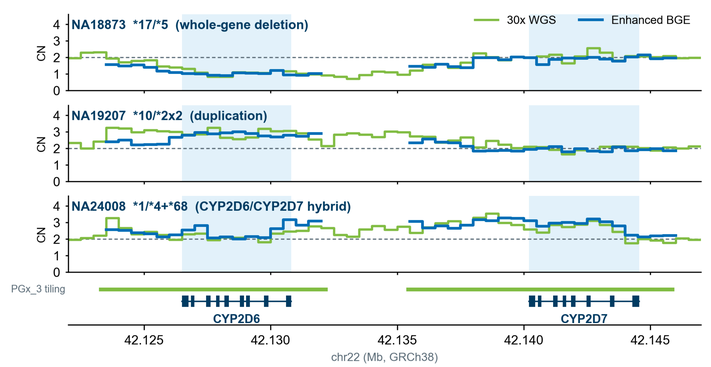


Figure 3. Copy number from read depth, enhanced BGE vs 30x WGS. Three samples with CYP2D6 structural variants. BGE (blue) is normalized to 15 copy-neutral libraries in the run; WGS (green) to its own flanking coverage. Computed by BCL from the CRAMs, independently of the caller.

Other 22 pharmacogenes vs 30x WGS (31 libraries)

- 642/642 assessable diplotypes concordant across 21 genes (100%); duplicate libraries were identical.

Coverage at PGx target sites

- All six added sites reached 20x in every enhanced library and in no standard library (Figure 4).
- CYP2B6 gene-body depth rose from 14x to 200x; in-exome sites were covered on both.

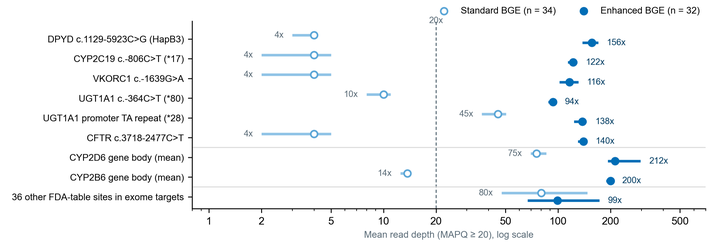


Figure 4. Depth at PGx target sites, standard (n = 34) vs enhanced BGE (n = 32). Medians and interquartile ranges. The enhanced run was sequenced somewhat deeper (exome mean 130x vs 104x).

Conclusions

- Enhanced BGE delivers comprehensive PGx across 24 genes from one exome-centric assay, concordant with 30x WGS.
- CYP2D6: 32/32, including deletions, duplications and hybrids. CYP2B6: 26/26 where truth existed.
- Other pharmacogenes: all 642 assessable calls across 21 genes concordant with 30x WGS.
- The PGx content adds about 0.2% to the exome target with no wet-lab change: one library, one run, three analyses.
- The PGx output is a technical deliverable; interpretation is out of scope.

Limitations

- Calls are limited to alleles in the DRAGEN definition set, and caveats from the clinical WGS validation apply, including DPYD haplotype matching in place of the CPIC activity score.
- Some CYP2B6 diplotypes cannot be phased from short reads, and no CYP2B6/CYP2B7 hybrid carriers were available to test hybrid detection.
- One SLCO1B1 call (NA17254) was excluded because the unvalidated DRAGEN 4.3.6 BGE pipeline filtered its defining variant, which passes in the validated DRAGEN 4.2.7 calls.
- Male G6PD calls were sometimes reported as two copies of the same allele; the allele always matched WGS.
- Results come from reference cell lines from 26 individuals; clinical specimen types will be covered in the planned validation.

Next steps

- Complete clinical validation (25 controls, 29 clinical samples) and release updated PGx probe mix for cBGE.
- Move to the DRAGEN release with the BGE targeted callers, and re-validate.
- Evaluate CNV-informed star alleles in genes other than CYP2D6 and CYP2B6; investigate phase-ambiguous CYP2B6 calls.

Acknowledgments

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