Comprehensive liquid biopsy profiling enabled by PGDx elio plasma™ complete to facilitate precision oncology through decentralized access to testing

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PGDx™ Personal Genome Diagnostics

Abstract 3112

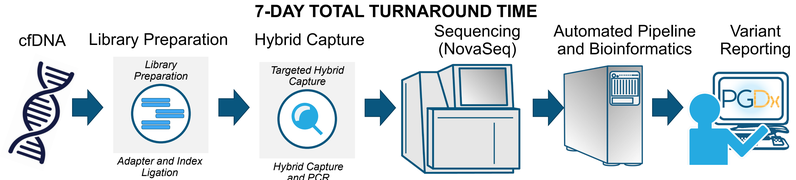
ABSTRACT

Background
Despite the growing body of clinical evidence to support the utility of comprehensive genomic profiling (CGP) for advanced cancer patients, only a small fraction of individuals receive precision oncology guided treatment strategies. We set out to develop the PGDx elio™ plasma complete assay in order to guide both translational biomarker discovery, monitoring response, and CGP-informed precision oncology strategies in cell free DNA (cfDNA) from the plasma of cancer patients.

Specific Objectives
- To develop a 500+ gene hybrid-capture next generation sequencing (NGS) assay as a decentralized kit for cell-free DNA from liquid biopsies
- To enable comprehensive detection of SNVs, indels, amplifications, translocations, MSI, and blood TMB
- To identify clinically relevant cancer variants, hotspots, and genomic signatures in plasma cfDNA
- To build an assay with high specificity, sensitivity, accuracy, precision, reproducibility, and robustness
- To enable compatibility on the NovaSeq 6000 Sequencer
- To build manual and automated versions of the assay for user flexibility

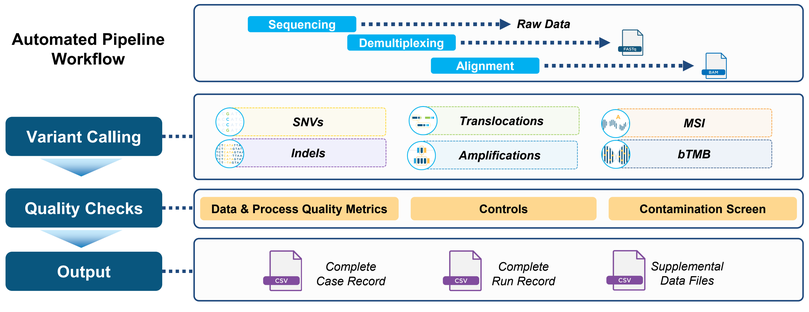
ASSAY WORKFLOW

7-DAY TOTAL TURNAROUND TIME



Automated liquid handler option for library preparation and hybrid capture

Improvements with PGDx Core Chemistry
- Improved ligase efficiency
- Higher library yield
- Enhanced sensitivity & read diversity
- Fewer touch points & faster processing time
- Multiplex capture improves usability and coverage uniformity
- Lower contamination risk from adapters
- Lower cost per sample



General Assay Specifications for PGDx elio plasma complete

Parameter	Specification
Panel Size	2.1 MB
Panel Content and Variant Type	521 genes for SNV & Indels 38 genes for amplifications 21 genes for translocations MSI Status bTMB (Muts/Mb) LOH Status
Reportable Range	SNVs & indels: ≥ 0.1% VAF Translocations: ≥ 2 fusion reads Amplifications: ≥ 1.15-fold
Sample Requirements	Plasma cfDNA
DNA input Requirements	25 ng recommended; 10 ng minimum
Sample Pass Rate	97.8% overall pass rate (271/277)
Sequencing Platform/ Flow Cell	NovaSeq 6000/S2 Flow cell
Sequence Run	2 x 150 bp
Cases per Sequencing Run	16
Workflow	Manual and Automated Available
Variant Reporting	Automated analytical pipeline and variant report generation
Average Total Coverage	~20,000x
Average De-duplicated Error-corrected Coverage	~2300x

METHODS

- Specificity was assessed using plasma cfDNA from 20 noncancerous donors at 25 ng input.
- Sensitivity was assessed in 3 series of dilutions of variant-positive DNA into wild-type DNA to multiple levels above and below the theoretical limit of detection
- Accuracy was assessed by comparing reported variant calls in clinical plasma samples and reference materials to orthogonal assays in 71 samples (including bladder, colorectal, gastric, lung, ovarian, and thyroid cancer) at 25 ng input.
- Precision, reproducibility, and repeatability was assessed in 2 well-characterized samples at 25 ng input in triplicate across 2 operators and 3 non-consecutive runs.
- DNA input was assessed at multiple inputs across 350 clinical samples. In addition, concordance between the 25 ng recommended input and the 10 ng minimum input was assessed in 15 samples.
- Comparison of manual and automated lab assays: 45 samples were processed manually and on the Tecan Freedom EVO 150.

RESULTS

Analytical Accuracy Primary Endpoint Results Compared to Targeted NGS Panels (n=64)

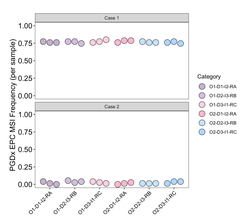
Analyte	PPA	NPA
SNVs	92.7%	99.9%
Indels	94.4%	99.9%
Translocations	82.4%	100%
Amplifications	89.3%	96.4%
MSI	100%	100%
bTMB	0.72 Spearman Correlation Coefficient	

Analytical Accuracy Primary Endpoint Results Compared to Competitor 500+ Gene cfDNA Assay (n=7)

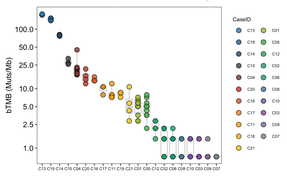
Analyte	PPA (2-sided 95% CI)	NPA (2-sided 95% CI)
SNVs	92.2% (95/103)	99.99% (8414930/8414933)
Indels	83.3% (10/12)	99.99% (8415023/8415024)
Translocations*	50% (2/4)	99.7% (366/367)
Amplifications‡	76% (19/25)	98.6% (141/143)
MSI	N/A	100% (7/7)
bTMB	0.73 Spearman Correlation Coefficient	

* Discordant calls: BRAF-ZC3HAV1 non-actionable, low fusion read count; EWSR1-RP11-9L18.2, filtered due to fusion with pseudogene
‡ All calls were < 2-fold except 1 concordant MET call

MSI Reproducibility



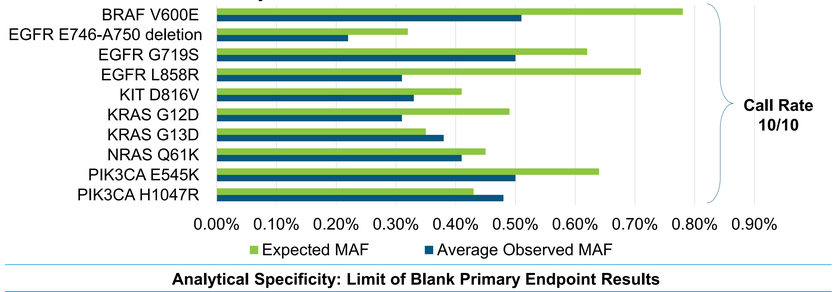
bTMB Reproducibility



SNV and Indel LoD Summary

Variant Category	Number of Variants	Observed Range[2]	Median LoD[3]
Clinically Relevant SNVs & Indels	10 (9 SNV; 1 indel)	0.32% - 0.78%	0.40% VAF
Panel-wide SNVs & Indels	263 (245 SNV; 18 indels)	0.34% - 1.75%	1.16% VAF
Translocations	2	0.22% - 0.82%	0.24% and 0.41% FRF
Amplifications	1	1.28 - 1.30-fold	1.29-fold

Clinically Actionable SNV and Indel LoD95 Performance



Analytical Specificity: Limit of Blank Primary Endpoint Results

Analyte Assessed	Specificity (n/N)
SNVs (clinically relevant)	100% (4260/4260)
Panel-Wide SNVs	99.9999% (28009535/28009540)
Indels (clinically relevant)	100% (1780/1780)
Panel-wide Indels	99.9999% (28009528/28009540)
Translocations	100% (420/420)
Amplifications	100% (760/760)
MSI	100% (20/20)
bTMB*	100% (20/20)

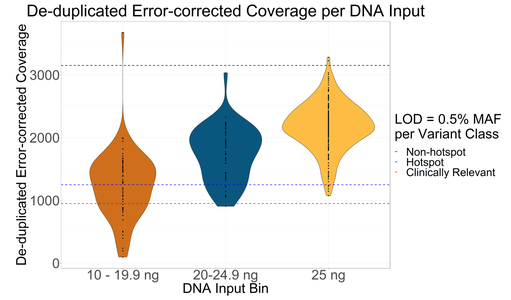
*Confirmation that non-cancerous samples bTMB score was below the established Limit of Blank of 1.0 Muts/Mb

RESULTS

Manual vs. Automated Assay Workflow

Variable Assessed	Result	
Library Preparation Yield	All samples had ≥ 108 ng/µL yield	
Total Coverage	Mean = 7.3% CV	
De-duplicated error-corrected coverage	Mean = 5.9% CV	
GC Bias	Mean = 0.42% CV	
Pass/Fail Status	100% success rate	
Clinically Relevant SNV & Indel Concordance	98.4% PPA	99.94% NPA
Panel-wide SNV & Indel Concordance	98.0% PPA (≥1.0% VAF)	92.2% PPA (all variants assessed)
Amplification Concordance	95.79% PPA	99.94% NPA
Translocation Concordance	100% PPA	99.78% NPA
MSI Concordance	100% PPA	100% NPA
bTMB Concordance	Mean = 6.5% CV	

De-duplicated Error-corrected Coverage per DNA Input



DNA Input Bin (ng)	N	Observed Pass Rate
10 - 19.9	68	85.3%
20-24.9	51	92.9%
25	231	98.2%
Total	350	

Precision and Reproducibility:
Average Positive Agreement (APA) and Average Negative Agreement (ANA) Results

Alteration Type	Overall		Inter-Operator		Inter-Day		Inter-Run		Within-Run	
Alteration Type	APA (%) 95% CI	ANA (%) 95% CI	APA (%) 95% CI	ANA (%) 95% CI	APA (%) 95% CI	ANA (%) 95% CI	APA (%) 95% CI	ANA (%) 95% CI	APA (%) 95% CI	ANA (%) 95% CI
MSI	100.0% (95.9%, 100.0%)	100.0% (95.9%, 100.0%)	100.0% (93.4%, 100.0%)	100.0% (93.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)
Amplifications (FGF4, FGF19, CCND1)	100.0% (95.9%, 100.0%)	100.0% (95.9%, 100.0%)	100.0% (93.4%, 100.0%)	100.0% (93.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)
Translocations (ALK, RET)	100.0% (95.9%, 100.0%)	100.0% (95.9%, 100.0%)	100.0% (93.4%, 100.0%)	100.0% (93.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)	100.0% (90.4%, 100.0%)
Clinically Relevant SNVs and Indels	100.0% (99.5%, 100.0%)	100.0% (99.6%, 100.0%)	100.0% (99.1%, 100.0%)	100.0% (99.3%, 100.0%)	100.0% (98.7%, 100.0%)	100.0% (98.9%, 100.0%)	100.0% (98.7%, 100.0%)	100.0% (98.9%, 100.0%)	100.0% (98.7%, 100.0%)	100.0% (98.9%, 100.0%)
Panel-wide SNVs and Indels	92.5% (91.5%, 93.5%)	94.0% (93.2%, 94.8%)	92.5% (91.0%, 93.7%)	93.9% (92.8%, 94.9%)	92.7% (91.0%, 94.1%)	94.1% (92.7%, 95.3%)	92.7% (91.0%, 94.1%)	94.1% (92.7%, 95.3%)	92.7% (91.0%, 94.1%)	94.1% (92.7%, 95.3%)

CONCLUSIONS

PGDx built a 500+ gene decentralized NGS panel for plasma with high sensitivity, specificity, and reproducibility with manual and automated lab assay options. Through pre-defined, robust design control processes, the product was designed, verified, and validated. The PGDx elio plasma complete assay was designed for ease of use both in the manual setting and automated setting (using the Tecan Freedom EVO 150), with a multiplex configuration that reduces lab steps and time. The assay is run with 16 samples per kit from library preparation through sequencing over the course of ≤ 3.5 days. While the recommended DNA input into the assay is 25 ng, the assay performs well down to 10 ng input. The NovaSeq instrument provides sufficient sequencing depth of coverage for high sensitivity over a large panel, which includes a genomic region of 2.1 Mb covering 521 genes. The automated software produces in-depth reports after an average of 3.5 days from completion of sequencing. The PGDx elio plasma complete reports provide information on single nucleotide variants (SNVs) and insertions and deletions (indels) for all 521 genes on the panel, as well as being able to report amplifications in 38 genes and translocations in 21 genes (see Appendix). The product also reports microsatellite instability (MSI) status, loss of heterozygosity (LOH) status, and a numerical value for blood tumor mutation burden (bTMB) for every case. Overall, elio plasma complete is a highly sensitive, specific, and reproducible plasma assay.