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Summary

Pharmacogenomics (PGx) evaluates genomic variation in drug-metabolizing genes to guide safer and more effective medication use. Genetic variants, commonly represented as *star alleles*, influence drug metabolism and are used to classify patients into metabolizer phenotypes (e.g., poor, intermediate, rapid), which can impact drug efficacy and risk of adverse events. The Illumina DRAGEN Star Allele Caller and Targeted Caller are used to generate PGx star allele data from clinical whole genome sequencing (cWGS).

This white paper presents the validation of the DRAGEN PGx Star Allele Caller and Targeted Caller (*CYP2D6* and *CYP2B6*) using cWGS. Across multiple validation dimensions—including accuracy, precision, repeatability, and limit of detection—the assay demonstrated high concordance (≥95%), robust reproducibility, and consistent performance across sample types.

These results support the use of DRAGEN-based PGx calling as a reliable component of cWGS-based pharmacogenomic workflows.

Background

PGx is rapidly becoming a core component of precision medicine, enabling more informed therapeutic decisions by linking genetic variation to drug response. As clinical adoption increases, there is a growing need for scalable, comprehensive, and accurate methods to generate PGx insights across diverse patient populations.

Star alleles represent haplotypes defined by specific combinations of genetic variants within pharmacogenes and are used to assign clinically relevant metabolizer phenotypes. These phenotypes directly influence drug selection, dosing, and risk of adverse events.

Historically, PGx testing has relied on targeted genotyping approaches such as SNP arrays. While effective for detecting common variants, these methods are limited in their ability to capture rare variants, copy number variations (CNVs), and structural variants (SVs). This limitation is particularly significant for complex pharmacogenes such as *CYP2D6* and *CYP2B6*, which exhibit high sequence homology with pseudogenes, gene duplications, deletions, and hybrid structures that complicate accurate genotype and phenotype determination.

The DRAGEN Star Allele Caller and Targeted Caller generate PGx genotypes and predicted metabolizer phenotypes directly from cWGS data. The Star Allele Caller evaluates 22 pharmacogenes included in FDA PGx recommendations or designated as CPIC Level A genes, including *CACNA1S, CFTR, CYP2C19, CYP2C9, CYP3A4, CYP3A5, CYP4F2, IFNL3, RYR1, NUDT15, SLCO1B1, TPMT, UGT1A1, VKORC1, DPYD, G6PD, MT-RNR1, BCHE, ABCG2, NAT2, F5,* and *UGT2B17*. Targeted Caller provides specialized analysis for *CYP2D6* and *CYP2B6*, enabling accurate characterization of these structurally complex loci.

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Genotype assignments are based on curated star allele definitions from resources such as PharmGKB. These diplotypes are translated into predicted metabolizer phenotypes using PharmCAT-based mappings, producing clinically relevant classifications such as poor, intermediate, and rapid metabolizers.

The DRAGEN PGx callers are primarily optimized for the hg38 reference genome and support the full set of pharmacogenes described above in this build. In addition, a subset of genes (*CACNA1S, CYP2C19, CYP2C9, CYP3A4, CYP3A5, CYP4F2, IFNL3, NUDT15, SLCO1B1, VKORC1, DPYD, ABCG2,* and *F5*) is supported on hg19/GRCh37 references.

This validation was designed to assess the analytical performance of the DRAGEN Star Allele Caller and Targeted Caller within a cWGS workflow, focusing on key performance characteristics including accuracy, precision, repeatability, and limit of detection across multiple sample types. Also, this validation pertains to Version 4.3.6 of DRAGEN, which is the current version that is validated for cWGS at BCL.

Validation Results Overview

Validation of the DRAGEN PGx Star Allele Caller and Targeted Caller demonstrated robust performance across multiple evaluation criteria, including accuracy, precision, repeatability, and limit of detection.

Across clinical samples, concordance between DRAGEN PGx calls and orthogonal array-based methods was high, with:

- **~99.2% agreement for PGx allele calls**
- **~99.3% agreement for phenotype assignments**

Precision studies showed:

- **100% concordance in intra-run testing**
- **100% concordance in inter-run testing**.

Limit of detection studies established that DNA inputs of **≥250 ng** consistently met performance criteria, while lower inputs (100 ng) did not reliably meet quality thresholds.

Overall, all validation metrics met or exceeded predefined acceptance criteria (≥95% concordance), supporting the analytical robustness of the DRAGEN PGx pipeline across multiple sample types and experimental conditions.

Output Files

List of output files of both the Star Allele Caller and the CYP2D6 and CYP2B6 Targeted Callers

Output Type	File Name Pattern	TDR schema name	Caller
JSON with targeted callers and PGx caller results	<prefix>.targeted.json	**pgx_targeted_json_path**	**PGx**

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TSV with gene/diplotype calls	<prefix>.star_allele.tsv	**pgx_star_path**	**PGx**
JSON with PGx results	<prefix>.star_allele.json	**pgx_star_json_path**	**PGx**
gVCF with PGx-relevant variants	<prefix>.select.gvcf	**pgx_gvcf_path**	**PGx**
TSV with metabolism status for CYP2D6	<prefix>.cyp2d6.tsv	**cyp2d6_path**	**CYP2D6**
TSV with metabolism status for CYP2B6	<prefix>.cyp2b6.tsv	**cyp2b6_path**	**CYP2B6**

FAQ

Which genes are included in the callers?

The DRAGEN Star Allele Caller evaluates 22 pharmacogenes (please see the background section for the full list) associated with drug metabolism and response. In addition, Targeted Caller provides specialized analysis for *CYP2D6* and *CYP2B6*, which require enhanced structural variant detection.

What types of genetic variation are detected?

The pipeline supports detection of:

- Single nucleotide variants (SNVs)
- Insertions and deletions (indels)
- Copy number variations (CNVs)
- Structural variants (SVs)

This enables accurate star allele assignment, including for complex genes that cannot be resolved using SNP-only approaches.

How accurate is the assay?

Analytical validation demonstrated:

- ≥95% overall concordance for both allele and phenotype calls
- ~99.2% allele agreement and ~99.3% phenotype agreement in clinical samples
- High concordance across all evaluated genes and sample types

How reproducible are the results?

The assay showed strong reproducibility:

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- 100% concordance in intra-run precision testing
- 100% concordance in inter-run precision testing
- Consistent results across reprocessed samples, with only minor, acceptable differences in quality scores

What sample types were validated?

Performance was validated using:

- Blood
- Saliva
- Buccal swabs

This supports flexibility in sample collection for clinical workflows.

Any material type accepted for cWGS can be used.

What is the recommended DNA input?

- ≥250 ng DNA input is recommended for reliable performance
- Inputs of 100 ng did not consistently meet quality thresholds and are below the validated limit of detection

How does WGS-based PGx compare to array-based testing?

WGS offers several advantages:

- Broader detection of rare and structural variants
- Improved resolution of complex genes (e.g., *CYP2D6*)
- Reduced dependence on predefined variant panels

Some discrepancies observed during validation were attributable to known limitations of array-based methods rather than WGS performance.

Are there any known limitations?

Yes, key limitations include:

- Limited orthogonal reference data for certain genes
- Differences in interpretation frameworks for specific genes (e.g., DPYD)
- Validation focused on technical performance rather than clinical interpretation
- Some genes were not fully assessed due to lack of comparison data (please see the validation report for additional details)

Are the PGx results clinically validated?

Yes, the PGx technical deliverables were clinically validated.

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Will BCL provide clinical interpretation?

Technical deliverable is the only deliverable that will be offered to customers and there will be no interpretation provided.

Why are specialized callers needed for CYP2D6 and CYP2B6?

These genes are located in complex genomic regions characterized by:

- High homology to pseudogenes
- Frequent CNVs and gene rearrangements

The DRAGEN Targeted Caller integrates multiple variant detection methods to accurately resolve these complexities and improve phenotype assignment.

Will there be a fee or change in price to receive these additional files?

No, there will not be any fee or change in price.

Do customers need to take action to receive these new deliverables?

For customers using an API that relies on a generic endpoint to retrieve all data files, the new outputs will be available immediately. However, if a customer's API uses a hardcoded endpoint to retrieve only specific outputs, the customer must update your endpoints to successfully capture the new files.

Access to the data remains available through the BCL Ordering portal (in the results dashboard), via API, and in your external Terra workspace (if applicable).

Will BCL offer re-processing of previously-run samples so customers can get PGx for these samples? What if a customer is willing to pay?

No, at this time this will not be a product offering. In the future, this may be a possibility for a fee.

Will BCL be offering PGx for BGE? When will BGE validation happen?

Yes, BCL is working with Illumina to offer PGx calling for BGE data. Clinical validation of PGx on BGE is TBD.

References

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