Validation of genomic profiling by PGDx plasma focus™ to facilitate precision oncology through cell-free DNA testing of solid tumors

Ellen L. Verner[1], Stephanie Hastings[2], Eric Severson[2], Shakti Ramkissoon[2], Prasanth Reddy[2], Mary Nesline[3], Kenneth C. Valkenburg[1], Amy Greer[1], Aanavi Karandikar[1]

[1]Personal Genome Diagnostics, Inc., 3600 Boston Street, Baltimore, MD 21224, USA
[2]LabCorp Oncology, Durham NC 27560, USA
[3]OmniSeq, Buffalo NY 14203, USA

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ABSTRACT

Introduction
Genomic profiling is critical for precision oncology to help guide treatment decisions and tissue isn't always available for testing. To overcome several challenges of tissue testing, an alternate and complimentary method is the assessment of cell-free DNA (cfDNA). The PGDx plasma focus assay is a custom Laboratory Developed Test (LDT), next generation sequencing cfDNA genomic profiling test intended to identify actionable variants in solid cancers with FDA-approved targeted therapies including NSCLC, CRC, melanoma, breast, esophageal, gastroesophageal junction (GEJ), and gastric carcinomas.

Methods
PGDx plasma focus is a hybrid capture-based assay that uses 25ng of cfDNA input. It interrogates all coding exons of 33 genes to detect single nucleotide variants (SNVs), insertions and deletions (Indels). The assay also reports copy number amplifications (CNAs) in 8 genes, gene fusions in 5 genes, and microsatellite instability (MSI). Targets were selected for clinical utility and include SNVs/Indels in AKT1, ALK, APC, ARID1A, ATM, BRAF, BRCA1/2, BRIP1, CCND1, CD274, CDH1, CSF1R, EGFR, ERBB2, EZH2, FGFR1/2, HRAS, KIT, KRAS, MET, MYC, NRAS, NTRK1, PDGFRA, PIK3CA, POLD1, POLE, RAF1, RET, ROS1, TP53; CNAs in CCND1, CD274, EGFR, ERBB2, FGFR2, KIT, MET, MYC and gene fusions in ALK, FGFR2, NTRK1, RET, and ROS1. Validation studies were performed with two Genome in a Bottle reference samples, 19 cell lines, and 94 clinical samples to assess assay performance against orthogonal cfDNA assays.

Results
Analytical sensitivity was determined using cell line blends and demonstrated a limit of detection (LoD) of 0.5-1.0% variant allele frequency (VAF) for SNVs, 0.3-1.3% for Indels, 1.3-1.4-fold for CNAs, and 0.1-1.4% VAF for gene fusions. Analytical specificity was assessed in two reference samples evaluated in duplicate, demonstrating specificity of >99% for SNVs/Indels (n=4.9x10^5), and 100% for amplifications (n=32), gene fusions (n=20), and MSI (n=4). Reproducibility was determined from sensitivity replicates with an average positive agreement (APA) of 97.5% and an average negative agreement (ANA) of 99.9%. Interim analysis of concordance was performed on data from 94 clinical specimens and 17 cell lines, with further testing underway. PPA was 98.7% for SNVs (220/223), 89.3% for Indels (25/28), 96.2% for CNAs (25/26), 100% for translocations (7/7), and 100% for MSI (8/8). NPA was >99% for SNVs (n=3.6x10^6), >99% for Indels (n=3.6x10^6), >99% for CNAs (244/246), >99% (162/163) for gene fusions, and 98.8% for MSI (81/82). Finally, across all testing, 91.8% (146/159) samples passed on the first attempt. After repeats, 100% (159/159) of sample libraries passed quality metrics.

Conclusions
Taken together, these data demonstrate that PGDx plasma focus is a sensitive, specific, accurate, reproducible, and robust approach for cfDNA genomic profiling to supplement tissue testing and inform precision medicine clinical decision making.

INTRODUCTION

Liquid Biopsy Background

Benefits of Liquid Biopsies
Minimally Invasive
Can offer a comprehensive profile of primary and metastatic lesions
Increases access to testing in community settings
Decreased TAT from sample collection
Able to detect tumor-specific mutations associated with clonal evolution, particularly in acquired resistance settings

ASSAY OVERVIEW

Intended Use

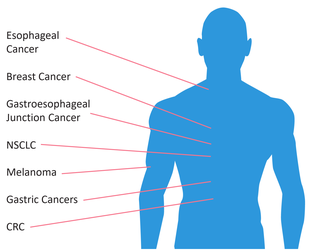


Figure 1
Overview of cancer types within the current intended use of the assay. PGDx plasma focus targets clinically actionable genes in all these cancer types.

Gene List

Gene	AKT1	ALK	APC	ARID1A	ATM	BRAF	BRCA1	BRCA2	BRIP1	CCND1	CD274	CDH1	CSF1R	EGFR	ERBB2	EZH2	FGFR1	FGFR2	HRAS	KIT	KRAS	MET	MYC	NRAS	NTRK1	PDGFRA	PIK3CA	POLD1	POLE	RAF1	RET	ROS1	TP53
SNV and Indels	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Amplifications										•	•			•	•			•		•		•	•										
Fusions		•																•							•						•	•	

ASSAY OVERVIEW

General Assay Specifications for PGDx plasma focus

Parameter	Specification
Panel Size	>240,000 bp
Panel Content and Variant Type	33 genes for SNV and Indels 8 genes for amplifications 5 genes for gene fusions MSI status
Reportable Range	SNVs and Indels: ≥ 0.1% VAF Gene fusions: ≥ 2 fusion reads Amplifications: ≥ 1.2-fold
Sample Type	Cell-free DNA (cfDNA) isolated from plasma
DNA input Requirements	25 ng
Sample Pass Rate	100% overall pass rate (159/159)
Sequencing Platform/ Flow Cell	NextSeq 550 RUO or Dx
Sequence Run	2 x 150 bp
Cases per Sequencing Run	7 samples (plus a run control)
Workflow	Manual, performed at PGDx laboratory in Baltimore
Variant Reporting	Automated analytical pipeline, clinically annotated variant report generated using QCI Interpret One
Intended Use Indications	NSCLC, CRC, Breast Cancer, Esophageal Cancer, Gastroesophageal Junction Cancer, Gastric Cancer, Melanoma
Average De-duplicated Error-corrected Coverage	~2,700x

ASSAY WORKFLOW

8-DAY TURNAROUND TIME

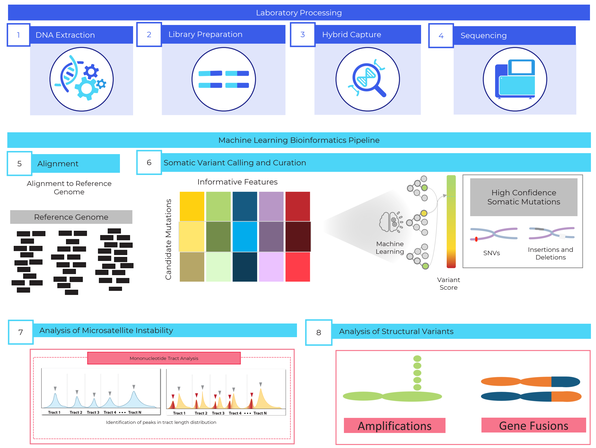


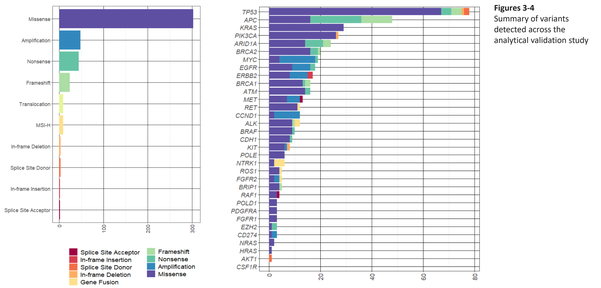
Figure 2
Overview of PGDx plasma focus lab and analytical processing (Figure adapted from Keefer et al., 2022 is licensed under CC BY)

METHODS

Study	Number of Unique Samples	Sample Composition
Accuracy	110	94 clinical samples, 17 cell lines, enriched for intended use indications Orthogonal Tests: PGDx PlasmaSELECT-64, PGDx elio™ plasma complete, PGDx elio plasma resolve, PGDx elio plasma focus (performed by an independent party), Pillar ONCO/Reveal PillarHS Multi-Cancer Panel
Analytical Sensitivity (LoD)	12	4 cell line blends diluted to three targeted levels (below, at, above established LoD), evaluated in triplicate
Analytical Specificity	2	Reference material (Genome in a Bottle), run in duplicate
Repeatability/Reproducibility/Precision	4	Nested study design within LoD analysis

RESULTS

Result Summary



Figures 3-4
Summary of variants detected across the analytical validation study

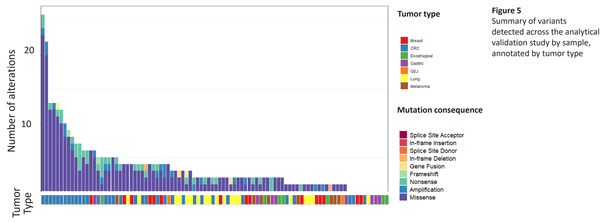


Figure 5
Summary of variants detected across the analytical validation study by sample, annotated by tumor type

Accuracy Analysis

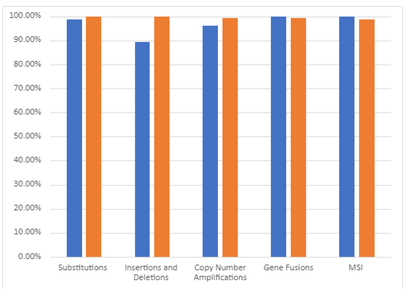


Figure 6
Summary of accuracy (concordance) results. Analysis includes all variants within the ROI of each assay and above LoD of both PGDx plasma focus and orthogonal (orth) assay.

PPA	+PF/+orth	220	25	25	7	8
	-PF/+orth	3	3	1	0	0
NPA	+PF/-orth	27	4	2	1	1
	-PF/-orth	3598804	3599023	244	162	81
Samples		110	110	110	85	89

RESULTS

Analytical Specificity Analysis

Variant Type	Specificity	Discrepant Positive Mutation Count
SNVs	99.99% (490,163/490,172)	9*
Indels	100.0% (490,172/490,172)	0
Copy Number Amplifications	100.0% (32/32)	0
Gene Fusions	100.0% (20/20)	0
Microsatellite Instability	100.0% (4/4)	0

*False positive SNVs were distributed across four samples included in the study. All <2.5% VAF.

Analytical Sensitivity Analysis

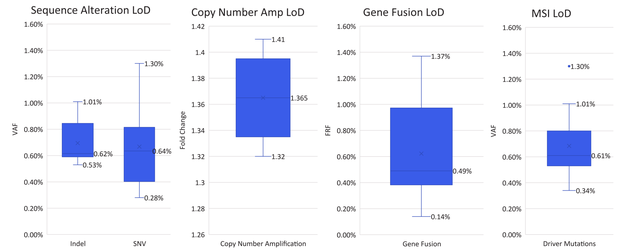


Figure 7
Summary of observed limit of detection across variant types. MSI-H detected with 100% sensitivity in this study. Ranged and medians reported.
Terms: VAF (Variant Allele Frequency), FRF (Fusion Read Fraction)

Repeatability, Reproducibility, and Precision Analysis

Variant Type		APA (%)	ANA (%)
Sequence Mutations	Overall	97.5%	99.9%
	Range Across Studies**	(97.0%-97.5%)	(99.9%-99.9%)
Copy Number Amplifications	Overall	88.9%***	98.4%
	Range Across Studies	(85.7%-92.3%)	(98.0%-99.0%)
Gene Fusions	Overall	100.0%	100.0%
	Range Across Studies	(100.0%-100.0%)	(100.0%-100.0%)
MSI	Overall	100.0%	100.0%
	Range Across Studies	(100.0%-100.0%)	(100.0%-100.0%)

**Evaluated overall (above), inter-lot, inter-instrument, inter-operator and within run.
***Lower APA for amplifications is the result of a CNA reported in one replicate (below LoD but above assay threshold).

CONCLUSIONS

PGDx plasma focus is a targeted LDT NGS assay designed for solid tumor profiling from cfDNA. The assay was designed, verified, and validated under pre-defined, robust design control processes. The assay configuration accommodates a throughput of 7 samples (plus a run control) and achieves sufficient sequencing depth of coverage for high sensitivity detection across a targeted panel, (~240,000 base pairs covering 33 genes) on a NextSeq 550 2x 150bp high output flow cell. PGDx plasma focus reports SNVs and Indels for all 33 genes in the panel, as well as amplifications in 8 genes and gene fusions in 5 genes, and also reports MSI status.

This validation study demonstrates that PGDx plasma focus is a highly sensitive, specific, accurate and reproducible assay. PPA evaluated in the accuracy study ranged from 89.3% (for Indels) to 100.0% (for translocations and MSI). NPAs were over 98% for all variant types. Specificity was 100.0% for all variants types except SNVs which had a specificity of >99%. SNV and Indels had an observed median LoD of 0.6% variant allele frequency, copy number amplifications at 1.4-fold, gene fusions at 0.5% fusion read fraction. MSI-high calls has a specificity of 100%. Samples repeated within runs, across runs, across operators, and across reagent batches had an APA of 97.5% and an ANA of >99%.