

PGDx^{elio}TM

tissue complete CDx

Manual

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Rx Only



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Part 1 – Product Insert and Performance Data



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Intended Use

PGDx elio tissue complete CDx is a qualitative next-generation sequencing-based in vitro diagnostic device that uses a high-throughput hybridization-based capture technology utilizing DNA isolated from formalin-fixed paraffin embedded tumor tissue. The PGDx elio tissue complete CDx targeted panel can detect single nucleotide variants, small insertions and deletions, copy number amplifications, and translocations. It is intended to be used as a companion diagnostic to identify melanoma patients who may benefit from treatment with the targeted therapies listed in **Table 1.1** below in accordance with the approved therapeutic product labeling.

Table 1.1 PGDx elio tissue complete CDx Companion Diagnostic Indications

Indication	Biomarker	Therapy
Melanoma	BRAF V600E	BRAF inhibitors approved by FDA or BRAF/MEK inhibitor combinations approved by FDA
Melanoma	BRAF V600K	BRAF inhibitors approved by FDA or BRAF/MEK inhibitor combinations approved by FDA

Summary and Explanation

Clinical Description

Precision medicine entails getting the right patients on the right treatment at the right time. This is underscored by the ability to identify oncogenic drivers and evaluate the patient's biomarker profile, to enable the treating oncologist to select the best treatment path with the highest likelihood of success. As the number of clinically relevant biomarkers increases, so too does the need for adequate tissue samples required for truly comprehensive testing. Availability of tissue samples for diagnostic testing however, is often limited particularly in late-stage cancer patients. This limitation has resulted in a need for pathologists and oncologists to triage biomarker detection assays (Lindeman et al., 2018).

Genomic profiling to characterize a patient's tumor through evaluation of their biomarker profile is predicted to become standard of care in oncology (Hartmaier 2017). Comprehensive genomic profiling (CGP) enabled by next generation sequencing (NGS) has been instrumental in enabling simultaneous and rapid detection of multiple genomic alterations for personalized tumor profiling, providing clinicians with more information from less sample (Thomas et al., 2007) while being able to provide reduced turn-around times compared to serial single-gene testing approaches. These molecular profiles provide critical information on biomarker status that may inform on a patient's potential eligibility for targeted therapy which may result in improved outcomes (Kris 2014).

Although melanoma comprises only about 1% of all skin cancer diagnoses, it is the leading cause of skin cancer-related mortality in the United States (American Cancer Society, 2025; Domingues et al., 2018; Castellani et al., 2023; Davis et al., 2019). For 40-50% of cases, the BRAF gene is mutated, 90% of which are V600 mutations (Pelosi et al., 2024). Targeted therapies against these genetic modifications have significantly improved the management of melanoma patients.

BRAF V600 mutations result in constitutive activation of the mitogen activated protein kinase (MAPK) pathway, driving tumor progression (Colombino et al., 2012; Davies et al., 2002). For patients with BRAF V600 mutant melanoma, dual inhibition of this pathway using BRAF and MEK inhibitors has become a standard of care, with clinical studies consistently demonstrating improvements in progression-free survival and overall survival, alongside a manageable safety profile (Ascierto et al., 2016; Larkin et al., 2014; Long et al., 2015). For patients with BRAF V600-mutant melanoma, confirmation of mutation status via NGS is essential prior to initiating first-line systemic therapy. The ability to detect co-occurring mutations further enhances diagnostic precision and supports individualized treatment planning.

The PGDx elio tissue complete CDx assay provides meaningful clinical benefits in the management of advanced melanoma through highly sensitive detection of BRAF V600 mutations, enabling identification of patients eligible for targeted BRAF/MEK inhibitor therapies and facilitating the timely initiation of these treatments. The analytical and clinical performance of PGDx elio tissue complete CDx provides evidence that the device is effective therapy selection for patients with advanced melanoma. Its

performance supports a class indication for BRAF/MEK inhibitor combinations, thereby expanding access to multiple approved therapeutic options. Clinical validation data from the PMA approval process demonstrate that the assay is non-inferior to currently FDA-approved companion diagnostics for the same indication, supporting its use as a reliable tool for guiding targeted therapy decisions.

Product Description

PGDx elio tissue complete CDx is a companion diagnostic test that uses NGS to detect tumor gene alterations in genomic DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue using a targeted panel (505 genes). The assay takes less than 7 days from DNA extraction to report tumor gene alterations.

Figure 1.1 describes components of the assay. A complete list of components, equipment and materials can be found in **Part 2** of this document.

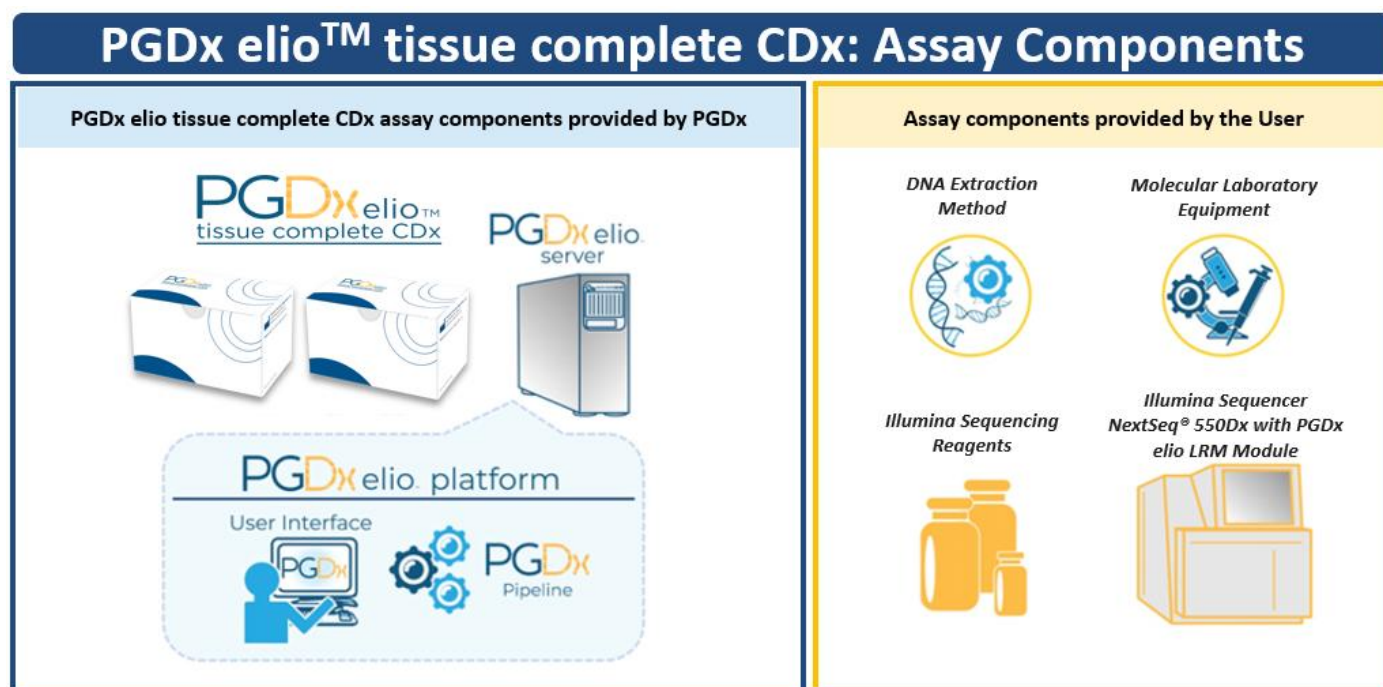


Figure 1.1 PGDx elio tissue complete CDx assay components. PGDx provided components include reagent kits, software for data analysis, and a server.

Principle of Procedure

The PGDx elio tissue complete CDx assay workflow consists of 6 sequential phases: Sample Preparation, Library Preparation, Hybrid Capture, Sequencing, Data Analysis, and Report Generation as further described below. Refer to **Part 2** of the PGDx elio tissue complete CDx Manual for detailed steps of the procedure. An overview of the procedural steps of the PGDx elio tissue complete CDx assay is provided in **Figure 1.2**.

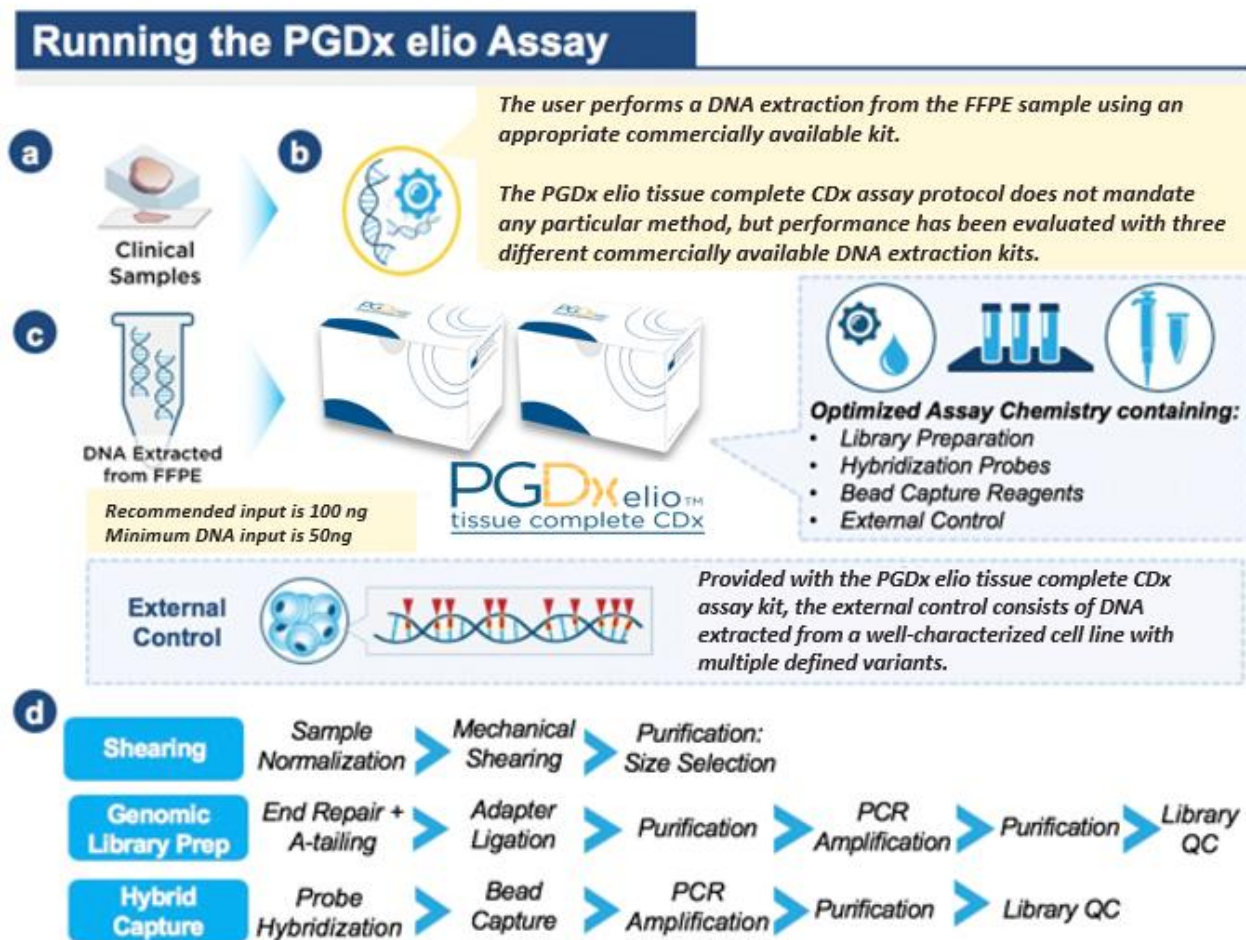


Figure 1.2 Laboratory Process of PGDx elio tissue complete CDx. (a) FFPE tissue specimen is collected. (b) DNA is extracted from FFPE tissue. (c) Extracted DNA is the input to the PGDx elio tissue complete CDx workflow, which includes an external control. (d) The PGDx elio tissue complete CDx laboratory workflow consists of shearing, followed by library preparation, then hybrid capture.

Sample Preparation

The PGDx elio tissue complete CDx assay requires genomic DNA isolated from FFPE tissue specimens using commercially available DNA extraction methods. The assay is validated for use with DNA recovered from tissue with a minimum of 20% viable tumor nuclei. If less than 100% of the tissue section contains $\geq 20\%$ tumor purity, the tissue should be macro-dissected to select as much viable tumor as possible and minimize the amount of adjacent non-tumor tissue. The recommended DNA input for the assay is 100 ng at a minimum concentration of 1 ng/ μ L; results can be obtained with inputs down to 50 ng.

Library Preparation

The PGDx elio tissue complete CDx assay workflow begins with genomic DNA. Genomic DNA is quantified using a fluorometer. DNA molecules are mechanically sheared to a target size of 200 bp and subjected to a magnetic bead purification step to remove smaller fragments and perform an exchange of buffer.

Fragmented DNA is end-repaired, phosphorylated, and A-tailed. Indexed adapters are then ligated to the A-tailed DNA molecules. Unincorporated adapters and reagents are removed by magnetic bead purification. Adapter-ligated DNA is enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Library quality is assessed using a DNA fragment analyzer prior to hybrid capture.

Hybrid Capture

The adapter-ligated library is hybridized with biotinylated RNA library baits, and targeted regions are captured using magnetic streptavidin coated beads. Captured libraries are purified to remove baits and incompletely hybridized DNA fragments. Captured libraries are enriched by PCR amplification. Primer dimers and residual reagents are removed by magnetic bead purification. Final library quality is assessed using a DNA fragment analyzer prior to sequencing.

Sequencing

Sample libraries are quantified and normalized into a sequencing pool of up to 15 samples and the external control. Pooled sample libraries are fluorometrically quantified, loaded on a sequencing flow cell and sequenced using a NextSeq® 550Dx instrument.

Data Analysis

Sequence data is processed using the PGDx elio platform software. The software contains a user interface that tracks sample status from sequencing through analysis and reporting. Users configure sequencing runs, and an automated pipeline of software for bioinformatic analysis identifies and reports genomic alterations. After processing, the software generates FASTQ files containing sequences and quality scores for each sample. The FASTQ files are then aligned to a reference genome to generate BAM files, which are processed for variant calling of different alteration types (SNVs, indels, amplifications, translocations, and MSI). SNVs and indels are then used to determine TMB scores reported as mutations per megabase.

Report Generation

The PGDx elio tissue complete CDx software generates reports for each sample and the respective sequencing run processed (**Figure 1.3**). There are 3 report outputs, all available for download from the PGDx elio platform user interface.

- **The Complete Run Record (CRR):** A run-level report providing summary data of the Analysis Plan and associated sequencing run metrics. This file is used to assess the quality of the sequencing run and is typically used for troubleshooting following details provided in this guide.
- **The Complete Case Record (CCR):** A case-level report providing aggregated data from the clinical sample with the external control to provide run-level, sample-level and external control QC status, as well as a list of variant calls.
- **The Case Report:** A case-level report organizing variants into two subsets consistent with reporting formats for FDA IVD tests: Companion Diagnostic Findings, Variants with Evidence of Clinical Significance, and Variants with Potential Clinical Significance. The Companion Diagnostic Findings section is limited to biomarkers approved by the FDA as a CDx in specific indications. The organization of Variants with Evidence of Clinical Significance is driven by selection of a specific tumor type by a qualified healthcare professional and consistent with Tier IA of AMP/ASCO/CAP guidelines (Li et al., 2017) and OncoKB levels of evidence (Suehnholz 2024 and Chakravarty 2017). Quality metrics are summarized, and results definitions are provided.

Following the completion of a sequencing run, the PGDx elio server, containing the PGDx analytical pipeline, begins processing automatically to generate reports.

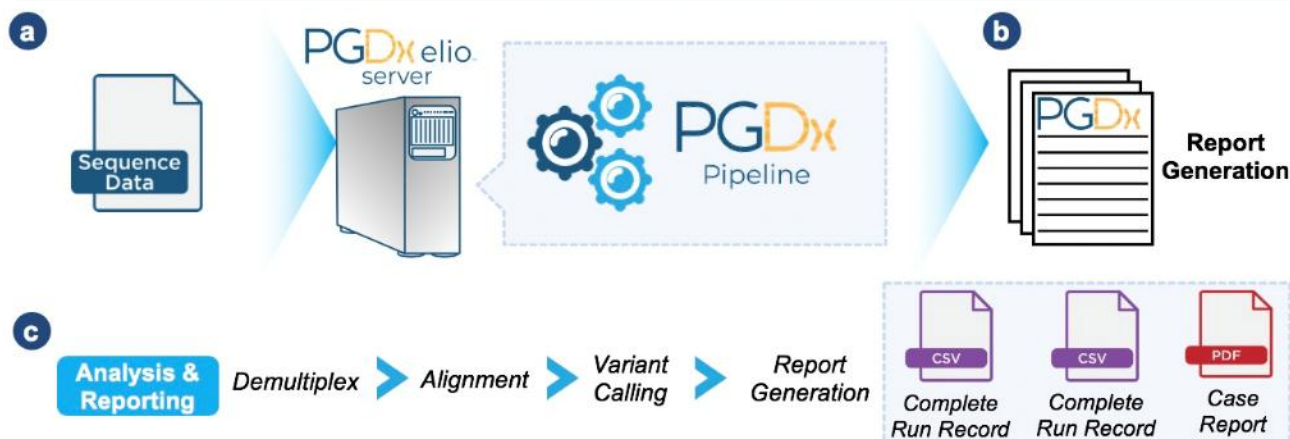


Figure 1.3 Report generation in PGDx elio tissue complete CDx. (a) Sequence data (b) is processed on the PGDx elio server to automatically generate reports (c) using the PGDx elio software.

Limitations of Procedure

1. For In vitro diagnostic use.
2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
3. PGDx elio tissue complete CDx has been validated for use with genomic DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue. Other sample types or fixation methods have not been evaluated.
4. The assay has been validated using samples with minimum of 20% tumor nuclei in the tissue area to be extracted.
5. This assay has not been validated for use with fine needle aspirates (FNA) as a specimen type.
6. PGDx elio tissue complete CDx is only cleared for use with Illumina NextSeq 550Dx instruments.
7. The test is designed to report out somatic variants and is not intended to report germline variants. This assay is designed to accept tumor-only samples (as opposed to tumor-normal pairs). Common germline variants are filtered using population databases. Rare and novel germline variants not listed in these databases may be reported and cannot be distinguished from somatic.
8. This device will not detect all instances of contamination when both sample and contaminant are from individuals of Asian ancestry. Extra care should be taken to avoid cross-contamination of such samples.
9. This device will not detect all instances of contamination when the contaminant is a high purity cell line that contains a translocation. Extra care should be taken to avoid cross-contamination of cell lines.
10. This device may not detect ERBB2 amplifications if the fold change is ≤ 6 at a tumor purity below 30%. The threshold for PGDx elio tissue complete CDx for an ERBB2 amplification positive call is 2.5-fold. Most FISH borderline cases with an ERBB2/Chromosome 17 ratio of 2-2.5 were negative by PGDx elio tissue complete CDx. The frequency of ERBB2 amplification with an ERBB2/Chromosome 17 FISH ratio of 2-2.5 is estimated to be 2.2%. Testing with an FDA-approved companion diagnostic should be performed to assess patients for selection of therapy.
11. The clinical significance of TMB measurement has not been established. Tumor Mutation Burden (TMB) is reported as mutations per megabase (Muts/Mb). TMB is a function of characteristics of a patient's specimen and testing

parameters; therefore, TMB may differ across specimens (e.g., primary vs metastatic, tumor content) and targeted panels. The TMB calculation will increase or decrease depending upon the variables listed below:

- The size and specific region to calculate TMB may result in different values. PGDx elio tissue complete CDx counts somatic, synonymous and non-synonymous variants in gene coding regions and divides it by the size of the region (1.3Mb).
 - The percentage of tumor in the sample – PGDx elio tissue complete CDx requires a minimum of 20% tumor purity.
 - Methods of germline filtering - PGDx uses ExAC, dbSNP, and gnomAD databases to remove germline variants.
 - The read depth and other bioinformatic test specifications.
12. Performance of PGDx elio tissue complete CDx has not been established for detection of insertions or deletions larger than 30 base pairs.
 13. This device is cleared for reporting amplifications only in ERBB2 and translocations only in ALK, NTRK2, NTRK3, and RET.
 14. Detection of non-hotspot SNVs, deletions and insertions that are Variants of Potential Clinical Significance showed decreased concordance (positive percent agreement just above 80%) when compared to predicate assays. False negatives may occur for non-hotspot SNVs, insertions, and deletions that are Variants of Potential Clinical Significance below 8% VAF.
 15. Detection of RET translocations achieved with this device showed decreased concordance (positive percent agreement of 55.6%) when compared to FISH. Direct comparison of translocation performance between PGDx elio tissue complete CDx and RET FISH is challenging, due to possible impact of tumor heterogeneity.
 16. NTRK3 reproducibility was established using cell line samples only, due to low prevalence of clinical samples.
 17. A negative result does not rule out the presence of a mutation below the Limits of Detection of the assay. Alterations at allele frequencies below the established Limit of Detection may not be detected consistently.
 18. DNA input at the minimum 50 ng amount may increase repeat rate of the assay and decrease specificity performance for SNVs and for TMB near the Limit of Blank.
 19. Overall pass rates for PGDx elio tissue complete CDx decrease with increasing age of FFPE block. The probability of samples passing from blocks aged roughly 14.5 years is approximately 75%.
 20. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
 21. Clinical validity performance of this test for predicting treatment effect has not been established.
 22. Phased indels with evidence of clinical significance (**Table 3.3**) will be eligible to be reported together only if they are within 10 bp.
 23. The sequencing "Run Name" does not allow special characters to be used in the naming.

Performance Characteristics

Note: Where indicated, results are portrayed in terms of hotspot or non-hotspot SNVs and indels. PGDx defines hotspots as > 25 exact hits in COSMIC version 72. A lower minimum variant allele frequency (VAF) is used when reporting these hotspot mutations. Summary data for ‘Variants with Evidence of Clinical Significance’ are provided in select tables. A discrete list of these variants is provided in **Part 3** of this Manual. Companion Diagnostic variants are named in **Table 1.1**.

Assay Quality Metrics

Analytical validation studies were conducted to validate performance of the assay using a DNA input of 100 ng, resulting thresholds and quality metrics are provided in **Table 1.2** below.

Table 1.2 Summary of PGDx elio tissue complete CDx Post-Sequencing Quality Control Metrics

Quality Metric	Level of Qualification	Passing Criteria
Cluster Density	Batch-level	Sequencer Cluster Density ≥ 130
Q30 Reads	Batch-level	%Q30 (Read 1 and Read 4) $\geq 80\%$ %Q30 (Read 2 and Read 3) $\geq 85\%$
External Control	Batch-level	All expected sequence mutations are detected and passes all other quality criteria
Percent Regions Covered	Sample-level	$\geq 90\%$ exons with > 100x Median Distinct Coverage
Percent Reads Identified	Sample-level	Percent Reads Identified 15%-35%
Contamination QC	Sample-level	Estimated contamination levels < 2%
Select SNVs and Indels with Evidence of Clinical Significance	Analyte-level	Mutant reads ≥ 4 VAF $\geq 0.4\%$
Hotspot SNVs and Indels	Analyte-level	Mutant reads ≥ 4 VAF $\geq 2\%$
Non-hotspot SNVs	Analyte-level	Mutant reads ≥ 6 VAF with lower bound 95% CI $\geq 5\%$
Non-hotspot Indels	Analyte-level	Mutant reads ≥ 6 VAF $\geq 5\%$
Homopolymer Indels	Analyte-level	Homopolymer regions < 5 bp or Homopolymer regions ≥ 5 bp with VAF $\geq 12\%$
Amplification(s)	Analyte-level	Fold change ≥ 2.5 in $\geq 25\%$ regions covered
Translocations	Analyte-level	Fusion reads ≥ 3

Laboratory Studies

The performance of PGDx elio tissue complete CDx test was established using DNA isolated from FFPE, including melanoma tissue, which were analyzed for BRAF V600E or V600K mutations, and then utilized to determine the eligibility for the appropriate treatment.

CDx Performance Summary – BRAF V600E/K in melanoma

BRAF V600E/K accuracy in melanoma was assessed in the clinical validation study using 389 intended use samples where BRAF V600E/K noninferiority was confirmed (see **Summary of Primary Clinical Studies** section). In addition, **Table 1.3** below is an overall performance summary for BRAF V600E and V600K across all studies conducted in support of companion diagnostic use.

Table 1.3 Overall Performance Summary for BRAF V600E and V600K

Study	Variants Observed	Total Unique Samples Positive for BRAF V600E/K
Clinical Validation for CDx	BRAF V600E/K in melanoma	152
Analytical Accuracy	BRAF V600E/K	20
Accuracy – In Silico	BRAF V600 (including V600E)	2
Specificity (Limit of Blank)	BRAF V600E/K	0
Sensitivity (LoD Establishment)	BRAF V600K in melanoma	1
	BRAF V600E in cell line	1
Sensitivity (Clinical Confirmation LoD)	BRAF V600K in melanoma	1
	BRAF V600E in melanoma	1
Interlaboratory Reproducibility ^{1,2}	BRAF V600E	2
Interlaboratory Reproducibility	BRAF V600E/K	4
BRAF V600E/K Reproducibility	BRAF V600E/K in melanoma	2
Lot-to-Lot Precision	BRAF V600E	1
Assay Guardbanding	BRAF V600E/K in melanoma	2
Real Time Stability	BRAF V600E	1
Transport Stability	BRAF V600E	1
Freeze/Thaw Stability	BRAF V600E	1
Exogenous Interfering Substances	BRAF V600E	1

¹ Data Not Shown

² Contrived Interlaboratory Reproducibility Study

Accuracy – CDx

Analytical accuracy for BRAF V600E/K was assessed using a dataset consisting of 146 pan-cancer FFPE samples (**Table 1.4**, 20 of which were melanoma), compared PGDx performance to 3 orthogonal methods: 2 NGS-based assays and 1 PCR-based assay. Samples were blinded to lab operator or data analyst until after the study was completed. Variant concordance was assessed between PGDx elio tissue complete CDx variant calls and the associated orthogonal method, which showed 100% PPA for both variants (**Table 1.5**).

Table 1.4 Cancer Types Assessed in BRAF V600E and V600K Analytical Accuracy Study

BRAF V600E/K Status	Samples	Cancer Types (n)
Positive	20	Melanoma (10); Colorectal (5); Brain (2); Thyroid (1), Skin (1); Cholangiocarcinoma (1)
Negative	126	Lung (25); Gastric (24); Breast (20); Colorectal (12); Endometrial (7); Brain (8); Ovarian (6); Prostate (6); Bladder (3); Adenocarcinoma (2); Renal (2); Cervical (1); Esophagus (1); Fallopian Tube (1); Liver (1); Mediastinum (1); Abdominal Granulosa (1); Pediatric with unknown primary (1); Serous Carcinoma (1); Pancreatic (1); Peritoneal (1); Thyroid (1)

Table 1.5 BRAF V600E and V600K Accuracy Results

Test	Variant	No. Samples	True Positives	False Negatives	False Positives	True Negatives	PPA	NPA
NGS Targeted Panel	V600E	70	7	0	0	63	100%	100%
NGS Targeted Panel	V600K	70	1	0	0	69	100%	100%
PCR	V600E	34	12	0	0	22	100%	100%
PCR	V600K	34	0	0	0	34	N/A	100%

Assay Acceptance Rates

Multiple factors can influence overall robustness and performance of complex molecular tests, including pre-analytical factors and overall sample quality. If key in-process or automated data quality metrics are not met, PGDx elio tissue complete CDx supports repeating samples through the workflow. Performance throughout verification and validation of the device was tracked and a summary of the rates for first pass (no repeat) and overall pass (allowing for a single repeat) are presented below.

Data aggregated for melanoma clinical FFPE samples (excluding the clinical validation study) showed an invalid rate of 5.6%. However, in the clinical validation study 379 samples were successfully tested of 389 total giving an invalid rate of 2.6% (assay success rate of 97.4%).

Reproducibility/Precision

Three studies conducted to assess precision are described below: interlaboratory reproducibility, BRAF V600E and V600K reproducibility, and lot-to-lot reproducibility.

BRAF V600E and V600K Reproducibility - CDx

An interlaboratory reproducibility study designed to specifically evaluate detection of BRAF V600E/K mutations was performed using 2 clinical FFPE melanoma samples (1 positive for V600E and 1 positive for V600K) diluted with normal FFPE DNA to 1-1.5x the confirmed limit of detection. These 2 blended clinical samples were then assessed across 2 sites, by 2 operators per site, and with 2 reagent lots, in triplicate, for a total of 24 replicates per variant (2 sites x 2 operators x 2 lots x 3 replicates = 24 replicates). The outcome of the study resulted in 100% concordance across all sample replicates for the CDx variants (**Table 1.6**). APA and ANA was also evaluated to assess the imprecision caused by different sources of variance across both sites (**Table 1.7**).

Additionally, an aggregate assessment of assay performance near LoD, assessing both the BRAF interlaboratory reproducibility study and the BRAF LoD clinical confirmation study, resulted in 100% (24 replicates in the interlaboratory study + 20 replicates in the LoD confirmation study = 44/44) call rate for both V600E and V600K variants in melanoma clinical samples. The aggregate mean VAF for BRAF V600E and V600K across the 48 replicates assessed were 3.8% and 5.7%, respectively, near the clinically confirmed LoDs of 3.1% VAF for V600E and 4.7% VAF for V600K.

Table 1.6 Interlaboratory reproducibility BRAF V600E/K overall concordance

Variant	Assessment	All Observations	Average Between Runs	Average Between Operators	Average Between Sites
BRAF V600E	PPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)
	NPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)

Variant	Assessment	All Observations	Average Between Runs	Average Between Operators	Average Between Sites
BRAF V600K	PPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)
	NPA (n/N), CI95	100% 24/24, (86.2%, 100.0%)	100% 3/3, (43.85%, 100.0%)	100% 6/6, (60.96%, 100.0%)	100% 12/12, (75.75%, 100.0%)

Table 1.7 BRAF V600E and V600K APA and ANA Summary

Comparison Type	V600E APA (%) n/N, (CI95)	V600E ANA (%) n/N, (CI95)	V600K APA (%) n/N, (CI95)	V600K ANA (%) n/N, (CI95)
Overall	100.0% 120/120, (96.9%, 100.0%)	100.0% 120/120, (96.9%, 100.0%)	100.0% 120/120, (96.9%, 100.0%)	100.0% 120/120, (96.9%, 100.0%)
Between Site	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)
Between Operator	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)
Between Lot	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)	100.0% 24/24, (86.2%, 100.0%)
Within Run	100.0% 48/48, (92.6%, 100.0%)	100.0% 48/48, (92.6%, 100.0%)	100.0% 48/48, (92.6%, 100.0%)	100.0% 48/48, (92.6%, 100.0%)

Interlaboratory Reproducibility

Interlaboratory reproducibility of the PGDx elio tissue complete CDx assay was assessed across 3 different sites, using DNA extracted from 13 FFPE tissue specimens from 8 tumor types and 1 cell line. These 14 samples included 3 BRAF V600E-positive CRC samples and 1 BRAF V600K-positive melanoma sample. Each of the 14 samples was tested in duplicate by 2 different operators on 12 sequencing runs across 3 non-consecutive days at each of the 3 independent laboratory sites using a single kit lot. The first pass rate was 90.3% (455/504) and the overall pass rate of the study was 98.2% (495/504) allowing a maximum of 1 round of repeat testing. Reproducibility was assessed 3 ways; (1) agreement for each positive variant detected across all replicates is reported (Positive call rate), and (2) Average Positive Agreement (APA) and Average Negative Agreement (ANA) and (3) modal analysis was used for per specimen reproducibility.

Reproducibility for BRAF V600E (3 CRC samples) from the interlaboratory reproducibility study was 100% PPA (107/107) and 100% NPA (388/388) for all samples with a passing status across all sites and other conditions. Reproducibility for BRAF V600K (1 melanoma sample) from the interlaboratory reproducibility study was 100% PPA (36/36) and 100% NPA (459/459) for all samples with a passing status across all sites and other conditions tested.

Lot to Lot Precision - CDx

Performance of PGDx elio tissue complete CDx was assessed across 3 unique kit lots by determining concordance of variant calls in FFPE tissue samples. The 3 unique kit lots were utilized to process 5 test cases (1 BRAF V600E-positive cell line, 1 cell line blend, and 3 FFPE samples in 3 tumor types) in triplicate for a total of 45 observations. All batches were sequenced on the same instrument. The overall pass rate of the study was 100% (45/45). BRAF V600E APA was 100% and the ANA was 100% for each of the three lots in the lot-to-lot precision study.

Analytical Specificity

Limit of Blank (LoB)

Non-cancerous FFPE tissues (n=42) were assessed for analytical specificity of BRAF V600E/K to confirm the reporting thresholds and quality metrics minimize false positives across 2 DNA input levels (50 ng and 100 ng) across 2 reagent lots, resulting in 89 replicates. Two reference standards (5 replicates each, 10 total replicates) from National Institute of Standards and Technology (NIST), NA24531 and NA24385, were evaluated by PGDx elio tissue complete CDx for variants reported at 100 ng DNA input.

The rate of false positives was 0% BRAF V600E and V600K in both the NIST standards and the FFPE tissue samples.

Cross Reactivity

An in silico cross-reactivity analysis was performed to evaluate specificity of capture baits based on sequence identity of target baits to the human reference genome. Bait sequences corresponding to gene regions of clinical significance demonstrated specificity to target regions based on mapping quality or unique sequence identity to the human reference genome. Any bait that had some sequence identity to regions other than the target region of interest was further investigated and were shown to be specific. This study demonstrated that the oligonucleotide baits in the PGDx elio tissue complete CDx assay are specific to the target regions of DNA intended to be reported.

Analytical Sensitivity

Limit of Detection (LoD)

LoD of the PGDx elio tissue complete CDx assay is defined as the mutant allele fraction (VAF) at which 95% of replicates for a variant type are reliably detected. The LoD study was comprised of two steps: LoD establishment using cell lines and LoD confirmation with FFPE clinical tumor samples from clinical cases across a diverse set of variants. Select specimens were used to evaluate specific mutations. Specimens were selected for allele frequencies near the claimed cut-offs. Details of the LoD confirmation results are discussed and shown below.

The limits of detection for BRAF V600E and V600K were established in a dilution series using a cell line (for V600E) and a melanoma FFPE sample (for V600K). The LoD was then confirmed in 1 unique melanoma FFPE sample per CDx variant and diluted to the established LoD level in noncancerous DNA derived from FFPE tissue. Each clinical case was tested with 10 replicates across 2 reagent kit lots for 20 replicates per case, and the confirmed LoD was the mean VAF between the 2 lots (Table 1.8).

Table 1.8 Analytical Sensitivity (LoD VAF) for CDx Variants in FFPE Tumor Tissue

Mutation Type	Mutation	Tumor Type	Average VAF (%)
SNV	BRAF V600E	Melanoma	3.1
MNV	BRAF V600K	Melanoma	4.7

DNA Extraction

Compatibility was assessed for BRAF V600E/K in 2 studies. First, an FFPE tissue sample containing BRAF V600E was extracted with 3 extraction methods and processed in duplicate. Second, an FFPE tissue sample containing BRAF V600E was extracted in duplicate by 2 operators with 3 extraction methods and processed in duplicate. In both studies, Method 2 (bead-based) and Method 3 (automated) were compared to the reference Method 1 (column-based). In the first study, BRAF V600E PPA was 100% (2/2) and NPA was 100% (12/12) when comparing to the reference method. In the second study, BRAF V600E PPA was 100% (16/16), and NPA was 100% (55/55) when comparing to the reference method. PGDx elio tissue complete CDx is compatible with genomic DNA extracted from FFPE samples using any appropriate commercially available FFPE extraction method.

DNA Input

To evaluate PGDx elio tissue complete CDx performance across a range of DNA inputs, BRAF V600E/K mutations were evaluated in 61 clinical FFPE samples (in 17 tumor types) previously tested in the analytical accuracy study that were randomly selected to represent all reportable variant types, including BRAF V600E. Each sample was blinded and tested at 50 ng DNA input with PGDx elio tissue complete CDx to test concordance (PPA and NPA) to the reference input of 100 ng. PGDx elio tissue complete CDx yielded concordant results for BRAF V600E with 100% PPA (7/7) and 100% NPA (49/49) observed in the 50 ng DNA input compared to the 100 ng reference. These data indicate PGDx elio tissue complete CDx is robust around the recommended 100 ng DNA input for all reported variant types, including BRAF V600E/K.

Interference (Endogenous and Exogenous)

Exogenous Interference

The impact of exogenous interfering substances on the performance of the PGDx elio tissue complete CDx assay was assessed by processing DNA from FFPE samples tested in the presence of each interfering substance at varying amounts (**Table 1.9**). The samples were evaluated for concordance of variant calls when compared to samples processed without the interfering substances. Replicates for 5 test cases were analyzed for 8 experimental and 2 baseline conditions.

Table 1.9 Exogenous Interfering Substances Tested

Substance	Amount in Excess of Standard Conditions
Proteinase K	2X and 3X
Indexed adapters	15% and 30%
Melanin	0.2 µg/mL and 1.6 µg/mL
Ethanol	2.5% and 5%

Analysis of BRAF V600E showed high PPA (100% 6/6) and NPA (100% 114/114) across 8 conditions. The results show minimal risk to assay performance from interfering exogenous substances.

Endogenous Interference

The impact of necrosis and FFPE block age on the performance of PGDx elio tissue complete CDx was evaluated by assessing the first pass and overall pass rates of samples processed in a panel-wide accuracy study. Of 521 samples enrolled for accuracy, 448 were evaluated (in 12 tumor types) for necrosis over a range of 0-75%, and 378 (in 12 tumor types) were evaluated for age of block over a range of 0-253 months.

Of the 521 clinical samples in the study, 46 were positive for BRAF V600E, and 16 of which were melanoma samples. Percent necrosis of these samples ranged from 0% to 50%, and block age ranged from 0 (DNA extracted same day as fixation) days to 189 days. A total of 19 of the BRAF V600E positive samples (10 were melanoma samples) had orthogonal data from the comparator assay with 100% PPA (19/19), demonstrating that CDx variant BRAF V600E is not impacted by either endogenous interferent. There were 4 BRAF V600K positive samples, all of which were melanoma samples. One BRAF V600K positive sample had orthogonal data from the comparator assay with 100% PPA (1/1). Percent necrosis for this sample was 0% and FFPE block age was 21 days. The data indicated there is no correlation between necrosis and pass rate.

Sample Carryover and Cross-Contamination

Cross-contamination (contamination from one sample to another within the same batch) and sample carryover (contamination from a previous sequencing run when using the same instrument) were assessed by evaluating false positive and false negative variant calls in 29 FFPE samples. Seven of the 29 cases had known positive variants; the remaining samples were known negative samples. All FFPE samples were assessed across 2 batches to test for contamination within and between runs. In batch 1, a checkerboard pattern within a 96-well plate was created by alternating the samples with representative positive variants and known negative samples. Batch 2 contained known negative samples and was pooled and sequenced directly after completion of batch 1 sequencing, following standard instrument cleaning procedures. No positive variant results were

observed in known negative samples tested. Therefore, the PGDx elio tissue complete CDx assay workflow presents minimal risk for contamination.

Assay Guardbanding

Systematic variations of reagent volumes, enzyme concentrations, and bead drying times in the laboratory workflow were tested to verify the robustness of PGDx elio tissue complete CDx. A total of 14 guardbanding conditions were tested, which included variations to the ER/AT step, the ligation step, the post-ligation PCR and cleanup steps, capture baiting, and post-hybridization PCR. A total of three cases (2 FFPE clinical cases in 2 tumor types, including melanoma, and 1 FFPE blend) were assessed in this study; these three cases also included a BRAF V600E-positive melanoma case and BRAF V600K-positive melanoma blend (this case was also positive for an ALK translocation). The PPA and NPA for BRAF V600E and V600K variants were 100% across all evaluated conditions.

Sample and Reagent Stability

Sample Stability

DNA stability was assessed by aging genomic DNA post-extraction and pooled libraries pre-sequencing stored at $\leq -20^{\circ}\text{C}$ and processing on PGDx elio tissue complete CDx. For genomic DNA stability, 2 BRAF V600E-positive samples (1 was in melanoma), and 2 BRAF V600K-positive samples (both were in melanoma) were evaluated. For pooled library stability, 2 BRAF V600E-positive melanoma samples were evaluated. The duration of DNA storage at the time of the evaluation ranged from 97 to 377 days post-extraction or post-library creation, with a median of 330 days (approximately 10.6 months) and a mean of 295 days (approximately 9.5 months). The 2 V600E samples were stored for 97 and 354 days, and the 2 V600K samples were stored for 328 and 330 days before reprocessing. In the genomic DNA samples, both BRAF V600E and V600K had 100% PPA (2/2) and 100% NPA (42/42). The pooled library samples the BRAF V600E PPA was 100% (3/3) and NPA was 100% (23/23). There were no observations of BRAF V600K in the pooled libraries, however 100% specificity (26/26) was observed.

Real Time Stability

The shelf-life of PGDx elio tissue complete CDx was assessed over time by evaluating variant concordance between the standard condition (0 months) and various timepoints (up to 19 months). A cell line blend with BRAF V600E was assessed in this study. The sample was tested in triplicate across three unique lots at seven different timepoints between 0 months (T0) and 19 months (T19). PGDx elio tissue complete CDx demonstrated concordant results for BRAF V600E with 100% PPA (3/3) and 100% NPA (12/12) for each reagent kit lot and each time point. Based on the results from this study, the PGDx elio tissue complete CDx kit has a shelf-life of 18 months.

Transport Stability

Transport stability was evaluated to demonstrate that the kits can withstand the intended storage and transport conditions. The kits were put under excessive heat and freezing temperature stresses. The kits were evaluated at baseline (T0), immediately post-stress (T1), and at a long-term storage timepoint (T16). T16 data was generated using kits that were stored under standard conditions for 16 months post manufacturing and then subjected to the stress conditions. One reagent kit lot was tested for each condition, for a total of three kits. A cell line blend containing BRAF V600E was assessed in triplicate across all five conditions. For all test conditions at all time points, BRAF V600E PPA was 100% (3/3) and NPA was 100% (12/12). PGDx elio tissue complete CDx demonstrated robust stability for shipment over 72 hours in transit.

Freeze/Thaw Stability

The in-use stability of PGDx elio tissue complete CDx kit components was determined by executing a controlled series of thaw/re-freeze cycles of -20°C and -80°C reagents, at 2 timepoints: 0 months (T0) and 18 months (T18). A cell line blend containing BRAF V600E was assessed in triplicate using kits that had undergone 0, 4, or 5 thaw/re-freeze cycles, across both timepoints. For all test conditions at time points, BRAF V600E PPA was 100% (3/3) and NPA was 100% (12/12). The assay demonstrated robust stability across 2 timepoints and up to 5 thaw/re-freeze cycles. Based on the results from this study, the PGDx elio tissue complete CDx kit can undergo 4 thaw/re-freeze cycles.

Summary of Primary Clinical Studies

PGDx elio tissue complete CDx Clinical Concordance Study for BRAF V600E and V600K Mutations in Melanoma

The safety and effectiveness of PGDx elio tissue complete CDx as a follow-on companion diagnostic (FCD) was evaluated for the detection of BRAF V600E and V600K mutations in patients with melanoma who may be eligible for treatment with FDA-approved BRAF inhibitor therapies or BRAF/MEK inhibitor combination therapies. A real-time PCR test intended for the detection of BRAF V600E and V600K mutations in FFPE melanoma tissue samples was used as the comparator CDx method (CCD).

A total of 379 unique FFPE melanoma tissue samples were tested twice using the CCD and once using PGDx elio tissue complete CDx. Non-inferiority was established by comparing the agreement between PGDx elio tissue complete CDx and CCD to the agreement observed between two replicates from the CCD.

To support the CDx indications listed in **Table 1.1**, the cohort was analyzed for amino acid concordance.

Definitions and calculations used in non-inferiority analyses are provided below:

- PPA_{C1F} is the proportion of CCD1 positive results in which FCD is positive.
- PPA_{C2F} is the proportion of CCD2 positive results in which FCD is positive.
- PPA_{C1C2} is the proportion of CCD1 positive results in which CCD2 is positive
- PPA_{C2C1} is the proportion of CCD2 positive results in which CCD1 is positive.
- NPA_{C1F} is the proportion of CCD1 negative results in which FCD is negative.
- NPA_{C2F} is the proportion of CCD2 negative results in which FCD is negative.
- NPA_{C1C2} is the proportion of CCD1 negative results in which CCD2 is negative.
- NPA_{C2C1} is the proportion of CCD2 negative results in which CCD1 is negative.

The differences in agreement (zetas) were calculated as follows:

- $\zeta PPA1$ ($PPA_{C1C2} - PPA_{C1F}$)
- $\zeta PPA2$ ($PPA_{C2C1} - PPA_{C2F}$)
- $\zeta NPA1$ ($NPA_{C1C2} - NPA_{C1F}$)
- $\zeta NPA2$ ($NPA_{C2C1} - NPA_{C2F}$)

Concordance data is provided below in **Table 1.10** (BRAF V600E results only), **Table 1.11** (BRAF V600K results only), and **Table 1.12** (BRAF V600E and V600K combined). High concordance was observed between the follow-on companion diagnostic (FCD) and the comparator device (CCD) for detection of both BRAF V600E and V600K mutations.

Concordance assessment of BRAF V600E variants yielded a PPA of 98.3% and an NPA of 99.2% (**Table 1.10**). In aggregate, BRAF V600E and V600K mutations demonstrated comparable performance of PGDx elio tissue complete CDx relative to the currently marketed CDx test, achieving 98.0% PPA and 99.1% NPA (**Table 1.12**).

These results provide strong evidence that PGDx elio tissue complete CDx can reliably identify melanoma patients harboring BRAF V600E or V600K mutations, aiding in the selection of patients who may benefit from targeted BRAF inhibitor therapy similar to the CCD.

Table 1.10 BRAF V600E Concordance

	CCD1+			CCD1-		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
FCD+	113	0	113	0	3	3
FCD-	2	0	2	0	261	261
Total	115	0	115	0	264	264
PPA C1F	98.3% 113/115 (93.9%, 99.5%)		NPA C1F		98.9% 261/264 (96.7%, 99.6%)	
PPA C2F	98.3% 113/115 (93.9%, 99.5%)		NPA C2F		98.9% 261/264 (96.7%, 99.6%)	
PPA C1C2	100% 115/115 (96.76%, 100.0%)		NPA C1C2		100% 264/264 (98.56%, 100.0%)	
PPA C2C1	100% 115/115 (96.76%, 100.0%)		NPA C2C1		100% 264/264 (98.56%, 100.0%)	
ζppa1 (CI95)	0.0174 (0, 0.045)					
ζppa2 (CI95)	0.0174 (0, 0.045)					
ζnpa1 (CI95)	0.0114 (0, 0.0256)					
ζnpa2 (CI95)	0.0114 (0, 0.0256)					

Table 1.11 BRAF V600K Concordance

	CCD1+			CCD1-		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
FCD+	35	0	35	0	0	0
FCD-	3	0	3	0	341	341
Total	38	0	38	0	341	341
PPA C1F	92.1% 35/38 (79.2%, 97.3%)		NPA C1F		100% 341/341 (98.88%, 100.0%)	
PPA C2F	92.1% 35/38 (79.2%, 97.3%)		NPA C2F		100% 341/341 (98.88%, 100.0%)	
PPA C1C2	100% 38/38 (90.81%, 100.0%)		NPA C1C2		100% 341/341 (98.88%, 100.0%)	
PPA C2C1	100% 38/38 (90.81%, 100.0%)		NPA C2C1		100% 341/341 (98.88%, 100.0%)	
ζppa1 (CI95)	0.0789 (0, 0.175)					
ζppa2 (CI95)	0.0789 (0, 0.175)					
ζnpa1 (CI95)	0 (0, 0)					
ζnpa2 (CI95)	0 (0, 0)					

Table 1.12 BRAF V600E and V600K Concordance

	CCD1+			CCD1–		
	CCD2+	CCD2-	Total	CCD2+	CCD2-	Total
FCD+	148	0	148	0	3	3
FCD-	5	0	5	0	602	602
Total	153	0	153	0	605	605
PPA C1F	96.7% 148/153 (92.6%, 98.6%)		NPA C1F		99.5% 602/605 (98.6%, 99.8%)	
PPA C2F	96.7% 148/153 (92.6%, 98.6%)		NPA C2F		99.5% 602/605 (98.6%, 99.8%)	
PPA C1C2	100% 153/153 (97.55%, 100.0%)		NPA C1C2		100% 605/605 (99.36%, 100.0%)	
PPA C2C1	100% 153/153 (97.55%, 100.0%)		NPA C2C1		100% 605/605 (99.36%, 100.0%)	
ζppa1 (CI95)	0.0327 (0.0068, 0.0633)					
ζppa2 (CI95)	0.0327 (0.0068, 0.0633)					
ζnpa1 (CI95)	0.005 (0, 0.0114)					
ζnpa2 (CI95)	0.005 (0, 0.0114)					

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Product Labeling

Table 1.13 Symbol Explanations

Label Symbol	Definition
	Danger: Specific Target Organ Toxicity (Oral - Central Nervous System) (Category 1)
	Warning: Skin Corrosion/Irritation (Category 2) Warning: Serious Eye Damage/Irritation (Category 2)
	Manufacturer
	Catalog Number
	Lot Number
	Expiration Date (DDMMYYYY)
	Consult Instructions for Use
	Storage Temperature Range
	For In Vitro Diagnostic Use
	Serial number

Revision History

Table 1.14 Part 1 Revision History

Date	ECR	Change Description
September 2026	ECR-001551	Initial release



Part 2 – User Guide



Rx Only



A0420100

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Product Components and Storage

Table 2.1 PGDx elio tissue complete CDx: Reagents and Storage (A0420100)

Storage Temp. (°C)	Component Name (P/N)	Volume	Cap Label
Library Preparation Kit, Box 1 of 2 (B12119)			
-25 to -15	ER/AT Buffer (20201)	302 µL	ER/AT Buffer
-25 to -15	ER/AT Enzyme (20202)	147 µL	ER/AT Enzyme
-25 to -15	Ligation Buffer (20203)	1.3 mL	Lig Buffer
-25 to -15	DNA Ligase (20204)	431 µL	DNA Ligase
-25 to -15	HotStart PCR Mix (2x) (20205)	1.0 mL	PCR Mix
-25 to -15	Primer Mix (10x) (20206)	204 µL	Primer Mix
-25 to -15	Nuclease-Free Water (20207)	4.9 mL	None
-25 to -15	MB_01 (20209)	20 µL	MB_01
-25 to -15	MB_02 (20210)	20 µL	MB_02
-25 to -15	MB_03 (20211)	20 µL	MB_03
-25 to -15	MB_04 (20212)	20 µL	MB_04
-25 to -15	MB_05 (20213)	20 µL	MB_05
-25 to -15	MB_06 (20214)	20 µL	MB_06
-25 to -15	MB_07 (20215)	20 µL	MB_07
-25 to -15	MB_08 (20216)	20 µL	MB_08
-25 to -15	MB_09 (20217)	20 µL	MB_09
-25 to -15	MB_10 (20218)	20 µL	MB_10
-25 to -15	MB_11 (20219)	20 µL	MB_11
-25 to -15	MB_12 (20220)	20 µL	MB_12
-25 to -15	MB_13 (20221)	20 µL	MB_13
-25 to -15	MB_14 (20222)	20 µL	MB_14
-25 to -15	MB_15 (20223)	20 µL	MB_15
-25 to -15	MB_16 (20224)	20 µL	MB_16
-25 to -15	MB_17 (20225)	20 µL	MB_17
Library Preparation Kit, Box 2 of 2 (B12120)			
2 to 8	Pre-PCR Beads (20301)	11.8 mL	None
Capture Kit, Box 1 of 4 (B12121)			
-25 to -15	Hyb Blocker 1 (20401)	47 µL	Hyb Blocker 1
-25 to -15	100 µM Primer 1 (20402)	27 µL	100 µM Primer 1
-25 to -15	100 µM Primer 2 (20403)	27 µL	100 µM Primer 2

Storage Temp. (°C)	Component Name (P/N)	Volume	Cap Label
Capture Kit, Box 1 of 4 (B12121) continued			
-25 to -15	Hyb Blocker 2 (20404)	208 µL	Hyb Blocker 2
-25 to -15	RNase Block (20405)	23 µL	RNase Block
-25 to -15	Hybridization Buffer (20406)	332 µL	Hyb Buffer
-25 to -15	DNA Pol Buffer (20407)	431 µL	Pol Buffer
-25 to -15	DNA Pol Enzyme (20408)	46 µL	Pol Enzyme
-25 to -15	dNTP Mix (20409)	23 µL	dNTP Mix
-25 to -15	Nuclease-Free Water (20207)	4.9 mL	None
Capture Kit, Box 2 of 4 (B12122)			
15 to 30	Binding Buffer (20501)	37.3 mL	None
15 to 30	Wash Buffer 1 (20502)	8.8 mL	None
15 to 30	Wash Buffer 2 (20503)	56.4 mL	None
Capture Kit, Box 3 of 4 (B12123)			
2 to 8	Post-PCR Beads (20601)	6.8 mL	None
2 to 8	Capture Beads (20602)	2.4 mL	None
Capture Kit, Box 4 of 4 (B12124)			
-85 to -65	Capture Baits (20701)	71 µL	Capture Baits
External Control (B12125)			
2 to 8	External Control (20802)	5 µL	Ext Control

PGDx elio Server and Software

The proprietary PGDx elio™ server contains analysis and reporting software necessary for the PGDx elio tissue complete CDx assay (software versions are displayed within the PGDx elio platform user interface and on reports).

The software is only compatible with NextSeq® 550Dx instruments with the PGDx elio LRM Module installed. The PGDx elio server saves reports only and does not provide storage or backup of raw sequencing data. The PGDx elio server should be placed in accordance with consensus standards. It is recommended that the PGDx elio server be placed in a server room. **Table 2.2** provides details of limits and conditions that the server was tested relative to the consensus standard. Please contact PGDx Tech Services at 1-855-PGDx-NOW (1-855-743-9669) or by email at PGDxTechService@labcorp.com to shut down or restart the server.

Table 2.2 Compliance Level for each Emission and Immunity Test

Phenomenon	Basic EMC Standard or Test Method	Test Level for PGDx elio Server
Electrostatic Discharge	IEC 61000-4-2	Contact discharge up to ± 8 kV Contact air up to ± 15 kV
Radiated RF EM Fields	IEC 61000-4-3	Test levels of A/m 80% at 1 kHz. 1) Swept test for 80-1000 MHz 3 V/m 2) Spot test for 1.8 GHz, 2.6 GHz, 3.5 GHz, 5.0 GHz: 3V/m

Phenomenon	Basic EMC Standard or Test Method	Test Level for PGDx elio Server
Proximity fields from RF wireless communications equipment	IEC 61000-4-3	N/A
RATED Power Frequency Magnetic Fields	IEC 61000-4-8	Test fields strength 1 A/m
Electrical Fast Transients / Bursts	IEC 61000-4-4	± 1 kV ± 0.5 kV for signals, Telecom
Surges	IEC 61000-4-5	± 2 kV and ± 1 kV
Conducted Disturbances Induced by RF Fields	IEC 61000-4-6	3 V 0.15 MHz – 10 MHz 3 V to 1V 10-30 MHz 1 V 30-80 MHz 1 V 80 MHz 80% A/m at 1 kHz
Voltage Dips, Short Interruptions and Voltage Variations	IEC 61000-4-11	Residual 4% Dips, 0.5 period Residual 70% Dips, 25 period Residual 4% Interruptions, 250 period

Required: NextSeq 550Dx Instrument, Not Provided

- Illumina NextSeq 550Dx instruments are not provided. Illumina instrument(s) and reagent kits can be purchased through Illumina, Inc.
- Illumina NextSeq 550Dx High Output Reagent Kit (300 Cycle) IVD is required for use with PGDx elio tissue complete CDx.
- Please contact PGDx Tech Services at 1-855-PGDx-NOW (1-855-743-9669) to install the required PGDx elio LRM (Illumina Local Run Manager) module.

Equipment, Consumables and Reagents

Reagents Required, Not Provided

- DNA extraction kits for FFPE tissue
- Molecular Biology Grade (MBG) water – Non DEPC-treated
- 1X Tris-EDTA (TE) Buffer, pH 7.6
- Molecular Biology Grade (MBG) 100% ethanol
- Tween® 20
- 10 mM Tris-HCl, pH 8.5
- 1 M Tris-HCl, pH 7.0
- 1 N NaOH
- DNA fragment analyzer reagents
- Fluorometer reagents
- Decontamination spray or wipes adequate for work with DNA
- Sequencing Reagent Kit: NextSeq 550Dx High Output Reagent Kit (300 Cycle)

- Use ancillary reagents only per the instructions for use contained in this package insert. Any assay problems or failures that are suspected to involve ancillary reagents should be reported to PGDx.

Recommended Reagents, Not Provided

- Sequencing filler: PGDx elio™ diversiPhi (P/N A0840101).
- **Note:** If pooling less than a complete batch of samples (15 samples and external control), use PGDx elio diversiPhi or a previously captured library with a unique sample index as a sequencing filler to maintain lane ratio. If sequencing a full batch, sequencing filler is not required.

Consumables Required, Not Provided

- Disposable divided reservoirs, sterile
- DNA fragment analyzer consumables
- DNA mechanical shearing consumables
- Standard, thin walled 96-well PCR plates
- 96-well deep well plates
- 0.5 mL microtubes (compatible with fluorometer)
- 1.5 or 2 mL DNA low binding microtubes
- Conical tubes (5 mL, 15 mL, and/or 50 mL)
- 96-well plate adhesive seals
- Ice or 96-well cold block
- 20, 200 and 1000 µL tips, filtered, low retention
- 8-strip tubes
- 8-strip domed caps
- Lint free tissues
- Alcohol wipes
- Appropriate PPE

Table 2.3 Required Equipment, Not Provided

Equipment	Notes
DNA shearing instrument	Mechanically shears DNA to the appropriate size.
DNA fragment analyzer	Automated sample processing determines size, quantity and purity for quick library QC.
Fluorometer	Uses detection of target-specific fluorescence to provide quantification of samples prior to library preparation and sequencing. Separate fluorometers are required in pre-PCR and post-PCR areas.
Magnetic stand	Designed for paramagnetic bead precipitation from standard and deep 96-well microplates. Separate magnetic stands are required in pre-PCR and post-PCR areas.
Mini-centrifuge or micro-centrifuge	Tabletop micro-centrifuge or mini-centrifuge capable of holding 0.5 mL to 2.0 mL tubes. Separate micro- or mini-centrifuges are required in pre-PCR and post-PCR areas.

Equipment	Notes
Thermal cycler	One 96-well dual-block thermal cycler (or two 96-well single block thermal cyclers) is required in the post-PCR areas.
Tabletop 96-well plate centrifuge	Any plate centrifuge capable of maintaining 280 x g for at least 1 minute is sufficient. Separate plate centrifuges are required for pre-PCR and post-PCR areas.
Thermomixer	Thermomixer capable of temperatures ranging from 20 °C to 70 °C and shaking at 1700 rpm. Two thermomixers or two thermal cyclers (or one thermal cycler with multiple thermal blocks) are required in the pre-PCR area and one thermomixer is required in the post-PCR area.
Tabletop vortex mixer	Separate vortex mixers are required in pre-PCR and post-PCR areas.
Single-channel pipettors (P-2, P-10, P-20, P-200, P-1000)	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.
Multi-channel pipettor (P-20, P-200)	Separate sets of pipettors are required in pre-PCR and post-PCR areas. Pipettors should be calibrated regularly and verified accurate within 5% of stated volume.

Specimen Type and Handling

The assay is compatible with genomic DNA isolated from formalin-fixed, paraffin-embedded (FFPE) samples using any appropriate commercially available FFPE extraction method. Tissue specimen preparation should follow standard ASCO-CAP protocols for formalin fixation (Sauter et al., 2009). The user should follow DNA extraction kit manufacturer instructions for specimen preparation and thickness for optimal DNA extraction. DNA extraction kits should be able to yield 50 ng of DNA with a minimum concentration of 1 ng/μL. The assay has been validated with extracted DNA stored at ≤ -20 °C for up to 9 months.

PGDx elio tissue complete CDx was validated with FFPE samples containing at least 20% tumor nuclei of the total viable nuclei (viable tumor nuclei + viable non-tumor nuclei) in the selected tumor area. If less than 100% of the tissue section contains ≥ 20% tumor purity, the tissue should be macro-dissected to select as much viable tumor as possible and minimize the amount of adjacent non-tumor tissue per the recommendations below.

Sample Preparation Recommendations:

Estimating tumor purity and preparing tissue sections for macro-dissection:

- Identify and locate area of tumor on H&E and outline/circle it with a permanent marker appropriate for glass
- Tips for tumor area selection:
 - Select as much viable tumor as possible.
 - If possible, avoid areas of necrosis.
 - If possible, minimize selection of peripheral inflammatory cells or areas of obscuring inflammatory cells if other less-obscured areas are present for selection.
 - If possible, when a sample is relatively large and tumor is multifocal, outline multiple distinct foci such that as much intervening stroma and/or inflammatory cells are excluded; doing so increases the tumor purity for the selected areas.
- Tips for assessing tumor purity:
 - Tumor purity is defined as the percentage of tumor cell nuclei in a solid tumor sample.
 - Manual visual assessment of tumor purity is a semi-quantitative estimate based on an assessment of the percentage of nucleated tumor cells to all nucleated cells in a selected tumor area. It is not assessed as percentage of area.

- Note that small lymphocytes or other inflammatory non-tumor cells, particularly when abundant in a selected tumor area, can “dilute” the tumor purity of the selected area; i.e., while the tumor may cover a significant portion of the selected tumor area, tumor purity may be lower than expected when many lymphocytes/lymphoid aggregates are present in the selected area.
- Estimates of tumor purity are most accurate and reproducible when provided in deciles or quartiles; i.e., there is more consistency and accuracy at these values and less so at values more granular than this, such as 35.

Warnings and Precautions – Assay Process

1. PGDx elio tissue complete CDx assay is designed for use by personnel trained in molecular diagnostic techniques including PCR and NGS.
2. This assay contains tetramethylammonium chloride, a mixture that is toxic and causes damage to organs. Personal injury may occur through ingestion, inhalation, or skin contact. Before handling all reagents provided in the kit, read and understand all applicable Safety Data Sheets and use appropriate personal protective equipment.
3. Handle all specimens carefully to avoid sample swap or cross-contamination. Use fresh pipette tips between samples and between dispensing of reagents. Mix samples with a pipettor and centrifuge the plate when indicated. Do not vortex plates unless instructed to do so. Use filtered tips to reduce the risk of amplicon carry-over and cross-contamination. Do not splash contents.
4. This device will not detect all instances of contamination when the contaminant is a high purity cell line that contains a translocation. Extra care should be taken to avoid cross-contamination of cell lines.
5. To avoid cross-contamination between samples, skip a column between samples in a 96-well plate layout.
6. Molecular laboratory techniques that minimize contamination are recommended to mitigate occurrence of PCR products from contaminating reagents, instrumentation, and genomic DNA samples. To prevent contamination, ensure that pre-PCR and post-PCR work areas have dedicated equipment (e.g., pipettors, pipette tips, vortex mixers, and centrifuges). PCR contamination may cause inaccurate and unreliable results.
7. Samples batched in the same sequencing run must have unique index adapters. Do not pool samples that have the same indices even when using previously captured library as a filler. Sequencing multiple samples prepared with the same index adapter will result in analysis failure.
8. Failure to follow the procedures as outlined may result in erroneous results or significant reduction in data quality.
9. PGDx elio tissue complete CDx does not store binary base call (BCL) files. Users should backup necessary files using appropriate local storage. Users should also backup all reports generated by the PGDx elio server for long-term storage. When data partitions exceed 80% capacity, a warning banner will appear instructing users to delete intermediate files or reach out to PGDx support to assist with storage cleaning. Once the server reaches full capacity, analysis plans will not complete, and cleaning will need to be performed.
10. Do not use any assay components beyond their stated expiration date. Do not mix reagent lots from different kits.
11. Store the assay components at the specified temperature on component labeling.
12. The instructions in this document and/or associated with the product must be strictly and explicitly followed by qualified and properly trained personnel to ensure the proper and safe use of the Product(s) described herein. The contents of this document must be fully read and understood prior to using such Product(s). **FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY. PGDx DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE).**

Procedural Notes

Definitions

- Batch: All samples that are within a single library preparation, capture and sequencing run
- Library: DNA fragments ligated with indexed adapters that can be sequenced
- Molecular biology grade water (MBG): Nuclease-Free Water that is not provided within the PGDx elio tissue complete CDx kit
- bp: Base pairs
- NaOH: Sodium hydroxide
- EDTA: Ethylenediaminetetraacetic acid
- NTC: No template control
- PCR: Polymerase chain reaction
- P/N: Part number
- Briefly centrifuge: 3-5 second centrifugation at maximum speed in a mini- or micro-centrifuge
- Room temperature (RT): 15-30°C
- Ice: May use wet ice or cold block
- n/a: Not applicable
- EC: External control

Guidelines for Mixing and Preparing Reagents

- There is enough reagent volume for 34 reactions. These can be processed in a minimum of two runs, but limit the number of kit freeze/thaw cycles to 4.
- Mixing steps that instruct “pipette up and down to mix” indicate that the user should mix by aspirating and dispensing 80% of the total volume 10 times to form a homogeneous solution unless specific directions are provided at that step.
- Vortex non-enzyme-containing reagents for approximately 5 seconds, followed by a brief centrifugation to collect contents. If precipitate is seen, vortex until precipitate is not seen. Enzymes should not be vortexed.
- Mix reagent bottles by inverting 5 times.
- Prepare fresh 80% ethanol daily. Ethanol may absorb water from the air, impacting results.
- Ensure that all ethanol is removed from the bottom of the wells during wash steps. Residual ethanol may impact results.
- Adhere to the specified drying times during bead wash steps to ensure complete ethanol evaporation. Residual ethanol may impact the performance of subsequent reactions.
- Calculate 10% overage if preparing reagent mixtures for more than one sample.
- Follow the recommended volumes and set ups for reaction mixtures.
- Avoid removing tube caps from more than one reagent at a time to reduce the risk of cap switching and possible reagent contamination.

Guidelines for DNA Requirements

- Optimal DNA input for library preparation is 100 ng.
- If 100 ng is not available, a minimum DNA input of 50 ng may be used.
- A minimum concentration of 1 ng/μL of DNA is required.
- The assay may require up to 10 slides at a minimum 5 micron thickness and 20% tumor purity.
- DNA must be quantified using a calibrated fluorometer following manufacturer's guidelines.

Batch Sizes

PGDx elio tissue complete CDx accommodates flexible batch sizes. The minimum and maximum batch sizes are shown in the table below:

Table 2.4 Batch Size Specifications for PGDx elio tissue complete CDx

	Batch Size	Clinical Samples	External Control	NTC
Maximum	17	15	1	1
Minimum	3	1	1	1

For a batch size of less than 16 samples (including the external control) in a sequencing run, use of a sequencing filler is required to maintain the target 6.25% lane ratio for each sample to be analyzed. Using PGDx elio diversiPhi (P/N A0840101) is recommended. Alternatively, previously captured libraries may be used as a sequencing filler if index adapter sequences remain unique.

Controls

Required – External Control: The external control must be processed in the same manner as tissue-derived genomic DNA (gDNA) – through library preparation, target enrichment, sequencing, and analysis – to monitor reagent performance within the assay. The external control is a cell-line derived reference standard product supplied by PGDx (P/N 20802). The external control contains multiple validated variants within the PGDx elio tissue complete CDx regions of interest (ROIs). The external control must be processed and sequenced with every batch of the PGDx elio tissue complete CDx assay or analysis will produce a failed report.

Recommended - No Template Control (NTC): A sample that does not contain DNA (1X TE, pH 7.6 or mechanical shearing buffer) is recommended during library preparation and target enrichment steps for use as a negative control. The NTC is recommended to detect in-process or reagent contamination. The presence of contamination (i.e. DNA) in the NTC after hybrid capture (average size of > 250 bp and having a peak concentration of > 5 ng/μL within the 180-800 bp region), as assessed by a DNA fragment analyzer, may indicate DNA contamination of reagents during library preparation and target enrichment steps has occurred. NTC is not meant to be sequenced.

Instructions for Use

Table 2.5 Kit and Reagent Lot Tracking

Reagent (Stored at RT unless specified)	Lot Number	Expiration Date
Normalization Lot Tracking (Section 1)		
Tris-EDTA Buffer, pH 7.6 (user supplied)		
Molecular biology grade water – non-DEPC (user supplied)		
External Control (B1207) (4 °C)		
Library Preparation Pre-PCR Reagent Tracking (Section 1)		
Library Preparation Kit, Box 1 of 2 (B1201) (-20 °C)		
Library Preparation Kit, Box 2 of 2 (B1202) (4 °C)		
100% ethanol, molecular biology grade (user supplied)		
Molecular biology grade water – non-DEPC (user supplied)		
Library Preparation Post-PCR Reagent Tracking (Section 1)		
Capture Kit, Box 1 of 4 (B1203) (-20 °C)		
Capture Kit, Box 3 of 4 (B1205) (4 °C)		
100% ethanol, molecular biology grade (user supplied)		
Molecular biology grade water – non-DEPC (user supplied)		
Target Enrichment Reagent Tracking (Section 2)		
Capture Kit, Box 1 of 4 (B1203) (-20 °C)		
Capture Kit, Box 2 of 4 (B1204) (room temperature)		
Capture Kit, Box 3 of 4 (B1205) (4 °C)		
Capture Kit, Box 4 of 4 (B1206) (-80 °C)		
100% ethanol, molecular biology grade (user supplied)		
Molecular biology grade water – non-DEPC (user supplied)		
Sequencing Reagent Tracking (Section 3)		
NextSeq Reagent Cartridge		
NextSeq Accessory Box		
NextSeq Buffer Cartridge		
NextSeq Flow cell		
1 N NaOH		
Molecular biology grade water – non-DEPC (user supplied)		
Tween 20		

Reagent (Stored at RT unless specified)	Lot Number	Expiration Date
1.0 M Tris-HCl, pH 7.0		
10 mM Tris-Cl, pH 8.5 (or equivalent)		
Sequencing Filler (if needed), e.g., PGDx elio diversiPhi		

1. Library Preparation

Note: All steps listed below should be carried out in a pre-PCR work area unless stated otherwise.

1.1 Sample Preparation and Shearing

1. _____ Quantify each DNA sample using a fluorometer and dilute 100 ng to a final volume of 130 μ L in 1X TE, pH 7.6 (not provided) in an appropriate shearing tube.
2. _____ Record information in **Table 2.6**. Skipping columns is recommended to avoid potential sample cross-contamination.

Table 2.6 Sample Preparation

Well	Sample ID	DNA Conc. (ng/ μ L)	DNA Input Volume (μ L) (ng of DNA / DNA Conc,)	1X TE, pH 7.6 (μ L) (130 μ L – Input DNA Volume)

3. _____ Prepare external control by combining 1 μ L external control DNA + 129 μ L 1X TE, pH 7.6 for a final volume of 130 μ L in an appropriate shearing tube. A no template control (NTC) containing only 130 μ L 1X TE, pH 7.6 is recommended.
4. _____ Mechanically shear the samples to a target of 200 bp using an appropriate shearing instrument.
5. _____ Briefly centrifuge the tubes containing the samples to collect the samples at the bottom of the tubes.
6. _____ Transfer the samples (approximately 130 μ L) to a deep-well plate, per the well positions defined in **Table 2.6** and purify the sheared DNA as follows:
 - a. _____ Allow Pre-PCR Beads (P/N 20301) to equilibrate at room temperature (RT) for 30 minutes. Vortex for 30 seconds at the highest setting before use.
 - b. _____ Allow Nuclease-Free Water (P/N 20207) to thaw at RT.
 - c. _____ Prepare fresh 80% ethanol using 100% molecular biology grade (MBG) ethanol (not provided) and MBG water (not provided).

Table 2.7 80% Ethanol Preparation

Volume 100% MBG Ethanol (mL)	Volume MBG Water (mL)

- d. _____ Fill a sterile, disposable reservoir with Pre-PCR beads with 10% overage volume.
Number of samples * 156 * 1.1 (μ L): _____
- e. _____ Add 156 μ L Pre-PCR beads to each sample. Pipette up and down 10 times to mix using P-200 multichannel pipettor set to 156 μ L.
- f. _____ Incubate at RT for 5 minutes.
- g. _____ Place the plate containing samples on a magnetic stand and wait until the solution is clear (3-5 minutes). Carefully remove and discard the supernatant without disturbing the beads using P-200 multichannel pipettor set to 156 μ L.
- h. _____ Allow beads to settle on the magnet (3-5 minutes), then carefully remove remainder of supernatant using a P-200 multichannel pipettor set to 156 μ L.
- i. _____ With the plate on the magnetic stand, add 150 μ L 80% ethanol without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard all the supernatant without disturbing the beads using a P-200.
- j. _____ Repeat the 80% ethanol wash (step 6.i.). Carefully remove all traces of ethanol after the second wash using a P-20 multichannel pipettor set to 20 μ L.
- k. _____ Air dry the beads for 5 minutes on the magnetic stand.
- l. _____ Fill a sterile, disposable reservoir with Nuclease-Free Water (P/N 20207) with 10% overage volume, from Library Preparation Kit, Box 1 of 2 (P/N B12119).
Number of samples * 51 * 1.1 (μ L): _____
- m. _____ Remove the plate from the magnetic stand and resuspend the beads in 51 μ L Nuclease-Free Water (P/N 20207).
- n. _____ Incubate at RT for 2 minutes.
- o. _____ Place the plate back on the magnetic stand until the solution is clear (approximately 2 minutes).
- p. _____ With the plate on the magnetic stand, transfer 50 μ L of clear supernatant to a new 96-well plate.

Note: This is a safe stopping point. The samples may be stored, sealed at 2 to 8 °C overnight.

Operator Name:

Signature:

Date:

1.2 End-Repair and A-Tailing (ER/AT)

1. ____ Set a thermomixer or thermal cycler to 20 °C for step 5 and another thermomixer or thermal cycler to 65 °C for step 6.
2. ____ To create the ER/AT master mix, thaw ER/AT Buffer (P/N 20201) at RT and ER/AT Enzyme (P/N 20202) on ice.
3. ____ On ice, prepare ER/AT master mix in a 1.5 to 2.0 mL tube according to **Table 2.8**. Pipette up and down 10 times to mix using P-200 pipettor set to 80% of the volume, and briefly centrifuge the tube to collect the mix at the bottom of the tube.

Table 2.8 ER/AT Master Mix Preparation

Reagent	1 Sample (μL)	# of Samples x 1.1	Reagent Volume (μL)
ER/AT Buffer	7		
ER/AT Enzyme	3		
Total Volume (μL)			
0.8 x Total Volume (μL)			

4. ____ Add 10 µL of the ER/AT master mix to each sample. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 50 µL. Seal the plate with an adhesive film and centrifuge at 230 x g for 30 seconds to collect the sample to the bottom of the well.
5. ____ Incubate the plate in a thermomixer or thermal cycler pre-set to 20 °C for 30 minutes.
6. ____ Immediately after step 5, transfer the plate to a thermomixer or thermal cycler pre-set to 65 °C and incubate for 30 minutes.
7. ____ Immediately after step 6, remove the plate from the thermomixer or thermal cycler and incubate at RT for 2 minutes.
8. ____ Centrifuge the plate at 230 x g for 30 seconds to collect the samples at the bottom of the wells.
9. ____ Proceed to Adapter Ligation immediately.

1.3 Adapter Ligation

1. ____ Assign a unique Index Adapter (MB_01 to MB_17) to each sample. Record Sample IDs and Index Adapters in Table 2.9.
2. ____ Thaw Index Adapters (P/N 20209 to 20225) and Ligation Buffer (P/N 20203) at RT and DNA Ligase (P/N 20204) on ice.

Table 2.9 Adapter Ligation

Well	Sample ID	Index Adapter

Well	Sample ID	Index Adapter

Note: Do not duplicate Index Adapters for samples that are intended for the same sequencing run.

3. ____ Add 7.5 μ L of the appropriate Index Adapter to each sample.
4. ____ On ice, prepare ligation master mix in a 1.5 to 2.0 mL tube according to Table 2.10. Pipette up and down 10 times to mix using a P-200 pipettor set to 80% of the volume and briefly centrifuge the tube to collect the mix at the bottom of the tube.

Table 2.10 Ligation Master Mix Preparation

Reagent	1 Sample (μL)	# of Samples x 1.1	Reagent Volume (μL)
Ligation Buffer	30		
DNA Ligase	10		
Nuclease-Free Water (provided)	2.5		
Total Volume (μL)			
0.8 x Total Volume (μL)			

5. ____ Add 42.5 μ L of the ligation master mix to each sample. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 100 μ L. Seal the plate with an adhesive film and centrifuge at 230 x g for 30 seconds to collect the samples at the bottom of the wells.
6. ____ Incubate the plate in a thermomixer or thermal cycler pre-set to 20 °C for 20 minutes.
7. ____ Purify the adapter-ligated DNA as follows:
 - a. ____ Allow Pre-PCR Beads (P/N 20301) to equilibrate at RT for 30 minutes (if beads were stored at 4 °C). Vortex for 30 seconds at the highest setting before use.
 - b. ____ Prepare fresh 80% ethanol using 100% MBG ethanol (not provided) and MBG water (not provided).

Table 2.11 80% Ethanol Preparation

Volume 100% MBG Ethanol (mL)	Volume MBG Water (mL)

- c. ____ Fill a sterile, disposable reservoir with Pre-PCR Beads with 10% overage volume.
Number of samples * 88 * 1.1 (μ L): _____
- d. ____ Add 88 μ L Pre-PCR Beads to each sample. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 88 μ L.

- e. _____ Incubate at RT for 5 minutes.
- f. _____ Place the plate containing samples on a magnetic stand and wait until the solution is clear (3-5 minutes). Carefully remove and discard all the supernatant without disturbing the beads using a P-200 multichannel pipettor set to 200 µL.
- g. _____ With the plate on the magnetic stand, add 150 µL 80% ethanol without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard the supernatant (about 188 µL) without disturbing the beads using a P-200 multichannel pipettor set to 150 µL.
- h. _____ Repeat the 80% ethanol wash (step 7.g.). Carefully remove all traces of ethanol after the second wash using a P-20 multichannel pipettor set to 20 µL.
- i. _____ Air dry the beads for 5 minutes on the magnetic stand.
- j. _____ Fill a sterile, disposable reservoir with Nuclease-Free Water (P/N 20207) with 10% overage volume from Library Preparation Kit, Box 1 of 2 (P/N B12119).
Number of samples * 21 * 1.1 (µL): _____
- k. _____ Remove the plate from the magnetic stand and resuspend the beads in 21 µL Nuclease-Free Water (P/N 20207).
- l. _____ Incubate at RT for 2 minutes.
- m. _____ Place the plate back on the magnetic stand until the solution is clear (approximately 2 minutes).
- n. _____ With the plate on the magnetic stand, transfer 20 µL of clear supernatant to a new 96-well plate.

Note: This is a safe stopping point. The samples may be stored, sealed at –25 to –15 °C for up to 7 days.

Operator Name:

Signature:

Date:

1.4 Library PCR

1. _____ Thaw HotStart PCR Mix (2X) (P/N 20205) and Primer Mix (10X) (P/N 20206) at RT.
2. _____ At RT, prepare library PCR master mix in a 1.5 to 2.0 mL tube according to **Table 2.12**. Pipette up and down 10 times to mix using a P-200 pipettor set to 80% of the volume and briefly centrifuge the tube to collect the mix at the bottom of the tube.

Table 2.12 Library PCR Master Mix Preparation

Reagent	1 Sample (μL)	# of Samples x 1.1	Reagent Volume (μL)
HotStart PCR Mix	25		
Primer Mix	5		
Total Volume (μL)			
0.8 x Total Volume (μL)			

3. _____ Add 30 μL of the library PCR master mix to each sample. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 30 μL. Seal the plate with an adhesive film and centrifuge at 230 x g for 30 seconds. Transfer the plate to post-PCR work area for all subsequent steps.

Note: The remaining steps should be carried out in post-PCR work area.

4. _____ Program a thermal cycler with heated lid on in the post-PCR work area using the cycling conditions in **Table 2.13**.

Table 2.13 Library PCR Cycling Conditions

Step	Temp.	Time	# of Cycles
1	98 °C	45 seconds	n/a
2	98 °C	15 seconds	13
	60 °C	30 seconds	
	72 °C	30 seconds	
3	72 °C	1 minute	n/a
4	4 °C	∞	n/a
Program Name:			
Instrument Number:			

5. _____ Transfer the plate containing samples to the thermal cycler and start the program.

Note: This is a safe stopping point. The samples may be incubated at 4 °C in the thermal cycler or stored at 2 to 8 °C overnight. Incubating samples at 4 °C in thermal cycler, particularly in areas of high humidity, may cause excessive condensation around the block.

Operator Name:

Signature:

Date:

6. _____ After Library PCR, centrifuge the plate at 230 x g for 30 seconds to collect samples at the bottom of the wells before proceeding to step 7.
7. _____ Purify the amplified libraries as follows:
- _____ Allow Post-PCR Beads (P/N 20601) to equilibrate to RT for 30 minutes. Vortex for 30 seconds at the highest setting before use.
 - _____ Prepare fresh 80% ethanol using 100% MBG ethanol (not provided) and MBG water (not provided).

Table 2.14 80% Ethanol Preparation

Volume 100% MBG Ethanol (mL)	Volume MBG Water (mL)

- _____ Fill a sterile, disposable reservoir with Post-PCR Beads with 10% overage volume.
Number of samples * 50 * 1.1 (μL): _____
- _____ Add 50 μL Post-PCR Beads to each sample. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 50 μL.
- _____ Incubate at RT for 5 minutes.
- _____ Place the plate containing samples on a magnetic stand and wait until the solution is clear (3-5 minutes). Carefully remove and discard the supernatant (about 100 μL) without disturbing the beads using a P-200 multichannel pipettor set to 150 μL.
- _____ With the plate on the magnetic stand, add 150 μL 80% ethanol without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard all the supernatant without disturbing the beads using a P-200 multichannel pipettor set to 150 μL.
- _____ Repeat the 80% ethanol wash (step 7.g.). Carefully remove all traces of ethanol after the second wash using a P-20 multichannel pipettor set to 20 μL.
- _____ Air dry the beads for 5 minutes on the magnetic stand.
- _____ Fill a sterile, disposable reservoir with Nuclease-Free Water (P/N 20207) with 10% overage volume from Capture Kit Box, 1 of 4 (P/N B12121).
Number of samples * 21 * 1.1 (μL): _____
- _____ Remove the plate from the magnetic stand and resuspend the beads in 21 μL Nuclease-Free Water.
- _____ Incubate at RT for 2 minutes.
- _____ Place the plate back on the magnetic stand until the solution is clear (approximately 2 minutes).
- _____ With the plate on the magnetic stand, transfer 20 μL of clear supernatant to a new 96-well plate.

Note: This is a safe stopping point. The samples may be stored, sealed at –25 to –15 °C for up to 7 days.

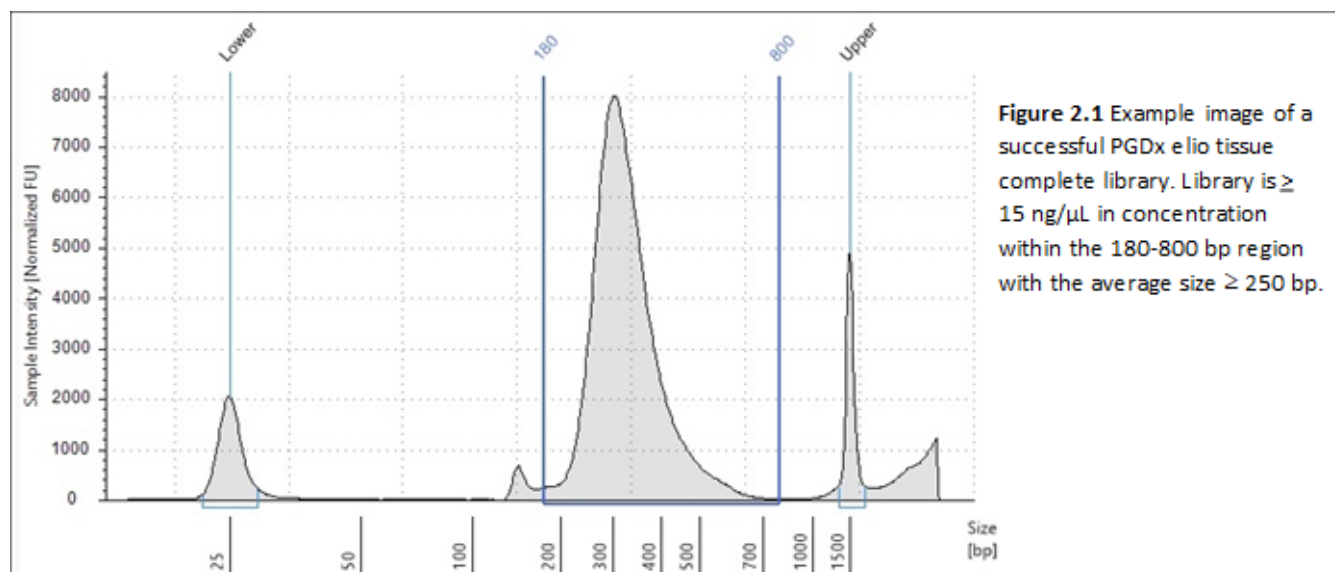
Operator Name:

Signature:

Date:

1.5 Library Preparation QC

1. _____ Use a DNA fragment analyzer to assess DNA libraries and controls by determining the concentration and average peak size (180–800 bp).



2. ____ Using the library QC criteria in **Table 2.16**, determine the PASS/FAIL status of each sample and each control. Record the average size [bp], concentration [ng/μL], and Pass/Fail status for each sample in **Table 2.15**.

Table 2.15 Library Preparation QC Results

[illegible]

Well	Sample ID	Average Size (bp)	Concentration (ng/μL)	Pass/Fail

Table 2.16 Library QC Criteria

Sample Type	Pass Criteria	Fail Criteria	Action if Fail
Sample Library	≥ 15 ng/μL; Average size ≥ 250 bp	< 15 ng/μL and/or Average size < 250 bp	Repeat library prep
External Control (EC)	≥ 15 ng/μL; Average size ≥ 250 bp	< 15 ng/μL and/or Average size < 250 bp	Repeat entire batch

Note: If External Control fails, library preparation should be repeated for all samples in the batch.

Note: A large adapter peak seen in the NTC may be the result of amplified adapter dimer.

Operator Name:

Signature:

Date:

2. Target Enrichment

Note: All steps listed below should be carried out in a post-PCR work area.

2.1 Hybridization

1. _____ Record Sample ID and Index Adapter (assigned in **Table 2.9**) in **Table 2.17**.

Table 2.17 Target Enrichment

[illegible]

- 2._____ If frozen, thaw samples at RT and transfer 12 μ L of each library to a new 96-well plate.
- 3._____ Thaw Hyb Blocker 1 (P/N 20401), Hyb Blocker 2 (P/N 20404), and Nuclease-Free Water (P/N 20207) at RT.
- 4._____ Prepare hybridization blocker mix in a 1.5 to 2.0 mL tube according to **Table 2.18**. Pipette up and down 10 times to mix using a pipettor set to 80% of the volume and briefly centrifuge to collect the mix at the bottom of the tube.

Table 2.18 Hybridization Blocker Mix

Reagent	1 Capture Sample (μL)	# of Samples x 1.1	Reagent Volume (μL)
Hyb Blocker 1	1		
Hyb Blocker 2	5		
Total Volume (μL)			
0.8 x Total Volume (μL)			

5. _____ Add 6 μ L of the hybridization blocker mix to each sample. Pipette up and down 10 times to mix using a P-20 multichannel pipettor set to 6 μ L. Seal the plate with an adhesive film and centrifuge at 230 x g for 30 seconds.
6. _____ Program a thermal cycler with heated lid on in the post-PCR work area according to **Table 2.19**.

Table 2.19 Capture Bait Hybridization Cycling Conditions

Step	Temp.	Time	# of Cycles
1	95 °C	5 minutes	1
2	65 °C	10 minutes	1
3	65 °C	1 minute	1, PAUSE
4	65 °C	1 minute	60
	37 °C	3 seconds	
5	65 °C	∞	n/a
Program Name:			
Instrument Number:			

Note: The thermal cycler MUST be paused during Step 3 of hybridization (65 °C for 1 minute) to allow the addition of reagents to the hybridization wells.

7. _____ Transfer the plate containing samples to the thermal cycler and start the program.
8. _____ During Step 1 (95 °C for 5 minutes) and Step 2 (65 °C for 10 minutes), thaw RNase Block (P/N 20405) and Capture Baits (P/N 20701) on ice and Hybridization Buffer (P/N 20406) at RT.
9. _____ Prepare the hybridization capture master mix according to **Table 2.20**. Pipette up and down 10 times to mix using a P-200 pipettor set to 80% of the volume and briefly centrifuge the tube to collect the mix at the bottom of the tube.

Table 2.20 Hybridization Capture Master Mix

Reagent	1 Capture Sample (μ L)	# of Samples x 1.1	Reagent Volume (μ L)
Hybridization Buffer	6		
Nuclease-Free Water	3.8		
Capture Baits	1.7		
RNase Block	0.5		
Total Volume (μ L)			
0.8 x Total Volume (μ L)			

10. _____ Aliquot the hybridization capture master mix into an 8-well strip tube, 12.5 μ L per sample in each plate row according to **Table 2.21**.

Table 2.21 Hybridization Capture Master Mix Aliquot

Plate Row	Number of samples in a row	Hybridization Capture Master Mix Volume (μ L) (Number of samples in a row x 12.5 μ L)
A		
B		
C		

Plate Row	Number of samples in a row	Hybridization Capture Master Mix Volume (μL) (Number of samples in a row x 12.5 μL)
D		
E		
F		
G		
H		

11. ____ Once Step 3 of the hybridization cycling condition (65 °C for 1 minute) starts on the thermal cycler, pause the program and remove the seal.
12. ____ With the plate containing samples in the thermal cycler, add 12 μL of the hybridization capture mix to each sample. Pipette up and down 10 times to mix using a P-20 multichannel pipettor set to 12 μL. Seal the plate with a new adhesive seal.
13. ____ Resume the program. Record the Hybridization start and end times.
14. ____ Hybridization start time: _____
15. ____ Hybridization end time: _____
16. ____ Prepare Capture Beads following steps in section **2.2 Capture Bead Preparation** while hybridization completes.
17. ____ Once the program is complete, centrifuge the plate at 230 x g for 30 seconds.

2.2 Capture Bead Preparation

1. ____ Vortex Capture Beads (P/N 20602) for 1 minute at the highest setting.
2. ____ Add 50 μL Capture Beads to each well of a new 96-well plate corresponding to sample plate wells.
3. ____ Wash the Capture Beads with Binding Buffer (P/N 20501) as follows:
 - a. ____ Fill a sterile, disposable reservoir with 800 μL per sample Binding Buffer with 10% overage volume (volume = # of samples * 800 * 1.1)
 - b. ____ Add 200 μL Binding Buffer to each well containing 50 μL Capture Beads. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 200 μL.
 - c. ____ Place the plate on a magnetic stand and wait until the solution is clear (1 minute).
 - d. ____ With the plate still on the magnetic stand, carefully remove and discard all the supernatant without disturbing the beads using a P-200 multichannel pipettor set to 200 μL.
 - e. ____ Remove the plate from the magnetic stand.
 - f. ____ Repeat steps 3 b-e twice for a total of 3 washes.
 - g. ____ After the final wash, resuspend beads in 200 μL Binding Buffer.

2.3 Hybridized DNA Library Capture

1. ____ Set a thermomixer to 23 °C at 1700 rpm for Step 3.
2. ____ After hybridization has completed, briefly centrifuge samples prior to addition of capture beads. Transfer 200 μL of cleaned and resuspended Capture Beads to the plate containing 30 μL hybridized DNA library. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 200 μL.

3. _____ Seal the plate with domed micro-amp strip caps and incubate on the thermomixer pre-set to 23 °C at 1700 rpm for 30 minutes.
4. _____ During the 30-minute shaking incubation, pre-warm Wash Buffer 2 (P/N 20503) at 65°C:
 - a. _____ For each sample, prepare 6 aliquots of 200 µL Wash Buffer 2 in 96-well plates.
 - b. _____ Seal the plates with adhesive film and incubate in a thermal cycler at 65 °C.

Note: Wash Buffer 2 must be kept incubated at 65 °C for step 10.

1. _____ After the 30-minute incubation, centrifuge the plate containing samples at 230 x g for 30 seconds.
2. _____ Place the plate on a magnetic stand and wait until the solution is clear (approximately 30 seconds).
3. _____ With the plate still on the magnetic stand, remove and discard all supernatant (about 230 µL) using a P-200 multichannel pipettor.
4. _____ Remove the plate from the magnetic stand and resuspend the beads in 200 µL Wash Buffer 1 (P/N 20502). Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 200 µL.
5. _____ Place the samples on the magnetic stand and wait until the solution is clear (approximately 30 seconds). Remove and discard all supernatant.
6. _____ Wash the beads containing the samples with Wash Buffer 2 as follows:
 - a. _____ Resuspend the beads in 200 µL pre-warmed Wash Buffer 2. Seal the plate with a new adhesive seal.
 - b. _____ Transfer the plate to a thermal cycler and incubate for 5 minutes at 65 °C.
 - c. _____ After the 5-minute incubation, remove the plate from the thermal cycler, place the plate on the magnetic stand and wait until the solution is clear (approximately 30 seconds).
 - d. _____ With the plate still on the magnetic stand, remove and discard all supernatant using a P-200 multichannel pipettor set to 200 µL.
 - e. _____ Repeat step 10 a-d for a total of 6 washes. Use a new adhesive seal for each wash. Check off each wash in **Table 2.22**.
 - f. _____ During washes, thaw PCR reagents and make PCR master mix during Wash 6 (see section **2.4 Target Enrichment PCR**, steps 1-2).

Table 2.22 Wash Buffer 2 Washes

Number of Washes	Wash 1	Wash 2	Wash 3	Wash 4	Wash 5	Wash 6
Wash Completed?						

- g. _____ Carefully remove and discard all supernatant after the final wash. Seal the plate with an adhesive film and centrifuge at 230 x g for 10 seconds to collect any remaining Wash Buffer 2, place the plate on the magnet and carefully remove and discard remaining supernatant using a P-20 multichannel pipettor set to 20 µL.
7. _____ Fill a sterile, disposable reservoir with Nuclease-Free Water (P/N 20207), with 10% overage volume, from Capture Kit Box, 1 of 4 (P/N B12121).

Number of samples * 26 * 1.1 (µL): _____
8. _____ Resuspend the bead pellet for each sample in the plate in 25 µL Nuclease-Free Water (P/N 20207) from Capture Kit Box, 1 of 4 (P/N B12121). Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 25 µL.

2.4 Target Enrichment PCR

1. _____ Thaw DNA Pol Buffer (P/N 20407), dNTP Mix (P/N 20409), 100 μ M Primer 1 (P/N 20402), and 100 μ M Primer 2 (P/N 20403) at RT. Thaw DNA Pol Enzyme (P/N 20408) on ice.
2. _____ On ice, prepare PCR master mix in a 1.5 to 2.0 mL tube according to **Table 2.23**. Pipette up and down 10 times to mix using a P-200 pipettor set to 80% of the volume and briefly centrifuge the tube to collect the mix at the bottom of the tube.

Table 2.23 Target Enrichment PCR Master Mix Preparation

Reagent	1 Reaction (μ L)	# of Samples x 1.1	Reagent Volume (μ L)
Nuclease-Free Water	12.5		
dNTP Mix	0.5		
100 μ M Primer 1	0.5		
100 μ M Primer 2	0.5		
DNA Pol Enzyme	1		
DNA Pol Buffer	10		
Total Volume (μ L)			
0.8 x Total Volume (μ L)			

3. _____ Add 25 μ L of the Target Enrichment PCR master mix to each sample. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 25 μ L. Seal the plate with an adhesive film and centrifuge at 230 x g for 10 seconds.
4. _____ Program a thermal cycler with heated lid ON according to the cycling conditions in **Table 2.24**.

Table 2.24 Target Enrichment PCR Cycling Conditions

Step	Temp.	Time	# of Cycles
1	98 °C	2 minutes	1
2	98 °C	30 seconds	18
	60 °C	30 seconds	
	72 °C	1 minute	
3	72 °C	10 minutes	1
4	4 °C	∞	n/a
Program Name:			
Instrument Number:			

5. _____ Transfer the plate containing the samples to the thermal cycler and start the program.

Note: This is a safe stopping point. The samples may be incubated at 4 °C in the thermal cycler or stored at 2-8 °C overnight in the 96-well PCR plate. Incubating samples at 4 °C in thermal cycler, particularly in areas of high humidity, may cause excessive condensation around the block.

Operator Name:

Signature:

Date:

6. ____ After the program has completed, remove the plate and centrifuge the plate at 230 x g for 30 seconds to collect samples at the bottom of the wells before proceeding to step 7.
7. ____ Purify the amplified, target enriched library as follows:
 - a. ____ Allow Post-PCR Beads (P/N 20601) to equilibrate at RT for 30 minutes. Vortex for 30 seconds at the highest setting before use.
 - b. ____ Prepare fresh 80% ethanol using 100% MBG ethanol and MBG water (not provided).

Table 2.25 80% Ethanol Preparation

Volume 100% MBG ethanol (mL)	Volume MBG Water (mL)

- c. ____ Fill a sterile, disposable reservoir with Post-PCR beads with 10% overage volume.
Number of samples * 90 * 1.1 (µL): ____
- d. ____ Add 90 µL Post-PCR beads to each sample. Pipette up and down 10 times to mix using a P-200 multichannel pipettor set to 90 µL.
- e. ____ Incubate at RT for 5 minutes.
- f. ____ Place the plate containing samples on a magnetic stand and wait until the solution is clear (3-5 minutes). Carefully remove and discard all supernatant (about 140 µL) without disturbing the beads using a P-200 multichannel pipettor set to 200 µL.
- g. ____ With the plate on the magnetic stand, add 150 µL 80% ethanol without disturbing the beads. Incubate for 30 seconds. Carefully remove and discard all the supernatant without disturbing the beads using a P-200 multichannel pipettor set to 200 µL.
- h. ____ Repeat the 80% ethanol wash (step 7.g.). Carefully remove all traces of ethanol after the second wash using a P-20 multichannel pipettor set to 20 µL.
- i. ____ Air dry the beads for 5 minutes on the magnetic stand.
- j. ____ Fill a sterile, disposable reservoir with Nuclease-Free Water (P/N 20207), with 10% overage volume, from Capture Kit Box, 1 of 4 (P/N B12121).
Number of samples * 26 * 1.1 (µL): ____
- k. ____ Remove the plate from the magnetic stand and resuspend the beads in 26 µL Nuclease-Free Water (P/N 20207).
- l. ____ Incubate at RT for 2 minutes.
- m. ____ Place the plate back on the magnetic stand until the solution is clear (approximately 1 minute).
- n. ____ With the plate on the magnetic stand, transfer 25 µL of clear supernatant to a labelled 1.5 mL low bind tube.

Note: This is a safe stopping point. Target enriched libraries may be stored at -25 to -15 °C prior to sequencing.

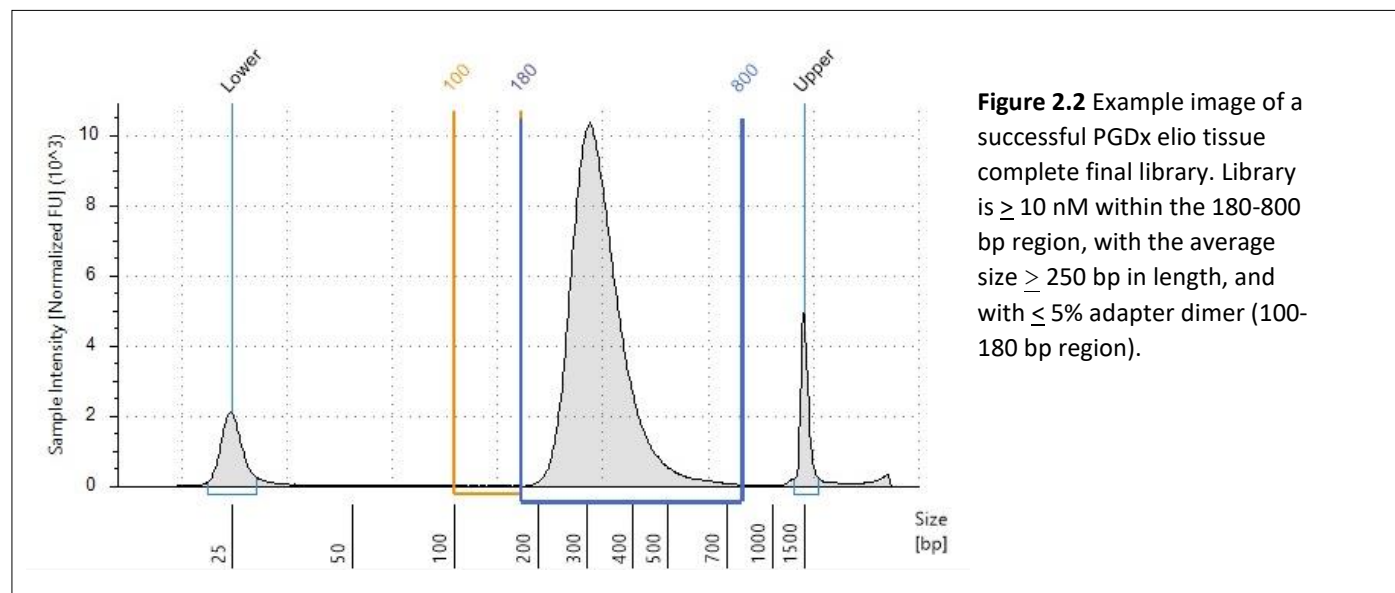
Operator Name:

Signature:

Date:

2.5 Final Library QC

1. _____ Use a DNA fragment analyzer to assess DNA libraries and controls by determining the concentration and average peak size (180–800 bp).



2. _____ Using the library QC criteria in **Table 2.27**, determine the PASS/FAIL status of each sample and each control. Record the average size [bp], concentration [ng/μL], and Pass/Fail status for each sample in **Table 2.26**.

Table 2.26 Final Library QC

[illegible]

Well	Sample ID	Average Size (bp)	Concentration (nM)	Pass/Fail

3. _____ Samples and external control must be ≥ 10 nM within the 180-800 bp range with the average size ≥ 250 bp in length.
4. _____ Check for primer/adaptor dimer peaks in the External Control and sample lanes (100-180 bp region). If the level of dimer in a sample library or External Control library is $> 5\%$ of the library yield, the library has failed QC. For failed samples, repeat processing from section **1. Library Preparation**. If there is insufficient material for library preparation, proceed to optional bead cleanup using the steps in **Appendix A2**. If the External Control contains $>5\%$ primer/adaptor dimer, proceed to optional bead clean up. See **Table 2.37** in **Assay Troubleshooting Guidelines** for more information.

Note: If criteria in steps 3 and 4 above are not met, do not proceed to sequencing steps. If the NTC has an average size ≥ 250 bp and has a peak concentration of > 5 ng/ μ L within the 180-800 bp region, the target enriched library preparation batch is considered contaminated and should be repeated from section 1 Library Preparation.

Table 2.27 Hybridization Capture Library QC Criteria

Sample Type	Pass Criteria	Fail Criteria	Action if Fail
Sample Library	≥ 10.0 nM; Average size ≥ 250 bp; Adapter Dimer $\leq 5\%$	< 10.0 nM and/or Average size < 250 bp and/or Adapter Dimer $> 5\%$	If < 10.0 nM and/or Average size < 250 bp, repeat library preparation using available DNA If Adapter Dimer $> 5\%$, repeat library preparation. If there is insufficient starting material available, proceed to optional bead cleanup (Appendix A2)
External Control	≥ 10.0 nM; Average size ≥ 250 bp; Adapter Dimer $\leq 5\%$	< 10.0 nM and/or Average size < 250 bp; Adapter Dimer $> 5\%$	If < 10.0 nM and/or Average size < 250 bp, batch-wide repeat using available DNA If Adapter Dimer $> 5\%$, proceed to optional bead cleanup (Appendix A2)
No Template Control (NTC)	Average size < 250 bp and/or ≤ 5.0 ng/ μ L	Average size ≥ 250 bp and > 5.0 ng/ μ L	Batch-wide repeat using available DNA

Operator Name:

Signature:

Date:

3. Sequencing

Warning: Do NOT start a new sequencing run if the prior sequencing run data has not been copied off the instrument.

Do not use the NextSeq 550Dx instrument for the PGDx assay without it being qualified by PGDx and listed as available for use in the PGDx elio user interface.

3.1 Shutdown and Quick Wash

Recommended: Follow the NextSeq 550Dx Instrument Reference Guide for instrument shutdown and wash schedules.

3.2 Preparation of NextSeq Reagents and Flow Cell

Recommended: Prepare NextSeq reagents according to the NextSeq 550Dx Instrument Reference Guide.

3.3 Library Normalization and Pooling

Note: If using previously captured libraries as sequencing filler, proceed to **Appendix A1** and follow instructions. Resume work at **3.4 Denature, Dilute and Load**.

Refer to PGDx elio tissue complete Library Normalization Calculator (LNC-ETC-09).

Note: For volumes lower than 2 µL, use a calibrated P-2 micropipette to avoid pipetting errors during normalization and pooling.

Note: Do not duplicate Index Adapter for samples that are intended for the same sequencing run.

1. _____ Thaw Illumina HT1 Buffer (from NextSeq sequencing kit) for use in **3.4 Denature Dilute and Load**.
2. _____ Prepare fluorometer reagents according to manufacturer instructions. All samples and initial sample pool should be in the range of 2 to 1000 ng/µL, pool dilutions and diversiPhi should be in the range of 0.2 to 100 ng/µL.
3. _____ Quantify each final library sample using a fluorometer and record values in **Table 2.28**.
4. _____ In a new 1.5 to 2.0 mL tube labeled with the run name, normalize and pool 150 ng of each sample library, up to 16 samples, using the formula below:

$$\text{Equation 1: Volume of sample library into initial pool (}\mu\text{L)} = \frac{150 \text{ ng}}{\text{Concentration (}\frac{\text{ng}}{\mu\text{L}}\text{) from step 3.}}$$

5. _____ Quantify the initial pool and enter the value in the field 'Initial Pool Concentration' in **Table 2.28**.
6. _____ Calculate the Sample Pool Molarity (nM) using the average of the average library sizes (bp) (**Table 2.26**) and the measured Initial Pool Concentration (ng/µL) (**Table 2.28**) using Equation 2 and record concentrations in **Table 2.28**.

$$\text{Equation 2: Molarity} = \left(\frac{\text{concentration in ng}/\mu\text{L}}{660 \text{ g/mol} * \text{avg maximum peak height in bp}} \right) * 10^6$$

Table 2.28 Library Normalization and Pooling

Well	Sample ID	Index Adapter	Average Fragment Analyzer Size (bp)	Concentration from Fluorometer (ng/µL)	Volume into Initial Pool (µL)

Well	Sample ID	Index Adapter	Average Fragment Analyzer Size (bp)	Concentration from Fluorometer (ng/μL)	Volume into Initial Pool (μL)
Sample Library Pool			Average Size (bp)	Initial Pool Concentration (ng/μL)	Total Pool Volume (μL)
			Calculated Sample Pool Molarity (nM)		

7._____ Dilute initial pool to 10 nM ($M1V1=M2V2$) using calculated Sample Pool molarity (from **Table 2.28**) and enter values in **Table 2.29**.

Table 2.29 10 nM Dilution

Volume initial pool (μL)	Volume 10 mM Tris-Cl pH 8.5 (μL)	Total Volume (μL)

8._____ Quantify 10 nM pool twice using a fluorometer and record values in **Table 2.30**.

Table 2.30 10 nM Pool Measured Concentrations

Fluorometer Reading 1 (ng/μL)	Fluorometer Reading 2 (ng/μL)	Average Concentration (ng/μL)	Average Size (bp)	Calculate Average Concentration (nM)

9._____ Dilute 10 nM pool to 4 nM ($M1V1 = M2V2$) using values from **Table 2.30**. Record dilution volumes in **Table 2.31**.

Table 2.31 4 nM Dilution

Volume 10 nM Dilution (μL)	Volume 10 mM Tris-Cl pH 8.5 (μL)	Total Volume (μL)

10._____ Quantify the newly made 4 nM sample library pool using a fluorometer and record values in **Table 2.32**.

Note: If pooling less than a complete batch of samples (15 cases + EC), the use of PGDx elio diversiPhi as a sequencing filler (targeting 6.25% lane ratio) is recommended. If sequencing a full batch, proceed to section **3.4 Denature, Dilute and Load**.

Table 2.32 nM Pool Measured Concentrations

Fluorometer Reading 1 (ng/μL)	Fluorometer Reading 2 (ng/μL)	Average Concentration (ng/μL)	Average Size (bp)	Calculated Average Concentration (nM)

11. _____ Quantify diversiPhi filler (P/N A0840101) and calculate volumes to dilute to 4 nM according to **Table 2.33**. diversiPhi average size (bp) can be found on the diversiPhi tube.

Table 2.33 Quantify and Dilute diversiPhi to 4 nM

Fluorometer Reading (ng/μL)	Average Size (bp)	Calculated Concentration (nM)	Volumes for 4 nM Dilution (total 10 μL)	
			Volume diversiPhi (μL)	Volume 10 mM Tris-Cl pH 8.5 (μL)

12. _____ Calculate the lane ratio of sample libraries and diversiPhi filler to be sequenced using Equation 3 and Equation 4 and record ratios in **Table 2.34**.

- a. _____ Each sample library should be 6.25% (0.0625) of the overall sequencing run.

Equation 3: Ratio of Libraries = (Number of Sample Libraries including EC)*0.0625

- b. _____ diversiPhi filler should be the remainder of the library pool.

Equation 4: Ratio of diversiPhi = 1- (Ratio of Sample Libraries from Equation 3)

13. _____ Calculate the volumes to be added to the final pool using equations 5 and 6 and record volumes in **Table 2.34**. Final Pool Volume should be > 5 μL. Volume of 4 nM Sample Library Pool and 4 nM diversiPhi should each be > 2 μL.

Equation 5: Volume 4 nM Sample Library Pool = (Final Volume) * (Ratio 4 nM Sample Library Pool)

Equation 6: Volume of 4 nM diversiPhi = (Final Volume) * (Ratio 4 nM diversiPhi)

14. _____ Combine 4 nM sample library pool with 4 nM diversiPhi according to **Table 2.34**.

Table 2.34 Combine Sample Library Pool and diversiPhi (4 nM)

	Ratio (from Equations 3 and 4)	Calculated Concentration (nM)	Volume into Final Pool (μL) (minimum 2 μL)
4 nM Sample Library Pool			
4 nM diversiPhi			
Final Pool Volume (μL)			

3.4 Denature, Dilute and Load

1. _____ In a 1.5 mL tube prepare 0.2 N NaOH:

- a. _____ Add 800 μL MBG water.

- b. _____ Add 200 μL 1 N NaOH, vortex and spin briefly.

2. _____ In a 1.5 mL tube prepare 200 mM Tris-HCl, pH 7.0:

- a. _____ Add 800 μL MBG water.

- b. _____ Add 200 μL 1 M Tris-HCl, pH 7.0 vortex and spin briefly.

3. _____ Label a 1.5 mL tube "20 pM denatured".

- 4._____ If sequencing a full batch of samples (15 cases + EC), use the 4 nM dilution from **Table 2.31** and proceed to step 6 below.
- 5._____ If sequencing less than a full batch of samples, use the 4 nM dilution from **Table 2.34**, (if using diversiPhi as a sequencing filler) or **Table A1.5 4 nM Pool Measured Concentrations** in **Appendix A1** (if using captured library as a sequencing filler) and proceed to step 6 below.
- 6._____ Add 5 µL of the 4 nM library pool to the '20 pM denatured' tube.
- 7._____ Add 5 µL of 0.2 N NaOH to the '20 pM denatured' tube. Vortex and quick spin in microcentrifuge.
- 8._____ Incubate for 5 minutes at RT.
- 9._____ While incubation proceeds, add 1228.5 µL pre-chilled HT1 Buffer in a new 1.5 mL tube labeled "1.1 pM loading pool".
- 10._____ After 5 minute incubation, add 5 µL of 200 mM Tris-HCl, pH 7.0 to the '20 pM denatured' tube, vortex and spin briefly.
- 11._____ Add 985 µL of pre-chilled HT1 to the '20 pM denatured' tube, vortex then quick spin in microcentrifuge.
- 12._____ Add 71.5 µL of denatured library from '20 pM denatured' tube to the '1.1 pM loading pool' tube. Invert several times to mix and quick spin in microcentrifuge.
- 13._____ Load 1300 µL of '1.1 pM loading pool' into the NextSeq sequencing cartridge per manufacturer's instructions.

Operator Name:

Signature:

Date:

3.5 Set Up and Load NextSeq

Note: The PGDx elio LRM Module must be installed on the sequencer in Dx mode.

Note: Repeat this section for each sequencing run.

Set up and load the NextSeq instrument according to the NextSeq 550Dx Instrument Reference Guide. Ensure the instrument mode indicator states “Welcome to NextSeq” with an orange color bar or vertical status icon orientation. If the NextSeq 550Dx instrument has a blue color bar, please reboot and change the mode to get the orange color bar.

1. ____ On the NextSeq Welcome screen, select ‘Local Run Manager’. Local Run Manager (LRM) will open in a new web browser window.
2. ____ Log in to Local Run Manager with your local LRM credentials (not provided by PGDx).
3. ____ From the LRM Run Dashboard screen, click ‘Create Run’ and then select ‘PGDx elio’.
4. ____ On the Create Run screen, enter the Run Name, Run Description (optional), then click ‘Save Run’.
5. ____ From the LRM Run Dashboard screen, click the options icon in the top right  , then click ‘Close’. The system should return to the NextSeq Control Software.
6. ____ From the NextSeq Welcome screen, select ‘Sequence’.
7. ____ Search for and select the previously entered Run Name, then click ‘Next’ to continue with loading the sequencing reagents.
8. ____ Load the new flow cell into the NextSeq and press Load, then Next.
 - a. If there is dust or debris on the glass of the flow cell, wipe with an alcohol wipe and dry with lint-free tissue or lens paper.
9. ____ Mix Buffer Cartridge by inverting 10 times.
 - a. If there is liquid waste in the spent reagents container, empty waste in appropriate container and remove/rinse Buffer Wash Cartridge.
 - b. Replace spent reagents container and add new Buffer Cartridge, then press Next.
10. ____ Mix Reagent Cartridge by inverting 10 times.
 - a. Use a P-1000 tip to pierce open the well of the Reagent Cartridge labeled “Load Library Here.”
 - b. Use a new tip to load 1300 µL from “Load: Pool” tube into well of the Reagent Cartridge labeled “Load Library Here.”
11. ____ Insert new Reagent Cartridge loaded with pool. Press Load, then press Next.
12. ____ Press Next and once system checks are complete, Start.
13. ____ Store the 10 nM sequencing pool labeled with the run description at -20 °C for 10 weeks and discard 4 nM sample pools.

3.6 Input Sample Information into PGDx elio platform

Refer to the **PGDx elio platform User Guide (UG-EP-03)** for detailed instructions on using the PGDx elio platform User Interface.

1. ____ Navigate to the URL provided by your local PGDx elio platform administrator. Enter valid credentials, select IVD Mode, and click Sign In.
2. ____ Select Create Analysis Plan option.

- 3._____ Select the appropriate assay version from the dropdown menu.
- 4._____ Enter a Plan Description, if desired. This can be any user-defined descriptive text.
- 5._____ Record Plan Description here: _____
- 6._____ Enter the Flow Cell ID for the sequencing run. Flow Cell ID: _____

Note: If the sequencing run corresponding to this batch of samples has already started or completed, toggle the 'Existing Sequencing Run' checkbox. The Flow Cell ID input field will become disabled, but the Existing Sequencing Run dropdown will become enabled. Select the existing run from the dropdown list (the dropdown lists sequencing runs on connected sequencers by Flow Cell ID).

- 7._____ Fill in sample information. Fields marked with an asterisk (*) are required.
- 8._____ If the pool is < 16 samples, then select 'Filler' as the final sample.
- 9._____ Check that the Index and Sample names are paired correctly. Click Save. Click Run.

Operator Name:

Signature:

Date:

Report Access and Results

Note: the PGDx elio platform User Guide (UG-EP-03) has detailed instructions and screenshots about accessing and downloading reports.

Accessing the Results

1. Log into the PGDx elio platform user interface using the URL, email address, and password provided by the PGDx elio site administrator.
2. Reports are available in two places:
 - a. Analysis Plan tab: General quality metric results about the entire sequencing run.
 - b. Cases tab: Specific sample results are available on the Cases tab once a tumor type has been selected. If no tumor type is selected, only the Complete Case Record will be available.
3. To review the sequencing run metrics: On the Analysis Plan tab, use the filter or search features to find the Analysis Plan of interest. If the Analysis Plan status is 'Completed', the reports are ready. Click on the Analysis Plan to display the details panel. On the details panel, click on the Reports tab to view and download the sequencing metric reports.
4. To see the case results, on the Cases tab, use the filter or search features to find the case of interest. Cases are named using the sample name entered into the Analysis Plan. If the case analysis status is 'Completed', the reports are ready. Selection of tumor type organizes the variant calls in the Case Report into either Variants with Evidence of Clinical Significance or Variants with Potential Clinical Significance for that tumor type. Tumor type selection should align with the clinical diagnosis and all available information. In the case of metastasis from a known primary site, the tumor type of the primary site should be selected. In the case of metastasis of unknown origin, unknown primary site, or uncertainty of the tumor type, 'Other' should be selected. For cell line sample types, 'Other' should be selected. See **Table 3.3** for a list of alterations that will be presented in the 'Variants with Evidence of Clinical Significance' table of the Case Report when 'Other' is selected. Click on the case to display the case details panel and click on the Reports tab to view and download Case Reports.

The following reports can be accessed from the Case Reports tab:

Case Report (once tumor type is selected): Download as .pdf and .csv file. This report contains:

- Quality metrics with PASS or FAIL indication
- Results for single nucleotide variants (SNVs) and insertions and deletions (indels), select amplifications and translocations and genomic signatures including microsatellite instability (MSI), and tumor mutation burden (TMB) in Muts/Mb. Variant calls are organized into Companion Diagnostic Findings (in approved indications), Variants with Evidence of Clinical Significance, or Variants with Potential Clinical Significance. Variants with Evidence of Clinical Significance align with Tier 1A of the AMP/ASCO/CAP guidelines (Li et al., 2017) based on the selected tumor type for use in tumor profiling.

Complete Case Record (always available): Downloaded as a .csv file. This report contains detailed quality metrics of the run, control and sample, as well as final variant call results.

Note: Opening CSV files in Excel and saving may result in unintended changes due to autoformatting or compatibility issues. It is recommended to use a text editor such as Notepad++ (PC) or TextEdit (Mac) for editing CSV files.

Interpreting the Case Results

Part 3 of the PGDx elio tissue complete CDx manual provides a detailed description of the report files. Briefly, TMB score values are reported as mutations per megabase denoted as Muts/Mb referring to mutations per megabase sequenced within the PGDx elio tissue complete CDx regions of interest. Microsatellite status is reported as Microsatellite Instability-high (MSI-H), Microsatellite Stable (MSS), or Indeterminate - microsatellite status. SNVs and indels are reported with gene, alteration,

consequence and variant allele frequency (VAF). Amplifications and translocations detected are reported with relevant gene information or partner if identified. Variants are marked as 'No Result' in situations where the sample, control, or run level quality metrics did not pass. In these situations, no variants are reported. Please refer to the Complete Case Record (CCR) for details on data quality metrics and refer to **Part 3** for details on Regions of Low Coverage.

The PGDx elio tissue complete CDx product provides users with multiple files for data and troubleshooting.

- The Complete Run Record (CRR) is a run-level report in comma separated-value (csv) format. The CRR provides summary data of the Analysis Plan and associated sequencing run metrics. This file is used to assess the quality of the sequencing run and is typically used for troubleshooting following details provided in **Part 3**.
- The Complete Case Record (CCR) is a case-level report in csv format. The CCR aggregates data from the clinical sample with the external control to provide run-level, sample-level and control QC status, as well as a list of variant calls organized by variant type, in alphabetical order by codon position. This file may be used to send data to annotation and interpretation services, or to aggregate data in internal customer database systems.
- The Case Report is a case-level report in csv and PDF format that requires a selection of tumor type to be generated by the system. Definitions of terms found in the header of the Case Report (PDF report) are provided in **Table 2.35**.
 - The PDF Case Report organizes variants by association with clinical evidence based on selection of tumor type. The PGDx elio tissue complete CDx product uses a positive reporting scheme only, meaning only variants detected and passing the pipeline filters and cutoffs will be reported in either the Companion Diagnostic Findings, Variants with Evidence of Clinical Significance or Variants with Potential Clinical Significance tables. This file may be used for medical records or appended to clinically interpreted reports.
 - The csv file contains the same information that can be integrated more readily into electronic systems.

Table 2.35 Key for Header Table in Case Report (PDF)

Term	Definition
Tumor Type	Chosen from a selection of 16 available tumor types. Including 15 tumor types aligning to NCCN guidelines: Breast, Central nervous system (CNS), Cholangiocarcinoma, Colon, Gastric, Gastrointestinal stromal tumors (GIST), Inflammatory Myofibroblastic Tumor (IMT), Melanoma, Non-small cell lung (NSCLC), Ovarian, Pancreatic, Prostate, Rectal, Uterine, and Thyroid.
Sample Type	The type of sample being processed (external control, specimen, or cell line). Classification is selected by the user during creation of the Analysis Plan.
Details	Free text field (max 29 characters). May be used to add histology information, diagnosis or SNOMED code.
Report Date	Date (YYYY-MM-DD) of report generation by the PGDx elio platform.
Run Quality Result	PASS/FAIL value indicating whether the associated sequencing run data passed quality checks. Please refer to the Complete Run Record (CRR) for details on sequencing run quality metrics.
Sample Quality Result	PASS/FAIL value indicating whether the sample data passed quality checks. Please refer to the Complete Case Record (CCR) for details on data quality metrics.
Control Quality Result	PASS/FAIL value indicating whether the control data passed quality checks. Please refer to the Complete Case Record (CCR) for details on data quality metrics.
Overall Case Quality Result	Overall PASS/FAIL value indicating the validity of the case data. It will fail if any of the other three quality metrics failed. Please refer to the Complete Case Record (CCR) for details on data quality metrics
Assay Name	Name of the PGDx elio assay that was run to generate the report.
Assay Version	Version of the assay that was run to generate the report.

Term	Definition
Platform Version	Software version of PGDx elio platform.
Flow Cell ID	Unique NextSeq flow cell ID.

Part 2 References

Sauter et al., Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. J Clin Oncol. 2009 Mar 10;27(8):1323-33.

Li M.M., Datto M., Duncavage E.J., Kulkarni S., Lindeman N.I., Roy S., Tsimberidou A.M., Vnencak-Jones C.L., Wolff D.J., Younes A., Nikiforova M.N. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.

Assay Troubleshooting Guidelines

Table 2.36 Library Troubleshooting

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
Sample or external control post library preparation concentration is too low (< 15 ng/μL, within 180-800 bp, with an average size ≥ 250 bp)	DNA quantification was inaccurate	Re-quantify DNA samples with a fluorometer	Repeat library preparation for the affected sample and external control, NTC recommended
	Sample DNA input was low	Ensure proper DNA input of 50-100 ng is used for library preparation	
	Sample DNA input was compromised or poor quality		
A large peak in the DNA fragment analyzer trace of the No template control (NTC).	Potential amplified adapter dimer	n/a	Proceed to target enrichment and monitor NTC

Table 2.37 Target Enrichment Troubleshooting

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
Sample post target enrichment library concentration too low (< 10 nM, within 180-800 bp range, with an average size ≥ 250 bp) and/or % primer dimer is too high (> 5% within 100-180 bp region)	DNA quantification was inaccurate	Re-quantify DNA samples with a fluorometer	Repeat library preparation and target enrichment for the affected samples, external control, and NTC (recommended)
	Sample DNA input was low	Ensure proper DNA input of 100 ng is used for library preparation	
	Sample DNA input was compromised or poor quality		
	PCR, ligation, or capture was inefficient	Ensure proper pipetting technique and mixing of reagents	
	Ethanol was carried over into PCR during library preparation		
	Post-PCR Beads were overdried	Do not dry the Pre/Post-PCR Beads more than the recommended time	
	Incorrect PCR cycling conditions were used	Use the recommended cycling conditions for PCR and hybridization	
	Capture Baits degraded	Ensure Capture Baits do not undergo more than 4 freeze-thaw cycles and RNase inhibitors are added	
% primer dimer is too high (> 5% within 100-180 bp region)	Insufficient starting material, poor quality sample, insufficient bead cleanup	Evaluate remaining material of sample	If sufficient sample material is available, prepare a new library starting from genomic DNA. If not enough sample material is available, perform an additional bead cleanup step to remove primer dimers and allow the sample to meet QC metrics (Appendix A2).

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
External control library concentration too low after target enrichment (< 10 nM, within 180-800 bp range, with an average size $\geq$ 250 bp)	Same possible causes as above	Same troubleshooting steps as above	Repeat library preparation and target enrichment for the entire batch
No template control (recommended) concentration > 5 ng/ μ L (within 180-800 bp range, with an average size $\geq$ 250 bp)	Reagent and/or equipment contamination	Repeat library preparation QC or decontaminate equipment as necessary	Repeat library preparation and target enrichment for the affected samples with external control and NTC (recommended)
Sample failed to display peak at recommended size ($\geq$ 250 bp within 180-800 bp range) on the DNA fragment analyzer after target enrichment	Expired reagents were used	Replace any expired reagents with fresh ones	Repeat library preparation and target enrichment for the affected sample with external control and NTC (recommended)
	Incorrect PCR cycling conditions were used	Use the recommended cycling conditions for PCR and hybridization	
External control failed to display peak at recommended size ($\geq$ 250 bp) on the DNA fragment analyzer after target enrichment	Same possible causes as above	Same troubleshooting steps as above	Repeat library preparation and target enrichment for all samples in the affected batch

Table 2.38 Sequencing Troubleshooting

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
Run Quality Result 'FAIL'		Review Complete Run Record or Complete Case Record and record metrics that failed	
	Wrong version of NextSeq sequencing software	Verify NextSeq sequencing software is compatible with PGDx elio platform.	Contact PGDx Tech Services at 1-855-PGDx-NOW or PGDxTechService@labcorp.com
	Q30 scores for read 1 and 4 are below 80%	Determine if run failed on Q30 or cluster density	Repeat sequencing
	Q30 scores for read 2 and 3 are below 85%		
	Cluster density is below 130 K/mm2		
Sample Quality Result 'FAIL'		Review Complete Case Record and record metrics that failed	
	Percentage of reads identified falls outside of acceptable range (15% - 35%)	Ensure input of initial DNA is correct	Repeat normalization and pooling of libraries, and repeat sequencing
		Ensure concentration of normalized libraries is correct	

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
		Ensure normalized libraries were correctly pooled	
		Ensure fluorometric quantification is accurate by using manufacturer controls	
	Percentage of regions covered is < 90%	Ensure input of initial DNA is correct	Repeat library preparation and target enrichment for the affected samples in the batch
	Incorrect index is assigned to sample (percentage of identified sequences is 0%)	Ensure the correct index was indicated for each sample in PGDx elio platform Analysis Plan	Repeat analysis with the correct index entered in PGDx elio platform
	Contamination detection software flagged sample as contaminated	Ensure laboratory process minimizes sources of sample cross-contamination, as outlined in Warnings and Precautions	Repeat library preparation and target enrichment for the affected samples in the batch
External control Quality Result 'FAIL'		Review Complete Case Record and record metrics that failed	
	External control QC check is marked as a fail because the verified sequence mutations in the external control are not detected	Ensure volume of initial external control DNA is correct	Repeat library preparation, target enrichment and sequencing of control and all samples in the same batch as the failed external control
	Percentage of regions covered is < 90%		
	Percentage of reads identified falls outside of acceptable range (15% - 35%)	Ensure concentration of normalized libraries is correct	
		Ensure normalized libraries were correctly pooled	
	Incorrect index is assigned to sample (percentage of identified sequences is 0%)	Ensure the correct index was indicated for the external control in PGDx elio platform	

Table 2.39 PGDx elio platform and server Troubleshooting

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
Missing output files	Network Issues	Ensure PGDx elio server is plugged in to network and has network access	If the Troubleshooting steps do not resolve the issue, then please contact PGDx Tech

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
	Browser Issues	Attempt viewing PGDx elio tissue complete CDx platform in a different web browser	Services at 1-855-PGDx-NOW or PGDxTechService@labcorp.com
Cannot access PGDx elio platform	Browser Certificate Issues	Use https:// for the URL & accept the security exception in your web browser	If the Troubleshooting steps do not resolve the issue, then please contact PGDx Tech Services at 1-855-PGDx-NOW or PGDxTechService@labcorp.com
	Browser Content i.e. Cookies	Clear the browsing privacy content and history of your browser and browser cache	
	Choice of License i.e. IVD, RUO	Ensure proper mode for PGDx elio tissue complete CDx kit is selected before attempting to log in to PGDx elio tissue complete CDx platform	
	User Credentials i.e. account, name and password do not exist or do not match	Check account credentials with local PGDx elio server Admin	
Sequencing Runs Status Does NOT Update	The sequencing run has not completed due to an issue with the sequencer	Check the sequencing run on the sequencer for its status	If sequencer has been running for longer than usual (about 30 hours), do not use sequencing data for downstream analysis and reporting. Contact PGDx Tech Services If the Troubleshooting steps do not resolve the issue, then please contact PGDx Tech Services at 1-855-PGDx-NOW or PGDxTechService@labcorp.com
	The sequencing run has not generated the file(s) indicating sequencing complete (see Section 4 of the elio Platform User Guide - IVD)	Check the sequencing output directory for the file	
	PGDx elio server is unable to process the sequencing run	Ensure PGDx elio server has access to the sequencing output directory	

Observation	Possible Causes	Troubleshooting Steps	Recommended Action
Case status does not update	The sequencing run has not completed due to an issue with the sequencer	Check the sequencing run on the sequencer for its status	If sequencer has been running for longer than usual (about 30 hours), do not use sequencing data for downstream analysis and reporting Contact PGDx Tech Services If the Troubleshooting steps do not resolve the issue, then please contact PGDx Tech Services at 1-855-PGDx-NOW or PGDxTechService@labcorp.com
	The sequencing run has not generated the file(s) indicating sequencing complete (see Section 4 of the elio Platform User Guide - IVD)	Check the sequencing output directory for the file	
	PGDx elio server is unable to process the sequencing run	Ensure PGDx elio server has access to the sequencing output directory	
	PGDx elio server cannot process the sequencing output data	Contact PGDx immediately	
Case Reports in Hold Status	Analysis does not complete. Sequencer is not qualified error message	Check that the PGDx elio partner module was installed on the LRM for the NextSeq 550Dx instrument and the correct PGDx elio assay was selected on the LRM for the sequencing run	Contact PGDx Tech Services at 1-855-PGDx-NOW or PGDxTechService@labcorp.com
Case Reports not available	Tumor type has not been selected	Check the Case Details Pane for tumor type selection.	Ensure the appropriate tumor type is selected for all cases. Tumor type selection should align with the clinical diagnosis and all available information.
	Analysis not complete	Check Sequencing Run status	Ensure reports are available after analysis has completed

Appendix A1

This appendix is to be followed when using previously captured library as a sequencing filler instead of diversiPhi.

Note: For volumes lower than 2 µL, use a calibrated P-2 micropipette to avoid pipetting errors during normalization and pooling.

1. _____ Thaw Illumina HT1 Buffer (from NextSeq sequencing kit) for use in **3.4 Denature Dilute and Load**.
2. _____ Prepare fluorometer reagents according to manufacturer instructions. All samples including sequencing filler and initial sample pool should be in the range of 2 to 1000 ng/µL, pool dilutions should be in the range of 0.2 to 100 ng/µL.
3. _____ Quantify each final library sample, including sample(s) to be used as sequencing filler, using a fluorometer and record values in **Table A1.1 Library Normalization and Pooling**.

Note: DO NOT duplicate Index Adapter for samples that are intended for the same sequencing run. Do NOT pool multiple samples, including filler(s), prepared with the same Index Adapter.

In a new 1.5 to 2.0 mL tube labeled with the run name, normalize and pool 150 ng of each sample library, up to 16 samples, using the formula below:

$$\text{Equation 1: Volume of sample library into initial pool (}\mu\text{L)} = \frac{150 \text{ ng}}{\text{Concentration (}\frac{\text{ng}}{\mu\text{L}}\text{) from step 1.}}$$

In the same labeled 1.5 or 2.0 mL tube, add each filler sample library using the formula below:

$$\text{Equation 2: Volume of filler sample(s) library into initial pool (}\mu\text{L)} = \left(\left(\frac{150 \text{ ng}}{\text{Concentration (}\frac{\text{ng}}{\mu\text{L}}\text{) from step 1.}} \right) \times \left(\frac{16 - \# \text{ samples}}{\# \text{ fillers}} \right) \right)$$

Note: Number of previously captured libraries required will depend on volume of sequencing filler available to compensate for the specific lane ratio that needs to be filled using this material.

Quantify the initial pool and enter the value in the field 'Initial Pool Concentration' in **Table A1.1 Library Normalization and Pooling**.

Calculate the Sample Pool Molarity (nM) using the average of the average library sizes (bp), including filler sample(s), and the measured Initial Pool Concentration (ng/µL) using Equation 2 and record concentration in **Table A1.1 Library Normalization and Pooling**.

$$\text{Equation 2: Molarity} = \left(\frac{\text{concentration in ng/uL}}{660 \text{ g/mol} * \text{avg maximum peak height in bp}} \right) * 10^6$$

Table A1.1 Library Normalization and Pooling

Well	Sample ID	Index Adapter	Average Fragment Analyzer Size (bp)	Concentration from Fluorometer (ng/μL)	Volume Into Initial Pool (μL)
Sample Library Pool			Average Size (bp)	Initial Pool Concentration (ng/μL)	Total Pool Volume (μL)
			Calculated Sample Pool Molarity (nM)		

Dilute initial pool to 10 nM ($M1V1=M2V2$) using calculated Sample Pool molarity (from **Table A1.1 Library Normalization and Pooling**) and enter values in **Table A1.2 10 nM Dilution**.

Table A1.2 10 nM Dilution

Volume initial pool (μL)	Volume 10 mM Tris-Cl pH 8.5 (μL)	Total Volume (μL)

Quantify 10 nM pool twice using a fluorometer and record values in **Table A1.3 10 nM Pool Measured Concentrations**.

Table A1.3 10 nM Pool Measured Concentrations

Fluorometer Reading 1 (ng/μL)	Fluorometer Reading 2 (ng/μL)	Average Concentration (ng/μL)	Average Size (bp)	Calculate Average Concentration (nM)

Dilute 10 nM pool to 4 nM ($M1V1 = M2V2$) using values from **Table A1.3 10 nM Pool Measured Concentrations**. Record dilution volumes in **Table A1.4 4 nM Dilution**.

Table A1.4 4 nM Dilution

Volume 10 nM Dilution (μL)	Volume 10 mM Tris-Cl pH 8.5 (μL)	Total Volume (μL)

Quantify the newly made 4 nM sample library pool using a fluorometer and record values in **Table A1.5 4 nM Pool Measured Concentrations**.

Table A1.5 4 nM Pool Measured Concentrations

Fluorometer Reading 1 (ng/μL)	Fluorometer Reading 2 (ng/μL)	Average Concentration (ng/μL)	Average Size (bp)	Calculated Average Concentration (nM)

For the remaining instructions, continue from 3.4 Denature, Dilute and Load in **Part 2**.

Appendix A2

This appendix is to be followed when repeating bead cleanup after **Step 4 of Final Library QC** following **Hybrid Capture** if there is insufficient material to repeat **Section 1. Library Preparation**.

I. Reagents and Consumables

1. _____ Complete **Table A2.1 Reagent Log**.

Table A2.1 Reagent Log

Reagent Name	Lot Number	Expiration Date
PGDx elio™ tissue complete CDx Kit		
Molecular Biology Grade Ethanol		
Molecular biology grade water – non-DEPC		

II. Dimer Cleanup

2. _____ Remove Post-PCR beads from 4°C storage and allow them to equilibrate to room temperature for at least 30 minutes.

3. _____ Purify samples using Post-PCR Beads:

- a. _____ Prepare an 80% ethanol solution with molecular biology grade water.
- b. _____ Briefly spin down the plate and remove the plate seal.
- c. _____ Vortex room temperature Post-PCR Beads for 30 seconds at max speed.
- d. _____ Add volume of Post-PCR beads **EQUAL** to the available sample volume, and pipette at least 10 times to mix.

Example: 25 µL of sample + 25 µL of Post-PCR beads.

- e. _____ Incubate samples for at least 5 minutes at room temperature.
- f. _____ Place the 96 well plate on magnetic stand and wait for the solution to clear (3-5 minutes).
- g. _____ With the plate still on the magnetic stand, carefully remove and discard supernatant.
- h. _____ With plate on magnetic stand, add 200 µL of 80% ethanol and incubate for 30 seconds.
- i. _____ Carefully remove and discard supernatant.
- j. _____ Repeat steps “h-i” one more time.
- k. _____ Ensure all ethanol is removed using a P20 pipettor set to 20 µL.
NOTE: Residual ethanol can impact the performance of downstream reactions.
- l. _____ Air-dry the beads on the magnetic stand for 5 minutes.
NOTE: Do not over dry beads.
- m. _____ Remove the plate from the magnetic stand and resuspend beads in 25.0 µL of Nuclease-Free water (P/N: 20207). Pipette up and down 10 or more times to mix, ensuring all beads are in solution.
- n. _____ Seal plate with adhesive seal and centrifuge at 230 x g for 5-10 seconds to collect the liquid.
- o. _____ Incubate for at least 2 minutes at room temperature.
- p. _____ Place sample on magnetic stand and wait for solution to clear (2 minutes).

q._____ Transfer 25 μ L of clear supernatant to labeled 1.5 mL tubes carefully to avoid transfer of beads

r._____ Sample(s) may be stored at -20°C.

III. Final Library QC and Quantification

- 1._____ Use a DNA fragment analyzer and fluorometer to quantify DNA libraries by determining the concentration and average peak size (180–800 bp), and record values in **Table A2.2 Final Library QC**. See the reference range above in **Figure 2.2** above.

Table A2.2 Final Library QC

Well	Sample ID	Average Size (bp)	DNA Fragment Analyzer Concentration (nM)	Fluorometer Concentration (ng/ μ L)	Pass/Fail

Part 3 – Report Interpretation Guide



Rx Only



A0420100

Contact Information



Personal Genome Diagnostics Inc.
3600 Boston Street, Suite 10,
Baltimore, MD 21224, USA
1-443-602-8833



PGDxTechService@labcorp.com
[PGDx elio® tissue complete CDx](#)
1-855-PGDX-NOW (1-855-743-9669)

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Introduction to Report Interpretation Guide

Intended Use

PGDx elio tissue complete CDx is a qualitative next-generation sequencing-based in vitro diagnostic device that uses a high-throughput hybridization-based capture technology utilizing DNA isolated from formalin-fixed paraffin embedded tumor tissue. The PGDx elio tissue complete CDx targeted panel can detect single nucleotide variants, small insertions and deletions, copy number amplifications, and translocations. It is intended to be used as a companion diagnostic to identify melanoma patients who may benefit from treatment with the targeted therapies listed in **Table 3.1** below in accordance with the approved therapeutic product labeling.

Table 3.1 PGDx elio tissue complete CDx Indications

Indication	Biomarker	Therapy
Melanoma	BRAF V600E	BRAF inhibitors approved by FDA or BRAF/MEK inhibitor combinations approved by FDA
Melanoma	BRAF V600K	BRAF inhibitors approved by FDA or BRAF/MEK inhibitor combinations approved by FDA

Purpose of This Interpretation Guide

This guide is intended to provide qualified healthcare professionals with an understanding of the technical features of the PGDx elio tissue complete CDx analysis and reporting software following library prep and sequencing. It will explain report structure and content and will provide sample preparation recommendations and information to troubleshoot sample workflows.

Detailed instructions on how to access and download reports can be found in the **PGDx elio platform guide (UG-EP-03)**.

Note: Product details within screen shots in this document are for demonstration purposes only and do not necessarily reflect details of any purchased product. Such product details include, but are not limited to: product names, product version numbers, product part numbers, lot numbers, IP addresses, file paths, URLs, use labeling, Analysis Plan names and details, case names and details, sequencing run names and details, user names and email addresses. See individual product packaging and labeling for all product details.

How is Next Generation Sequencing (NGS) Used in the Patient Care Continuum?

In recent years, single-target detection assays have been replaced by comprehensive NGS-based methods allowing for the simultaneous evaluation of many genes and genomic signatures (e.g., MSI) in parallel (Li et al., 2017). Today, over 75% of oncologists report using NGS to guide decisions (Freedman et al., 2018) ranging across disease stage and for purposes including clinical trial enrollment, among others.

The age of precision oncology is enabled by the development of a myriad of targeted therapies, including those against immune checkpoints, and has revolutionized the treatment of cancer. Practicing precision oncology mandates the need for highly accurate and sensitive assays able to identify key biomarkers (La Thangue et al., 2011; Shabani et al., 2018). Conventional technologies often lead to complex iterative testing paradigms that are highly time and resource consuming. NGS assays provide a comprehensive genomic profile used to guide decision making (Freedman et al., 2018).

The rapid adoption of NGS into routine clinical oncology practice provides a comprehensive genomic profiling solution (Julka et al., 2018) that is both tissue and time sparing. NGS panels can be widely inclusive, thereby eliminating the need for multiple assays. Additionally, NGS panels can be designed to be independent of cancer type, enabling laboratory efficiencies and providing patients with information pertinent to patient management.

What Information Will Be Available in PGDx elio tissue complete CDx Reports?

Note: the **PGDx elio platform User Guide (UG-EP-03)** has detailed instructions and screenshots about accessing and downloading reports.

The PGDx elio tissue complete CDx product provides users with multiple reports for data and troubleshooting. More information about the reports listed below can be found in **Part 2** of the PGDx elio tissue complete CDx Manual.

- The Complete Run Record (CRR) is a run-level report in comma separated-value (csv) format. The CRR provides summary data of the Analysis Plan and associated sequencing run metrics. This file is used to assess the quality of the sequencing run and is typically used for troubleshooting following details provided in this guide.
- The Complete Case Record (CCR) is a case-level report in csv format. The CCR aggregates data from the clinical sample with the external control to provide run-level, sample-level and external control QC status, as well as a list of variant calls organized by variant type in alphabetical order and in order of codon position. This file may be used to send data to annotation and interpretation services, or to aggregate data within internal customer database systems.
- The Case Report is a case-level report in csv and PDF format.
 - The PDF Case Report organizes variants by association with clinical evidence per Tier IA of AMP/ASCO/CAP guidelines (Li et al., 2017). The PGDx elio tissue complete CDx product uses a positive reporting scheme only, meaning only variants detected and passing the pipeline filters and cutoffs will be reported in the Companion Diagnostic Findings (in Melanoma), Variants with Evidence of Clinical Significance (Tier IA), and Variants with Potential Clinical Significance (Tier IB-IV) tables. This file may be used for medical records or appended to clinically interpreted reports.
 - The csv file can be integrated more readily into electronic systems.
 - Case Report, both PDF and csv, are only available when a tumor type is selected in the user interface.

Variants are positively reported, if found when cases pass all QC metrics. If no variant within a specific variant type is found in analysis, or if a variant found is below reporting thresholds, the report will notify users that no result was found for that variant type.

What Information Is Not Provided in PGDx elio tissue complete CDx reports?

Clinical interpretation of variants is not provided in PGDx reports. Test results should be interpreted in the context of other laboratory and clinical data, such as pathological evaluation of tumors, treatment history and clinical findings and results of other laboratory tests. All clinical interpretations of the variants detected in this assay should be made by a board certified Pathologist or equivalent. Additional testing and reporting information may be obtained from NCCN Guidelines and AMP/ASCO/CAP NGS Guidelines (Li et al., 2017).

This assay is designed to accept tumor-only samples (as opposed to tumor-normal pairs) and reports somatic mutations that meet reporting requirements. While the assay filters out common germline mutations, some rare germline alterations may be reported. Similarly, certain somatic variants may be present in population databases and may be filtered out of the final reports. More information on germline mutation filtering can be found below in **Thresholds and Annotation**.

Limitations and Warnings

Refer to the **Limitations of Procedure** in **Part 1** and **Warnings** in **Part 2** of this manual.

Reports Description

What Information Can Be Found in the Complete Run Record (CRR)?

The CRR is the first report available after an Analysis Plan goes through demultiplexing. This file provides a preview of the sequencing run quality metrics to inform whether the plan has passed or failed QC metrics (look for 'Run Quality Metrics' in the CRR). See **Figure 3.1** for an example. If a sequencing metric has failed in the CRR, troubleshooting steps can either be found

below in the **Quality Control (QC) and Troubleshooting** section of this guide or in **Part 2** of the PGDx elio tissue complete CDx Manual.

27	[Run Quality Metrics]			
28	Metric	Value	Threshold	Pass/Fail
29	Cluster Density (K/mm2)	127	130 - N/A	FAIL
30	Bases >=Q30 in Read 1 (%)	94	80 - N/A	PASS
31	Bases >=Q30 in Read 2 (I) (%)	99	85 - N/A	PASS
32	Bases >=Q30 in Read 3 (I) (%)	99	85 - N/A	PASS
33	Bases >=Q30 in Read 4 (%)	93	80 - N/A	PASS

Figure 3.1 Summary of Run Quality Metrics from CRR.

What Information Can Be Found in the Complete Case Record (CCR)?

The CCR is a csv file enabling movement of data from the case record into laboratory information systems, data warehouses or third-party annotation and interpretation services. The CCR summarizes all run-level, control-level and sample-level information and quality metrics as well as presents variant call results for the Case (sample + control). Detailed information on how to troubleshoot using the QC metrics can be found in **Quality Control (QC) and Troubleshooting** section below.

Case results are presented by variant types starting with 'Sample Genomic Signatures' (see **Figure 3.2**). Variants are organized by variant type with named sections that contain detailed information required for annotation, such as genomic position and transcript ID, that align with CAP guidelines. Data in the CCR is organized by variant type, in alphabetical order and by genomic position and is not organized by levels of significance. See **Figure 3.2** for an example screenshot of a CCR.

108	[Sample Genomic Signatures]								
109	Signature	Status/Score							
110	Microsatellite Analysis	MSI-H							
111	TMB Muts/Mb	83.9							
112									
113	[Sample Sequence Mutation Analysis]								
114	Gene	Genomic Location	Reference	Mutant	Amino Acid Change	Coding DNA Change	Transcript	Consequence	MAF (%)
115	ABL1	Chr9:133738378-133738378	G	A	V260M	778G>A	CCDS35166.1	Missense	36.6
116	PIK3R2	Chr19:18280036-18280036	G	A	A707T	2119G>A	CCDS12371.1	Missense	12.6
117	POLD1	Chr19:50920455-50920455	G	T	E1049D	3147G>T	CCDS12795.1	Missense	26.4
118	TP53	Chr17:7577121-7577121	G	A	R273C	817C>T	CCDS11118.1	Missense	16.9
119									
120	[Sample Amplification Analysis]								
121	Gene	Status	Genomic Location						
122	ERBB2	Amplified	Chr17:37844392-37884915						
123									
124									
125	[Sample Translocation Analysis]								
126	Gene	Genomic Location	Consequence	Partner	Partner Genomic Location				
127	ALK	Chr2:29447061	Gene fusion	SSX2IP	Chr1:85117185				

Figure 3.2 Examples of variant calls provided in the CCR.

What Information Can Be Found in the Case Report (PDF)?

The Case Report presents a PDF view of case results. Case Reports will be available in the PGDx elio user interface only after a tumor type for the sample has been selected. Tumor type selection should align with the clinical diagnosis and all available information. In the case of metastasis from a known primary site, the tumor type of the primary site should be selected. In the case of metastasis of unknown origin, unknown primary site, or uncertainty of the tumor type, 'Other' should be selected. Tumor type selection drives the appearance of Variants with Evidence of Clinical Significance on the first page of the Case Report. Variants with Evidence of Clinical Significance in alternative tumor types may appear on subsequent pages and should always be reviewed in the context of all available clinical information. For cell line sample types, 'Other' should be selected. See **Table 3.3** for a list of alterations that will be presented in the 'Variants with Evidence of Clinical Significance' table of the Case

Report when 'Other' is selected. Sample, assay and run details are provided in the header of every page (**Figure 3.3** and **Table 3.2**).

Sample Name: braf_v600_sample			
Tumor Type: Melanoma	Run Quality Result: PASS	Assay Name: PGDx elio tissue complete CDx (04)	
Sample Type: Cell Line	Sample Quality Result: PASS	Assay Version: 3.0.0.X	
Details: (blank)	Control Quality Result: PASS	Platform Version: v1.8.2-4	
Report Date: 2025-05-29	Overall Case Quality Result: PASS	Flow Cell ID: HTLHTBGX9	
<i>Please refer to the Complete Case Record (CCR) for details on quality metrics.</i>			

Figure 3.3 Screenshot of Case Report Header.

Table 3.2 Key for Header Table in PDF Case Report

Term	Definition
Tumor Type	Chosen from a selection of 16 available tumor types, including 15 tumor types aligning to NCCN guidelines: Breast, Central nervous system (CNS), Cholangiocarcinoma, Colon, Gastric, Gastrointestinal stromal tumors (GIST), Inflammatory Myofibroblastic Tumors (IMT), Melanoma, Non-small cell lung (NSCLC), Ovarian, Pancreatic, Prostate, Rectal, Thyroid, and Uterine.
Sample Type	The type of sample being processed (external control, specimen, or cell line). Classification is selected by the user during creation of the Analysis Plan.
Details	Free text field (max 29 characters). May be used to add histology information, diagnosis or SNOMED code.
Report Date	Date (YYYY-MM-DD) of report generation by the PGDx elio platform.
Run Quality Result	PASS/ FAIL value indicating whether the associated sequencing run data passed quality checks.
Sample Quality Result	PASS/ FAIL value indicating whether the sample data passed quality checks.
Control Quality Result	PASS/ FAIL value indicating whether the control data passed quality checks.
Overall Case Quality Result	Overall PASS/ FAIL value indicating the quality of the case data. It will fail if any of the other three quality metrics failed.
Assay Name	Name of the PGDx elio assay that was run to generate the report.
Assay Version	Version of the assay that was run to generate the report.
Platform Version	Software version of PGDx elio platform.
Flow Cell ID	Unique NextSeq flow cell ID.

The Case Report summarizes the results for variants approved as companion diagnostics (CDx) for PGDx elio tissue complete CDx in approved tumor types. These variants are reported under the section titled "Companion Diagnostic Findings". Variants are also classified according to the three-tier evidence framework defined by AMP/ASCO/CAP guidelines (Li et al., 2017). Specifically, variants meeting Tier 1A evidence (those associated with FDA-approved therapies or explicitly included in professional clinical guidelines) are presented in the section labeled "Variants with Evidence of Clinical Significance".

Figure 3.4 is an example case report where the 'Melanoma' tumor type was selected.

PGDx elio™ tissue complete CDx IVD Report

Sample Name: LVA000301T1_TMB2

Tumor Type: Melanoma
Sample Type: Specimen
Details: N17_0052
Report Date: 2025-06-19

Run Quality Result: PASS
Sample Quality Result: PASS
Control Quality Result: PASS
Overall Case Quality Result: PASS

Assay Name: PGDx elio tissue complete CDx (04)
Assay Version: 3.0.1.0
Platform Version: v1.8.2-4
Flow Cell ID: HHJKWBXY

Please refer to the Complete Case Record (CCR) for details on quality metrics.

Companion Diagnostic Findings in Melanoma[§]

Sequence Mutation Analysis				
Gene	Alteration	Consequence	VAF (%)	FDA-Approved Therapeutic Options
BRAF	V600E	Missense	9.1	BRAF Inhibitors approved by FDA or BRAF/MEK Inhibitor Combinations approved by FDA

[§]PGDx elio tissue complete CDx is intended to be used as a companion diagnostic to identify cancer patients who may benefit from treatment with the therapeutics listed in accordance with the approved therapeutic product labeling.

Variants with Evidence of Clinical Significance in Melanoma^{§§}

Genomic Signatures	
Signature	Status/Score
Microsatellite Analysis	MSS
TMB Muts/Mb (Sequenced)	10.0

Sequence Mutation Analysis			
Gene	Alteration	Consequence	VAF (%)
No Sequence Mutations with Evidence of Clinical Significance were detected.			

Amplification Analysis	
Gene	Alteration
No Amplifications with Evidence of Clinical Significance were detected.	

Translocation Analysis		
Gene	Alteration	Partner
No Translocations with Evidence of Clinical Significance were detected.		

Figure 3.4 Page 1 of Melanoma Case Report illustrating 'Companion Diagnostic Findings' and 'Variants with Evidence of Clinical Significance' section of report. The Companion Diagnostic Findings section will only appear in 'Melanoma' or 'Other' tumor types.

Sample Name: LVA000301T1_TMB2

Tumor Type: Melanoma
Sample Type: Specimen
Details: N17_0052
Report Date: 2025-06-19

Run Quality Result: PASS
Sample Quality Result: PASS
Control Quality Result: PASS
Overall Case Quality Result: PASS

Assay Name: PGDx elio tissue complete CDx (04)
Assay Version: 3.0.1.0
Platform Version: v1.8.2-4
Flow Cell ID: HHJKWBDXY

Please refer to the Complete Case Record (CCR) for details on quality metrics.

Variants with Potential Clinical Significance

Sequence Mutation Analysis			
Gene	Alteration	Consequence	VAF (%)
AXIN1	S665C	Missense	45.6
CHEK2	L467F	Missense	46.9
CSF1R	G333S	Missense	9.6
DNMT3A	W297*	Nonsense	12.8
MAP2K4	R281Q	Missense	14.9
MITF	L442V	Missense	48.3
PIK3C2G	L282R	Missense	10.2
SNCAIP	E627K	Missense	9.6
TERT	n/a	Promoter	6.6
TET2	I1236N	Missense	14.0
TET2	R1354Sfs*46	Frameshift	5.4

Amplification Analysis	
Gene	Alteration
No Amplifications with Potential Clinical Significance were detected.	

Translocation Analysis		
Gene	Alteration	Partner
No Translocations with Potential Clinical Significance were detected.		

Figure 3.5 Page 2 of a Case Report in which 'Melanoma' was selected as the tumor type, illustrating the 'Variants with Potential Clinical Significance' section of the report. Variants not annotated as Tier 1A can be found in the section titled 'Variants with Potential Clinical Significance.'

Figure 3.6 shows an example case report with the 'Non-Small Cell Lung Cancer (NSCLC)' tumor type selected.

PGDx elio™ tissue complete CDx IVD Report			
Sample Name: LVA000301T1_TMB2			
Tumor Type: NSCLC	Run Quality Result: PASS	Assay Name: PGDx elio tissue complete CDx (04)	
Sample Type: Specimen	Sample Quality Result: PASS	Assay Version: 3.0.1.0	
Details: N17_0052	Control Quality Result: PASS	Platform Version: v1.8.2-4	
Report Date: 2025-06-19	Overall Case Quality Result: PASS	Flow Cell ID: HHJJKWBDXY	
Please refer to the Complete Case Record (CCR) for details on quality metrics.			

Variants with Evidence of Clinical Significance in NSCLC§

Genomic Signatures			
Signature			Status/Score
Microsatellite Analysis			MSS
TMB Muts/Mb (Sequenced)			10.0
Sequence Mutation Analysis			
Gene	Alteration	Consequence	VAF (%)
BRAF	V600E	Missense	9.1
Amplification Analysis			
Gene	Alteration		
No Amplifications with Evidence of Clinical Significance were detected.			
Translocation Analysis			
Gene	Alteration	Partner	
No Translocations with Evidence of Clinical Significance were detected.			

Figure 3.6 Page 1 of 'NSCLC' Case Report illustrating 'Variants with Evidence of Clinical Significance' section of report. In this example, BRAF V600E is not an approved companion diagnostic for NSCLC by the PGDx elio tissue complete CDx assay. Therefore, only the 'Variants with Evidence of Clinical Significance' table will appear.

Figure 3.7 is an example of the ‘Variants with Potential Clinical Significance’ section of a case report in which ‘NSCLC’ was selected.

Variants with Potential Clinical Significance

Sequence Mutation Analysis			
Gene	Alteration	Consequence	VAF (%)
AXIN1	S665C	Missense	45.6
CHEK2	L467F	Missense	46.9
CSF1R	G333S	Missense	9.6
DNMT3A	W297*	Nonsense	12.8
MAP2K4	R281Q	Missense	14.9
MITF	L442V	Missense	48.3
PIK3C2G	L282R	Missense	10.2
SNCAIP	E627K	Missense	9.6
TERT	n/a	Promoter	6.6
TET2	I1236N	Missense	14.0
TET2	R1354Sfs*46	Frameshift	5.4

Amplification Analysis	
Gene	Alteration
No Amplifications with Potential Clinical Significance were detected.	

These results are intended to provide pan cancer tumor profiling information for use by qualified healthcare professionals in accordance with professional guidelines and are not conclusive or prescriptive for labeled use of any specific therapeutic product unless reported in the Companion Diagnostic Findings section. Qualified healthcare professionals should confirm the clinical diagnosis and all available information, including whether the sample is from a primary or a metastatic site. Therapies listed in this report have been approved by the US FDA.

§Variants with evidence of clinical significance appear on the first page based on the selected tumor type. Variants with evidence of clinical significance in alternative tumor types may appear on subsequent pages.

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Rx only

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Figure 3.7 Page 2 of a Case Report in which ‘NSCLC’ was selected as the tumor type, illustrating the ‘Variants with Potential Clinical Significance’ section of the report.

In both the ‘Variants with Evidence of Clinical Significance’ and ‘Variants with Potential Clinical Significance’ sections, when cases pass all QC metrics, variants are positively reported if found. If no variants within a specific variant type (e.g., amplification) are found in analysis, the report will read ‘No Amplifications with Potential Clinical Significance were detected’ in the appropriate variant type section.

Results definitions for all terminology used in reporting can be found on the last page of the Case Report (**Figure 3.8**).

Results Definition

Companion Diagnostic Findings: PGDx elio tissue complete CDx has been demonstrated to be essential for the safe and effective use of the labeled therapeutic product.

Variants with Evidence of Clinical Significance: Aligns to AMP/ASCO/CAP NGS reporting Guidelines for Tier IA.

Variants with Potential Clinical Significance: Aligns to AMP/ASCO/CAP NGS reporting Guidelines for Tier IB-IV evidence.

- AMP/ASCO/CAP NGS Reporting Guidelines: Li, Marilyn M. et al (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer. The Journal of Molecular Diagnostics, Volume 19, Issue 1, 4-23.

Genomic Signatures

- TMB Score
 - Muts/Mb: the mutations per megabase sequenced within the PGDx elio tissue complete targeted region of interest.
- Microsatellite Analysis
 - MSI-H: Microsatellite Instability High.
 - MSS: Microsatellite Stable (MSS).
 - Indeterminate - microsatellite status: Tract unstable frequency ≤ 0.1 and mutation signature score ≥ 20 OR Tract unstable frequency ≥ 0.25 and mutation signature score ≤ -10

Sequence Mutation Analysis

- VAF: Mutant Allele Fraction
- M1?: Start codon loss, translation of this codon will likely not occur making an amino acid change not applicable.

Amplification Analysis

- Amplification: an increase in copy number was detected.
- Indeterminate - amplification: tumor purity could not be estimated to support the detection of copy number alterations of this gene.

Translocation Analysis

- Gene Fusion: a translocation event was detected where gene coding strand continuity is maintained (this may result in transcription of the coding strand of one gene into the coding strand of another gene).
- Rearrangement: a translocation event was detected where gene coding strand continuity is not maintained (this may result in transcription of the coding strand of one gene into the template strand of another gene).

Regions of Low Coverage

- No signal detected; low coverage at this position: Coverage in this region is insufficient to determine results, no evidence of alteration at this position.
- Low signal detected; insufficient coverage to report: Coverage in this region is insufficient to determine results.

Sample Reporting

- No Result: 'No Result' may be reported if the sample, control, or run have not met quality standards resulting in the inability to report results (please see Complete Case Record (CCR) for details on data quality metrics).

Indication

- CNS: Central Nervous System
- GIST: Gastrointestinal Stromal Tumor
- IMT: Inflammatory Myofibroblastic Tumor
- NSCLC: Non-Small Cell Lung Cancer

Figure 3.8 Results definitions found toward the end of the Case Report.

Limitations of Approach

For mutations listed in Companion Diagnostic Findings, clinical validation for companion diagnostic use has been established for variants in accordance with the approved therapeutic labeling.

For mutations listed in the Variants with Potential Clinical Significance section of the PGDx elio tissue complete report, the clinical significance has not been demonstrated with this test.

For mutations listed in the Variants with Evidence of Clinical Significance section of the PGDx elio tissue complete report, please refer to the Report Interpretation Guide for information concerning conclusions about the level of clinical evidence.

Next generation sequencing approaches may provide incorrect sequence or mutational data due to insufficient coverage in specific regions of the genome, inability to distinguish highly related human sequences, and sequencing errors and the analysis of sequence specific alterations can also be hampered by limitations related to the tumor DNA. A negative result does not rule out the presence of a mutation below the limits of detection of the assay.

The information contained in this report does not constitute medical advice. While this report is believed to be accurate and complete as of the date issued, Personal Genome Diagnostics Inc. is not responsible for updating the report to incorporate newly obtained information or new interpretations of the assay results contained in the report.

Disclaimer

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Figure 3.9 Limitations of Approach and Disclaimers are found on the last page of the Case Report.

Thresholds and Annotation

PGDx uses patented analysis methodologies (Leary et al., 2010, Wang et al., 2002, Wood et al., 2018) for somatic mutation identification to optimize sensitivity and specificity for detection of true alterations.

PGDx elio tissue complete CDx software applies a unique threshold on a gene-by-gene basis to known clinically actionable variants and high frequency mutations commonly found in cancer. The variants found in **Table 3.3** below will be presented in the 'Variants with Evidence of Clinical Significance' tables of the Case Report on the basis of tumor type selection. Only variants for a selected tumor type will appear on page 1 of the Case Report in 'Variants with Evidence of Clinical Significance', while remaining variants of significance in alternative tumor types will appear on subsequent pages in the 'Variants with Potential Clinical Significance' tables.

Table 3.3 Variants with Evidence of Clinical Significance

Tumor Type	Gene	Alteration†
Pan-cancer	BRAF	V600E
Pan-cancer	NTRK2	Translocations
Pan-cancer	NTRK3	Translocations
Pan-cancer	MSI	High
Pan-cancer	RET	Translocations
Pan-cancer	TMB	Muts/Mb
Breast	ERBB2	Amplification
Breast	PIK3CA	C420R, E542K, E545A, E545D, E545G, E545K, Q546E, Q546R, H1047L, H1047R, H1047Y
Cholangiocarcinoma	IDH1	R132

Tumor Type	Gene	Alteration†
CNS	IDH1	R132
CNS	IDH2	R140Q, R172
Colon	KRAS	G12/13, A59, Q61, K117, A146
Colon	NRAS	G12/13, A59, Q61, K117, A146
Gastric	ERBB2	Amplifications
GIST	KIT	V825A, Exon9 and 11 mutations
GIST	PDGFRA	V561D, D842V
IMT	ALK	Translocations
Melanoma	BRAF	V600‡
Melanoma	KIT	D816H, D816Y, V825A
NSCLC	MET	Exon 14 splice site mutations
NSCLC	KRAS	G12C
NSCLC	ERBB2	V659E, A771_Y772insYVMA, Y772_A775dup, G776delinsVC, V777_G778insGSP, G778_P780dup
NSCLC	EGFR	G719, S768I, T790M, L858R, L861Q, Exon 19 Deletions, Exon 20 Insertions
NSCLC	ALK	Translocations
Ovarian	BRCA1	Deleterious Alterations +
Ovarian	BRCA2	Deleterious Alterations +
Pancreatic	BRCA1	Deleterious Alterations +
Pancreatic	BRCA2	Deleterious Alterations +
Prostate	BRCA1	Deleterious Alterations +
Prostate	BRCA2	Deleterious Alterations +
Rectal	KRAS	G12/13, A59, Q61, K117, A146
Rectal	NRAS	G12/13, A59, Q61, K117, A146
Thyroid	RET	A883F, C634, M918T#
Uterine	ERBB2	Amplification

† If no specific amino acid is listed under Alteration, any substituted amino acid is considered significant.

RET alterations are clinically relevant in medullary thyroid cancer and not all thyroid cancers.

+ See **Table 3.4** for deleterious alterations in BRCA1 and BRCA2 genes.

‡ In melanoma, BRAF V600E and K will appear in the Companion Diagnostic Findings table. Any other BRAF V600 variants will be reported as Variants with Evidence of Clinical Significance.

What Databases are Used Within PGDx elio tissue complete CDx?

Human Reference Genome

hg19/GRCh37

Hotspot Mutations

Sequence mutations in both cancer hotspots and non-hotspots are reported. PGDx defines hotspots as > 25 exact hits in COSMIC version 72. Hotspot mutations and Variants with Evidence of Clinical Significance have lower thresholds to improve sensitivity.

Functional Annotation

Mutations are annotated with a gene and predicted functional consequence using Variant Effect Predictor (Ensembl v75) and Consensus Coding Sequences (CCDS) transcripts where available.

Germline Databases

Germline mutations, including common polymorphisms in the population, present in dbSNP v150, ExAC v0.3.1, and gnomAD v2.0.2 are identified and excluded from reports. These databases are widely used in the field, with well-characterized cohorts and publicly available data. While the assay filters out these common germline mutations, some rare germline alterations may be reported. SNVs and indels that are not Variants with Evidence of Clinical Significance or hotspots will also be removed from reporting if they have ≥ 3 ExAC hits and have a VAF $\geq 20\%$.

What Filters are Used in PGDx elio tissue complete CDx?

The PGDx elio tissue complete CDx assay applies minimum coverage thresholds for reporting to support the verified performance labeling of sensitivity at the LoD₉₅. This assay will report positive alterations below the LoD₉₅ when criteria for positive variants are met, including passing mutant observations and quality scores. A summary of filtering for each variant type is described below.

SNVs and Indels

The PGDx elio tissue complete CDx pipeline identifies sequence mutations, including single nucleotide variants (SNVs) and insertions or deletions (indels) up to 42 bp. Candidate sequence mutations are identified in genome alignments using PGDx software and assigned a confidence score that is used for filtering. Hotspot mutations require 4 mutant observations and variant allele frequency (VAF) $\geq 2\%$ to be reported with the exception of BRCA nonsense mutations, which require VAF $\geq 3\%$. Non-hotspot mutations require 6 mutant observations and VAF $\geq 5\%$. Additionally, variants with emerging clinical significance in ALK, BRAF, EGFR, KIT, and MET require 4 mutant observations and VAF $\geq 3\%$.

Candidate mutations are also evaluated for characteristics of high confidence somatic variants, including sequence coverage and quality, genomic context, functional annotation, germline status, and prevalence in databases of normal controls. SNVs classified as Variants with Potential Clinical Significance that are not hotspots are removed from reporting if the lower bound of the confidence interval of the VAF of the variant is < 5%. Indels classified as 'Variants with Potential Clinical Significance' that are non-hotspots are removed from reporting if they are a homopolymer of length ≥ 5 and have a VAF < 12%. SNVs and indels that are not Variants with Evidence of Clinical Significance or hotspots will not be reported if found in regions listed in **Table A3.3 Low Complexity and Repeat Genomic Regions Excluded from Reporting of Non-hotspot SNVs and Indels Classified as Variants with Potential Significance** SNVs and indels classified as Variants with Potential Clinical Significance will also be removed from reporting if they have ≥ 3 ExAC hits and have a VAF $\geq 20\%$.

Translocations

The PGDx elio tissue complete CDx pipeline identifies select intra-chromosomal events (inversions, deletions, or duplications) and intra-chromosomal translocations greater than 2 kilobase pairs in length and inter-chromosomal translocations for the following genes: ALK, NTRK2, NTRK3, and RET. Alterations are annotated for gene partner and fusion status to determine if a resulting gene product is expected to maintain a coding strand. Gene products maintaining the coding strands are referred to as a Gene Fusion, whereas those resulting in a coding strand of one partner arranged with the non-coding strand of the second partner are referred to as a Rearrangement. Filtering is used to remove low-quality observations and common structural variations present in the human genome or normal controls.

Amplifications

The PGDx elio tissue complete CDx pipeline identifies regions of ERBB2 for amplifications and reports a qualitative status. A fold change equal to or greater than 2.5 (5 copies in diploid genome) is reported as amplified. A tumor purity estimate is derived from predicted somatic driver mutations and used to determine the fold change.

Tumor Mutation Burden Filtering

TMB is a measure of the number of somatic coding mutations in a tumor to characterize potential neoantigen load (Rizvi, 2015, Rosenberg, 2016, Snyder, 2014) as a significant association between neoantigen load and response to immunotherapeutics has been observed. The PGDx elio tissue complete CDx pipeline reports TMB based on detected sequence mutations and indels. Low VAF mutations, common somatic driver mutations, and common germline mutations are excluded from consideration when calculating TMB scores.

While whole-exome sequencing (WES) analysis can interrogate up to 50 Mb of genomic sequence for somatic mutations, due to the high cost of the large WES panels, most clinical testing is conducted with targeted panels ranging in size from 0.5 – 2 Mb and is reported in mutations per megabase (Muts/Mb) of interrogated exonic sequence present in the targeted panel. Many methodological variables influence the TMB score including size of targeted panel, tumor purity, methods of filtering and other bioinformatic specifications. PGDx has worked with the diagnostic community to come up with standards for TMB score (Vega, D.M. *et al.* 2021) and presents TMB scores in Muts/Mb.

Microsatellite Status

The PGDx elio tissue complete CDx pipeline identifies microsatellite instability (MSI) based on select mononucleotide tracts and the genomic context signatures of sequence mutations. Mononucleotide tracts are evaluated for changes relative to reference sequences. Sequence mutations are also evaluated to determine the likelihood of association with an MSI-H phenotype.

Deleterious Alterations in Homologous Recombination Repair Genes

Deleterious alterations in homologous recombination repair (HRR) genes are loss of function mutations (**Table 3.4**). These include nonsense, frameshift, and splice variants, as well as specific missense mutations in some genes. Alterations in BRCA1/2 include rare germline variants that are not present in public germline databases, and the assay does not distinguish from somatic mutations. BRCA1/2 alterations defined as deleterious are listed in **Table 3.4** and will be reported as Variants with Evidence of Clinical Significance for the appropriate tumor types.

Table 3.4 PGDx elio tissue complete CDx Deleterious Alterations in BRCA1 and BRCA2 Genes

BRCA1	BRCA2
All nonsense alterations	All nonsense alterations except with the exception of K3326* and downstream alterations
M1?, C61G, C64Y, R71G, R71K, R1516M, E1580K, D1713N, D1713H, R1720W, A1729E	M1?, V159M, V211L, V211I, R2336P, R2336H
Splice-site alterations within +/- 2 bp of intron-exon junctions	

Low Coverage Assessment

For select genes and regions, quality metrics are assessed to check for low coverage or incomplete data needed to identify an alteration. Low Coverage status is reported when 1) no evidence of the alteration was found, but minimum coverage was not met to support the verified limit of detection, or 2) some evidence of the alteration was observed, but minimum coverage thresholds were not met to report the variant. Supporting evidence of detected alterations and coverage in read data is available in the Complete Case Record (CCR). Details on the specific alterations and coverage targets are described below.

Sequence Mutations

A status of “Low signal detected, insufficient coverage to report” is reported when evidence of an alteration is observed in regions of low coverage below 80x distinct coverage. Positions that fail this metric may have decreased sensitivity and specificity.

A status of “No signal detected, low coverage at this position” is reported when an alteration is not detected, but there is insufficient coverage. Positions that fail this metric may have decreased sensitivity. Minimum coverage thresholds range from 125x – 260x distinct coverage.

Select genes and regions that are assessed for coverage in reporting “Low Coverage” variant calls are provided in **Table 3.5**.

Table 3.5 Regions Assessed for Sequence Mutation Low Coverage Status

Gene	Alteration	Gene	Alteration	Gene	Alteration
BRAF	V600	BRCA1	R71	KRAS	A59
BRCA1	Splice-site category	BRCA2	Splice-site category	KRAS	G12
BRCA1	Nonsense category	BRCA2	Nonsense category	KRAS	G13
BRCA1	A1729	BRCA2	M1	KRAS	K117
BRCA1	C61	BRCA2	R2336	KRAS	Q61
BRCA1	C64	BRCA2	V159	NRAS	A146
BRCA1	D1713	BRCA2	V211	NRAS	A59
BRCA1	E1580	EGFR	Exon 19 Deletions	NRAS	G12
BRCA1	M1	EGFR	L858	NRAS	G13
BRCA1	R1516	EGFR	T790	NRAS	K117
BRCA1	R1720	KRAS	A146	NRAS	Q61

Translocations

Low Coverage status is reported for translocations when low coverage (<100x) is observed at intron and coding sequence (CDS) breakpoint regions for specific genes listed in **Table 3.6**.

Table 3.6 Regions Assessed for Translocation Low Coverage Status

Gene	Intron/CDS Breakpoint Regions Assessed
ALK	Intron/CDS 19, CDS 20
NTRK2	Intron/CDS 9, CDS 10, Intron/CDS 12, CDS 13
NTRK3	Intron/CDS 11, Intron/CDS 12, CDS 13
RET	Intron/CDS 9, Intron/CDS 10, Intron/CDS 11

Amplifications

Reported Amplification status depends on the gene.

For ERBB2, "Amplified" will be reported if the tumor-adjusted foldchange is $\geq 2.5x$. "Indeterminant- amplification" will be reported when tumor purity cannot be predicted and observed fold change is elevated but below amplification cutoff. This range is between $1.3x - 2.5x$.

MSI

Microsatellite status is determined from two sources 1) observed microsatellite tract changes and 2) likelihood that variants detected are associated with an MSI-H phenotype. Indeterminate status is reported for MSI when there is a significant disagreement in status between the sources.

What Gene Annotations are Reported by PGDx elio tissue complete CDx?

PGDx elio tissue complete CDx reports gene annotation for sequence mutations, including a gene name, codon number, and amino acid changes in a format such as BRAF V600E. A predicted functional consequence is also reported, such as Missense, Nonsense, Frameshift, In-frame Insertion, In-frame Deletion, Splice Site Acceptor, or Splice Site Donor.

Reporting at Start Codon (M1) Positions

Due to start codon loss, an amino acid change is not applicable and therefore is reported as 'M1?' (aligning with Human Genome Variation Society (HGVS) nomenclature). Since translation of this codon will likely not occur, the question mark signifies this consequence is unclear.

What Factors Might Contribute to Discrepancies When Comparing NGS Methods?

When comparing PGDx elio tissue complete CDx to other NGS assays the following factors may contribute to seemingly discrepant results.

Region of Interest (ROI) Intersection

PGDx elio tissue complete CDx analyzes the full coding region of > 500 genes, with a subset of 4 driver genes for translocations and 1 gene for amplification. See **Table A3.1 PGDx elio tissue complete CDx Targeted Regions of Interest** in the appendix for PGDx elio tissue complete CDx targeted regions of interest and **Table A3.2 Regions of The PGDx elio tissue complete CDx Panel that are Excluded from Variant Analysis Due to Consistent Below-Target Coverage** for regions excluded from analysis due to low coverage. When comparing two assays, wherever possible, concordance analysis should be done on the genomic level as there may be differences in gene names or aliases (e.g., MLL3 vs. KMT2C), or there may be differences within the same gene ROI due to capture bait design, coverage or performance.

Reference Genome

PGDx elio tissue complete CDx genomic coordinates are based on reference genome assembly hg19/GRCh37. When evaluation results from other testing methodologies, ensure that the reference genomes match or convert genomic locations between reference genomes.

Annotation

The presence of alternative transcripts within the same gene may give rise to annotation differences between assays. PGDx elio tissue complete CDx provides transcript IDs in the CCR. Other discrepancies in annotation between assays may include left versus right alignment of alterations (e.g., indels) or HGVS notation differences (e.g., frameshift short). An annotation table of the PGDx elio tissue complete CDx gene list mapped to Ensembl and HGNC ID (HUGO Gene Nomenclature Committee) is provided in **Appendix A3 (Table A3.1 PGDx elio tissue complete CDx Targeted Regions of Interest)**.

Filtering Differences

Assays with integrated bioinformatics analysis likely utilize different verified thresholds for reporting that can result in either novel or masked variant calls. Examples of such thresholds include minimum VAF cutoffs, germline filtering rules or coverage and quality standards.

Sequencing platform differences may also contribute to differences in error profiles and error correction schemes used by assays with integrated bioinformatics analysis.

Please contact Customer Service and Support at PGDx with any questions or recommendations for evaluating PGDx elio tissue complete CDx (PGDxTechService@labcorp.com, 1-855-PGDx-NOW).

Quality Control (QC) and Troubleshooting

What Automated QC Metrics are Used by PGDx elio tissue complete CDx?

PGDx elio tissue complete CDx evaluates 5 main quality metrics: 1) in-process lab QC (e.g., library quality), 2) Run or Analysis Plan-level QC, 3) external control QC, 4) sample-level QC and 5) overall case (sample + external control) QC. Each metric is assessed independently, with pass/fail status output in the 'Pass/Fail' column for all metrics except in-process lab QC. If any metric fails, the metric itself will be flagged as 'FAIL.' If any QC metrics cause a sample to fail, this status will be output in the 'Case Summary' section of the PDF Case Report or csv CCR file.

In Process Lab QC (Manual Process)

Samples failing the lab process QC should not be included in Analysis Plans, nor sequenced. If not included as recommended, no reports are generated from the system for samples excluded from Analysis Plans.

Sequencing Run or Analysis Plan-Level QC

Analysis Plans are evaluated for run-level QC metrics including cluster density and individual read Q30 scores. These metrics prevent poor sequencing quality from impacting results. Run-level quality metrics are assessed in the section 'Run Quality Metrics'.

External Control QC

The external control provided with the PGDx elio tissue complete CDx kit contains verified mutations at known VAF levels. The pipeline examines the external control to ensure: 1) a discrete set of 7 verified mutations are found, 2) expected lane ratio (% reads identified) is achieved, and 3) panel-wide coverage metrics were achieved (% regions covered).

Sample-Level QC

All samples are evaluated for expected lane ratio (% reads identified) and panel-wide coverage minimums (% regions covered) to support reporting TMB and panel-wide variants. Select regions are evaluated for independent regional coverage metrics to support reporting of Variants with Evidence of Clinical Significance (see the Regions of Low Coverage section in **Thresholds and Annotation**). Samples are also evaluated with a proprietary contamination checking method to mitigate false positive or false negative reporting.

Overall Case QC

Clinical samples are reported back to the user as a 'Case' that evaluates both the batch-level external control and sample quality metrics before reporting results. Variant calls and genomic signatures will not be reported for cases where the external control or the sample-level QC metrics have failed.

At What Stage Should a Sample Be Re-Processed in the Event of a Failure?

Table 3.7 Recommended Steps for Reprocessing if QC Metrics Fail. provides recommended steps for reprocessing samples to overcome QC failure.

Table 3.7 Recommended Steps for Reprocessing if QC Metrics Fail.

QC Metric Failure	Stage Where Re-processing Should Take Place
External Control - % Reads Identified	Sequencing
External Control - % Regions Covered	Library preparation
External Control - QC Check	Library preparation, if source of failing criteria cannot be identified as a sample swap
Contamination ¹	DNA extraction or library preparation
Run Quality Metrics - Q30 or Cluster Density	Sequencing
% Reads Identified	Sequencing
% Regions Covered	Library preparation
Low Coverage Regions	Library preparation

¹**Note:** contamination may occur upstream of the PGDx elio tissue complete CDx assay, such as during sample collection or extraction.

How Should the Regions of Low Coverage Section Be Used to Troubleshoot?

If a variant has low coverage, it is reported in the PDF Case Report and it is classified in a separate section in the CCR. Refer to the 'Sample Variants of Low Coverage' section in the CCR (**Figure 3.9**). Use of the 'observed distinct coverage' and 'supporting reads (or distinct pairs)' columns can further classify Low coverage regions into: 1) Variant calls for which no evidence of the alteration was found but minimum coverage was not met to support the verified LoD, and 2) Variant calls for which minimum coverage thresholds were not met to report the variant, though some supporting read evidence was observed. See **Table 3.5** for a full list of genes or regions assessed for low coverage.

[Sample Regions of Low Coverage]						
Gene	Alteration	Observed Distinct Coverage	Minimum Distinct Coverage for Limit of Detection (negative call)	Minimum Distinct Coverage for Positive call	Supporting reads (or distinct pairs)	Status
BRAF	V600	10	248	80	none	No signal detected

Figure 3.10 Example Sample Variants of low coverage from CCR.

Regions or variants with 'Low signal detected, insufficient coverage to report' may be considered to have insufficient coverage with some supporting evidence of alteration. That is some evidence (e.g., supporting reads) of the variant was detected, but coverage quality thresholds were not met to support reporting this variant (see 'observed distinct coverage' compared to 'minimum distinct coverage for positive call' value = 80).

Regions or variants with 'No signal detected, low coverage at this position' in the 'Status' column can be considered as a low confidence call for which no evidence of the alteration was found. That is, PGDx elio tissue complete CDx saw no evidence that would support reporting that variant. However, minimum coverage thresholds were not achieved to meet labeled LoD requirements (see 'observed distinct coverage' compared to 'minimum distinct coverage for validated limit of detection' values).

Sample Preparation Recommendations

Please refer to **Part 2 ‘Specimen Type and Handling’** for more information about sample preparation.

Definitions

- **Analysis Plan:** Outlines a set of specimen samples to be analyzed after a sequencing run to generate run level and case level reports within the PGDx elio user interface.
- **CCR:** Complete Case Record (CCR) is a case-level report in csv format. The CCR aggregates data from the clinical sample with the external control to provide run-level, sample-level and control QC status, as well as a list of variant calls organized by variant type, in alphabetical order by codon position.
- **CRR:** Complete Run Record (CRR) is a run-level report in csv format. The CRR provides summary data of the Analysis Plan and associated sequencing run metrics. This file is used to assess the quality of the sequencing run and is typically used for troubleshooting.
- **Confidence score:** A score generated by the PGDx software to determine if a mutation is a true mutation or an artifact. This score is based on over 60 characteristics of a mutation.
- **CSV:** comma-separated value
- **HGNC:** HUGO Gene Nomenclature Committee
- **HGVS:** Human Genome Variation Society
- **HUGO:** Human Genome Organization
- **LoD:** Limit of Detection. The level for an analyte at which there is 95% confidence that a negative call is not a type II error.
- **MSI-H:** Microsatellite instability-high
- **MSS:** Microsatellite stable
- **ROI:** Region of interest
- **SNV:** Single nucleotide variant
- **TMB:** Tumor mutation burden
- **VAF:** Variant allele frequency

Part 3 References

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Appendix A3 PGDx elio tissue complete CDx Additional Details

Table A3.1 PGDx elio tissue complete CDx Targeted Regions of Interest

Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
ABL1	chr9	ENSG00000097007	76	ABL2	chr1	ENSG00000143322	77
ACVR1	chr2	ENSG00000115170	171	ACVR1B	chr12	ENSG00000135503	172
ADORA2A	chr22	ENSG00000128271	263	AKT1	chr14	ENSG00000142208	391
AKT2	chr19	ENSG00000105221	392	AKT3	chr1	ENSG00000117020	393
ALK	chr2	ENSG00000171094	427	ALOX12B	chr17	ENSG00000179477	430
AMER1	chrX	ENSG00000184675	26837	APC	chr5	ENSG00000134982	583
AR	chrX	ENSG00000169083	644	ARAF	chrX	ENSG00000078061	646
ARFRP1	chr20	ENSG00000101246	662	ARID1A	chr1	ENSG00000117713	11110
ARID1B	chr6	ENSG00000049618	18040	ARID2	chr12	ENSG00000189079	18037
ARID5B	chr10	ENSG00000150347	17362	ASXL1	chr20	ENSG00000171456	18318
ASXL2	chr2	ENSG00000143970	23805	ATM	chr11	ENSG00000149311	795
ATR	chr3	ENSG00000175054	882	ATRX	chrX	ENSG00000085224	886
AURKA	chr20	ENSG00000087586	11393	AURKB	chr17	ENSG00000178999	11390
AXIN1	chr16	ENSG00000103126	903	AXIN2	chr17	ENSG00000168646	904
AXL	chr19	ENSG00000167601	905	B2M	chr15	ENSG00000166710	914
BAP1	chr3	ENSG00000163930	950	BARD1	chr2	ENSG00000138376	952
BBC3	chr19	ENSG00000105327	17868	BCL2	chr18	ENSG00000171791	990
BCL2L1	chr20	ENSG00000171552	992	BCL2L11	chr2	ENSG00000153094	994
BCL2L2	chr14	ENSG00000129473	995	BCL6	chr3	ENSG00000113916	1001
BCOR	chrX	ENSG00000183337	20893	BCORL1	chrX	ENSG00000085185	25657
BCR	chr22	ENSG00000186716	1014	BIRC2	chr11	ENSG00000110330	590
BLM	chr15	ENSG00000197299	1058	BMPR1A	chr10	ENSG00000107779	1076
BRAF	chr7	ENSG00000157764	1097	BRCA1	chr17	ENSG00000012048	1100
BRCA2	chr13	ENSG00000139618	1101	BRD4	chr19	ENSG00000141867	13575
BRIP1	chr17	ENSG00000136492	20473	BTG1	chr12	ENSG00000133639	1130
BTG2	chr1	ENSG00000159388	1131	BTK	chrX	ENSG00000010671	1133
BUB1B	chr15	ENSG00000156970	1149	C11orf30	chr11	ENSG00000158636	18071
CALR	chr19	ENSG00000179218	1455	CARD11	chr7	ENSG00000198286	16393
CASP8	chr2	ENSG00000064012	1509	CBFB	chr16	ENSG00000067955	1539
CBL	chr11	ENSG00000110395	1541	CCND1	chr11	ENSG00000110092	1582
CCND2	chr12	ENSG00000118971	1583	CCND3	chr6	ENSG00000112576	1585
CCNE1	chr19	ENSG00000105173	1589	CD22	chr19	ENSG00000012124	1643
CD274	chr9	ENSG00000120217	17635	CD276	chr15	ENSG00000103855	19137
CD70	chr19	ENSG00000125726	11937	CD79A	chr19	ENSG00000105369	1698
CD79B	chr17	ENSG00000007312	1699	CDC73	chr1	ENSG00000134371	16783

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Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
CDH1	chr16	ENSG00000039068	1748	CDK12	chr17	ENSG00000167258	24224
CDK4	chr12	ENSG00000135446	1773	CDK6	chr7	ENSG00000105810	1777
CDK8	chr13	ENSG00000132964	1779	CDKN1A	chr6	ENSG00000124762	1784
CDKN1B	chr12	ENSG00000111276	1785	CDKN1C	chr11	ENSG00000129757	1786
CDKN2A	chr9	ENSG00000147889	1787	CDKN2B	chr9	ENSG00000147883	1788
CDKN2C	chr1	ENSG00000123080	1789	CEBPA	chr19	ENSG00000230259	1833
CHD2	chr15	ENSG00000173575	1917	CHD4	chr12	ENSG00000111642	1919
CHEK1	chr11	ENSG00000149554	1925	CHEK2	chr22	ENSG00000183765	16627
CIC	chr19	ENSG00000079432	14214	CREBBP	chr16	ENSG00000005339	2348
CRKL	chr22	ENSG00000099942	2363	CSF1	chr1	ENSG00000184371	2432
CSF1R	chr5	ENSG00000182578	2433	CSF2	chr5	ENSG00000164400	2434
CSF3	chr17	ENSG00000108342	2438	CSF3R	chr1	ENSG00000119535	2439
CTCF	chr16	ENSG00000102974	13723	CTLA4	chr2	ENSG00000163599	2505
CTNNA1	chr5	ENSG00000044115	2509	CTNNB1	chr3	ENSG00000168036	2514
CUL3	chr2	ENSG00000036257	2553	CUL4A	chr13	ENSG00000139842	2554
CXCR2	chr2	ENSG00000180871	6027	CXCR4	chr2	ENSG00000121966	2561
CYLD	chr16	ENSG00000083799	2584	CYP17A1	chr10	ENSG00000148795	2593
DAXX	chr6	ENSG00000204209	2681	DCUN1D1	chr3	ENSG00000043093	18184
DDB2	chr11	ENSG00000134574	2718	DDR1	chr6	ENSG00000204580	2730
DDR2	chr1	ENSG00000162733	2731	DICER1	chr14	ENSG00000100697	17098
DIS3	chr13	ENSG00000083520	20604	DNMT1	chr19	ENSG00000130816	2976
DNMT3A	chr2	ENSG00000119772	2978	DNMT3B	chr20	ENSG00000088305	2979
DOT1L	chr19	ENSG00000104885	24948	E2F3	chr6	ENSG00000112242	3115
EED	chr11	ENSG00000074266	3188	EGFL7	chr9	ENSG00000172889	20594
EGFR	chr7	ENSG00000146648	3236	EIF1AX	chrX	ENSG00000173674	3250
EP300	chr22	ENSG00000100393	3373	EPAS1	chr2	ENSG00000116016	3374
EPCAM	chr2	ENSG00000119888	11529	EPHA2	chr1	ENSG00000142627	3386
EPHA3	chr3	ENSG00000044524	3387	EPHA5	chr4	ENSG00000145242	3389
EPHA7	chr6	ENSG00000135333	3390	EPHB1	chr3	ENSG00000154928	3392
EPHB4	chr7	ENSG00000196411	3395	ERBB2	chr17	ENSG00000141736	3430
ERBB3	chr12	ENSG00000065361	3431	ERBB4	chr2	ENSG00000178568	3432
ERCC1	chr19	ENSG00000012061	3433	ERCC2	chr19	ENSG00000104884	3434
ERCC3	chr2	ENSG00000163161	3435	ERCC4	chr16	ENSG00000175595	3436
ERCC5	chr13	ENSG00000134899	3437	ERCC6	chr10	ENSG00000225830	3438
ERCC8	chr5	ENSG00000049167	3439	ERG	chr21	ENSG00000157554	3446
ERRFI1	chr1	ENSG00000116285	18185	ESR1	chr6	ENSG00000091831	3467
ETV1	chr7	ENSG00000006468	3490	ETV4	chr17	ENSG00000175832	3493
ETV5	chr3	ENSG00000244405	3494	ETV6	chr12	ENSG00000139083	3495

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Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
EWSR1	chr22	ENSG00000182944	3508	EXT1	chr8	ENSG00000182197	3512
EXT2	chr11	ENSG00000151348	3513	EZH2	chr7	ENSG00000106462	3527
FAM175A	chr4	ENSG00000163322	25829	FAM46C	chr1	ENSG00000183508	24712
FANCA	chr16	ENSG00000187741	3582	FANCB	chrX	ENSG00000181544	3583
FANCC	chr9	ENSG00000158169	3584	FANCD2	chr3	ENSG00000144554	3585
FANCE	chr6	ENSG00000112039	3586	FANCF	chr11	ENSG00000183161	3587
FANCG	chr9	ENSG00000221829	3588	FANCI	chr15	ENSG00000140525	25568
FANCL	chr2	ENSG00000115392	20748	FANCM	chr14	ENSG00000187790	23168
FAS	chr10	ENSG00000026103	11920	FAT1	chr4	ENSG00000083857	3595
FBXW7	chr4	ENSG00000109670	16712	FGF10	chr5	ENSG00000070193	3666
FGF12	chr3	ENSG00000114279	3668	FGF14	chr13	ENSG00000102466	3671
FGF19	chr11	ENSG00000162344	3675	FGF23	chr12	ENSG00000118972	3680
FGF3	chr11	ENSG00000186895	3681	FGF4	chr11	ENSG00000075388	3682
FGF6	chr12	ENSG00000111241	3684	FGFR1	chr8	ENSG00000077782	3688
FGFR2	chr10	ENSG00000066468	3689	FGFR3	chr4	ENSG00000068078	3690
FGFR4	chr5	ENSG00000160867	3691	FH	chr1	ENSG00000091483	3700
FLCN	chr17	ENSG00000154803	27310	FLT1	chr13	ENSG00000102755	3763
FLT3	chr13	ENSG00000122025	3765	FLT4	chr5	ENSG00000037280	3767
FOXA1	chr14	ENSG00000129514	5021	FOXL2	chr3	ENSG00000183770	1092
FOXP1	chr3	ENSG00000114861	3823	FRS2	chr12	ENSG00000166225	16971
FUBP1	chr1	ENSG00000162613	4004	GABRA6	chr5	ENSG00000145863	4080
GATA1	chrX	ENSG00000102145	4170	GATA2	chr3	ENSG00000179348	4171
GATA3	chr10	ENSG00000107485	4172	GATA4	chr8	ENSG00000136574	4173
GATA6	chr18	ENSG00000141448	4174	GID4	chr17	ENSG00000141034	28453
GLI1	chr12	ENSG00000111087	4317	GNA11	chr19	ENSG00000088256	4379
GNA13	chr17	ENSG00000120063	4381	GNAQ	chr9	ENSG00000156052	4390
GNAS	chr20	ENSG00000087460	4392	GPC3	chrX	ENSG00000147257	4451
GPR124	chr8	ENSG00000020181	17849	GREM1	chr15	ENSG00000166923	2001
GRIN2A	chr16	ENSG00000183454	4585	GRM3	chr7	ENSG00000198822	4595
GSK3B	chr3	ENSG00000082701	4617	H3F3A	chr1	ENSG00000163041	4764
H3F3B	chr17	ENSG00000132475	4765	H3F3C	chr12	ENSG00000188375	33164
HDAC1	chr1	ENSG00000116478	4852	HDAC2	chr6	ENSG00000196591	4853
HDAC6	chrX	ENSG00000094631	14064	HGF	chr7	ENSG00000019991	4893
HIST1H1C	chr6	ENSG00000187837	4716	HIST1H2BD	chr6	ENSG00000158373	4747
HIST1H3B	chr6	ENSG00000124693	4776	HNF1A	chr12	ENSG00000135100	11621
HRAS	chr11	ENSG00000174775	5173	HSD3B1	chr1	ENSG00000203857	5217
HSP90AA1	chr14	ENSG00000080824	5253	HSP90AB1	chr6	ENSG00000096384	5258
ICOSLG	chr21	ENSG00000160223	17087	ID3	chr1	ENSG00000117318	5362

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Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
IDH1	chr2	ENSG00000138413	5382	IDH2	chr15	ENSG00000182054	5383
IFNGR1	chr6	ENSG00000027697	5439	IGF1	chr12	ENSG00000017427	5464
IGF1R	chr15	ENSG00000140443	5465	IGF2	chr11	ENSG00000167244	5466
IGF2R	chr6	ENSG00000197081	5467	IKBKE	chr1	ENSG00000143466	14552
IKZF1	chr7	ENSG00000185811	13176	IL10	chr1	ENSG00000136634	5962
IL7R	chr5	ENSG00000168685	6024	INHBA	chr7	ENSG00000122641	6066
INPP4A	chr2	ENSG00000040933	6074	INPP4B	chr4	ENSG00000109452	6075
INSR	chr19	ENSG00000171105	6091	IRF2	chr4	ENSG00000168310	6117
IRF4	chr6	ENSG00000137265	6119	IRS1	chr2	ENSG00000169047	6125
IRS2	chr13	ENSG00000185950	6126	JAK1	chr1	ENSG00000162434	6190
JAK2	chr9	ENSG00000096968	6192	JAK3	chr19	ENSG00000105639	6193
JUN	chr1	ENSG00000177606	6204	KAT6A	chr8	ENSG00000083168	13013
KDM5A	chr12	ENSG00000073614	9886	KDM5C	chrX	ENSG00000126012	11114
KDM6A	chrX	ENSG00000147050	12637	KDR	chr4	ENSG00000128052	6307
KEAP1	chr19	ENSG00000079999	23177	KEL	chr7	ENSG00000197993	6308
KIT	chr4	ENSG00000157404	6342	KLF4	chr9	ENSG00000136826	6348
KLHL6	chr3	ENSG00000172578	18653	KMT2A	chr11	ENSG00000118058	7132
KMT2C	chr7	ENSG00000055609	13726	KMT2D	chr12	ENSG00000167548	7133
KRAS	chr12	ENSG00000133703	6407	LATS1	chr6	ENSG00000131023	6514
LATS2	chr13	ENSG00000150457	6515	LMO1	chr11	ENSG00000166407	6641
LRP1B	chr2	ENSG00000168702	6693	LTK	chr15	ENSG00000062524	6721
LYN	chr8	ENSG00000254087	6735	LZTR1	chr22	ENSG00000099949	6742
MAF	chr16	ENSG00000178573	6776	MAGI2	chr7	ENSG00000187391	18957
MAML1	chr5	ENSG00000161021	13632	MAP2K1	chr15	ENSG00000169032	6840
MAP2K2	chr19	ENSG00000126934	6842	MAP2K4	chr17	ENSG00000065559	6844
MAP3K1	chr5	ENSG00000095015	6848	MAP3K13	chr3	ENSG00000073803	6852
MAPK1	chr22	ENSG00000100030	6871	MAX	chr14	ENSG00000125952	6913
MCL1	chr1	ENSG00000143384	6943	MDC1	chr6	ENSG00000137337	21163
MDM2	chr12	ENSG00000135679	6973	MDM4	chr1	ENSG00000198625	6974
MED12	chrX	ENSG00000184634	11957	MEF2B	chr19	ENSG00000213999	6995
MEN1	chr11	ENSG00000133895	7010	MERTK	chr2	ENSG00000153208	7027
MET	chr7	ENSG00000105976	7029	MITF	chr3	ENSG00000187098	7105
MKNK1	chr1	ENSG00000079277	7110	MLH1	chr3	ENSG00000076242	7127
MLH3	chr14	ENSG00000119684	7128	MPL	chr1	ENSG00000117400	7217
MRE11A	chr11	ENSG00000020922	7230	MSH2	chr2	ENSG00000095002	7325
MSH3	chr5	ENSG00000113318	7326	MSH6	chr2	ENSG00000116062	7329
MST1R	chr3	ENSG00000164078	7381	MTAP	chr9	ENSG00000099810	7413
MTOR	chr1	ENSG00000198793	3942	MUTYH	chr1	ENSG00000132781	7527

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Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
MYB	chr6	ENSG00000118513	7545	MYC	chr8	ENSG00000136997	7553
MYCL	chr1	ENSG00000116990	7555	MYCN	chr2	ENSG00000134323	7559
MYD88	chr3	ENSG00000172936	7562	MYOD1	chr11	ENSG00000129152	7611
NBN	chr8	ENSG00000104320	7652	NCOA3	chr20	ENSG00000124151	7670
NCOR1	chr17	ENSG00000141027	7672	NF1	chr17	ENSG00000196712	7765
NF2	chr22	ENSG00000186575	7773	NFE2L2	chr2	ENSG00000116044	7782
NFKBIA	chr14	ENSG00000100906	7797	NKX2-1	chr14	ENSG00000136352	11825
NKX3-1	chr8	ENSG00000167034	7838	NOTCH1	chr9	ENSG00000148400	7881
NOTCH2	chr1	ENSG00000134250	7882	NOTCH3	chr19	ENSG00000074181	7883
NOTCH4	chr6	ENSG00000204301	7884	NPM1	chr5	ENSG00000181163	7910
NRAS	chr1	ENSG00000213281	7989	NSD1	chr5	ENSG00000165671	14234
NTSC2	chr10	ENSG00000076685	8022	NTRK1	chr1	ENSG00000198400	8031
NTRK2	chr9	ENSG00000148053	8032	NTRK3	chr15	ENSG00000140538	8033
NUP93	chr16	ENSG00000102900	28958	NUTM1	chr15	ENSG00000184507	29919
PAK1	chr11	ENSG00000149269	8590	PAK3	chrX	ENSG00000077264	8592
PAK7	chr20	ENSG00000101349	15916	PALB2	chr16	ENSG00000083093	26144
PARK2	chr6	ENSG00000185345	8607	PARP1	chr1	ENSG00000143799	270
PARP2	chr14	ENSG00000129484	272	PARP3	chr3	ENSG00000041880	273
PAX5	chr9	ENSG00000196092	8619	PAX8	chr2	ENSG00000125618	8622
PBRM1	chr3	ENSG00000163939	30064	PDCD1	chr2	ENSG00000188389	8760
PDCD1LG2	chr9	ENSG00000197646	18731	PDGFRA	chr4	ENSG00000134853	8803
PDGFRB	chr5	ENSG00000113721	8804	PDK1	chr2	ENSG00000152256	8809
PDPK1	chr16	ENSG00000140992	8816	PHOX2B	chr4	ENSG00000109132	9143
PIK3C2B	chr1	ENSG00000133056	8972	PIK3C2G	chr12	ENSG00000139144	8973
PIK3C3	chr18	ENSG00000078142	8974	PIK3CA	chr3	ENSG00000121879	8975
PIK3CB	chr3	ENSG00000051382	8976	PIK3CD	chr1	ENSG00000171608	8977
PIK3CG	chr7	ENSG00000105851	8978	PIK3R1	chr5	ENSG00000145675	8979
PIK3R2	chr19	ENSG00000105647	8980	PIK3R3	chr1	ENSG00000117461	8981
PIM1	chr6	ENSG00000137193	8986	PLCG2	chr16	ENSG00000197943	9066
PLK2	chr5	ENSG00000145632	19699	PMAIP1	chr18	ENSG00000141682	9108
PMS1	chr2	ENSG00000064933	9121	PMS2	chr7	ENSG00000122512	9122
PNRC1	chr6	ENSG00000146278	17278	POLD1	chr19	ENSG00000062822	9175
POLE	chr12	ENSG00000177084	9177	POLH	chr6	ENSG00000170734	9181
POT1	chr7	ENSG00000128513	17284	PPARG	chr3	ENSG00000132170	9236
PPP2R1A	chr19	ENSG00000105568	9302	PPP2R2A	chr8	ENSG00000221914	9304
PRDM1	chr6	ENSG00000057657	9346	PREX2	chr8	ENSG00000046889	22950
PRKAR1A	chr17	ENSG00000108946	9388	PRKCI	chr3	ENSG00000163558	9404
PRKDC	chr8	ENSG00000253729	9413	PRSS1	chr7	ENSG00000204983	9475

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Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
PRSS8	chr16	ENSG00000052344	9491	PTCH1	chr9	ENSG00000185920	9585
PTEN	chr10	ENSG00000171862	9588	PTK2	chr8	ENSG00000169398	9611
PTPN11	chr12	ENSG00000179295	9644	PTPRD	chr9	ENSG00000153707	9668
PTPRO	chr12	ENSG00000151490	9678	PTPRS	chr19	ENSG00000105426	9681
PTPRT	chr20	ENSG00000196090	9682	QKI	chr6	ENSG00000112531	21100
RAC1	chr7	ENSG00000136238	9801	RAD21	chr8	ENSG00000164754	9811
RAD50	chr5	ENSG00000113522	9816	RAD51	chr15	ENSG00000051180	9817
RAD51B	chr14	ENSG00000182185	9822	RAD51C	chr17	ENSG00000108384	9820
RAD51D	chr17	ENSG00000185379	9823	RAD52	chr12	ENSG00000002016	9824
RAD54B	chr8	ENSG00000197275	17228	RAD54L	chr1	ENSG00000085999	9826
RAF1	chr3	ENSG00000132155	9829	RANBP2	chr2	ENSG00000153201	9848
RARA	chr17	ENSG00000131759	9864	RASA1	chr5	ENSG00000145715	9871
RB1	chr13	ENSG00000139687	9884	RBM10	chrX	ENSG00000182872	9896
RECQL4	chr8	ENSG00000160957	9949	REL	chr2	ENSG00000162924	9954
RET	chr10	ENSG00000165731	9967	RFWD2	chr1	ENSG00000143207	17440
RHOA	chr3	ENSG00000067560	667	RICTOR	chr5	ENSG00000164327	28611
RIT1	chr1	ENSG00000143622	10023	RNF43	chr17	ENSG00000108375	18505
ROS1	chr6	ENSG00000047936	10261	RPA1	chr17	ENSG00000132383	10289
RPS6KA4	chr11	ENSG00000162302	10433	RPS6KB2	chr11	ENSG00000175634	10437
RPTOR	chr17	ENSG00000141564	30287	RUNX1	chr21	ENSG00000159216	10471
RUNX1T1	chr8	ENSG00000079102	1535	RYBP	chr3	ENSG00000163602	10480
SBDS	chr7	ENSG00000126524	19440	SDHA	chr5	ENSG00000073578	10680
SDHAF2	chr11	ENSG00000167985	26034	SDHB	chr1	ENSG00000117118	10681
SDHC	chr1	ENSG00000143252	10682	SDHD	chr11	ENSG00000204370	10683
SETD2	chr3	ENSG00000181555	18420	SF3B1	chr2	ENSG00000115524	10768
SGK1	chr6	ENSG00000118515	10810	SH2D1A	chrX	ENSG00000183918	10820
SHQ1	chr3	ENSG00000144736	25543	SLIT2	chr4	ENSG00000145147	11086
SLX4	chr16	ENSG00000188827	23845	SMAD2	chr18	ENSG00000175387	6768
SMAD3	chr15	ENSG00000166949	6769	SMAD4	chr18	ENSG00000141646	6770
SMARCA4	chr19	ENSG00000127616	11100	SMARCB1	chr22	ENSG00000099956	11103
SMARCD1	chr12	ENSG00000066117	11106	SMO	chr7	ENSG00000128602	11119
SNCAIP	chr5	ENSG00000064692	11139	SOCS1	chr16	ENSG00000185338	19383
SOX10	chr22	ENSG00000100146	11190	SOX17	chr8	ENSG00000164736	18122
SOX2	chr3	ENSG00000181449	11195	SOX9	chr17	ENSG00000125398	11204
SPEN	chr1	ENSG00000065526	17575	SPOP	chr17	ENSG00000121067	11254
SPTA1	chr1	ENSG00000163554	11272	SRC	chr20	ENSG00000197122	11283
STAG2	chrX	ENSG00000101972	11355	STAT3	chr17	ENSG00000168610	11364
STAT4	chr2	ENSG00000138378	11365	STK11	chr19	ENSG00000118046	11389

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Gene Name	Chr	Ensembl Gene ID	HGNC ID	Gene Name	Chr	Ensembl Gene ID	HGNC ID
STK40	chr1	ENSG00000196182	21373	SUFU	chr10	ENSG00000107882	16466
SUZ12	chr17	ENSG00000178691	17101	SYK	chr9	ENSG00000165025	11491
TAF1	chrX	ENSG00000147133	11535	TBX3	chr12	ENSG00000135111	11602
TEK	chr9	ENSG00000120156	11724	TERC	chr3	ENSG00000270141	11727
TERT	chr5	ENSG00000164362	11730	TET1	chr10	ENSG00000138336	29484
TET2	chr4	ENSG00000168769	25941	TGFBR1	chr9	ENSG00000106799	11772
TGFBR2	chr3	ENSG00000163513	11773	TIPARP	chr3	ENSG00000163659	23696
TLR4	chr9	ENSG00000136869	11850	TLR7	chrX	ENSG00000196664	15631
TLR8	chrX	ENSG00000101916	15632	TLR9	chr3	ENSG00000239732	15633
TMEM127	chr2	ENSG00000135956	26038	TMPRSS2	chr21	ENSG00000184012	11876
TNFAIP3	chr6	ENSG00000118503	11896	TNFRSF14	chr1	ENSG00000157873	11912
TOP1	chr20	ENSG00000198900	11986	TOP2A	chr17	ENSG00000131747	11989
TP53	chr17	ENSG00000141510	11998	TP53BP1	chr15	ENSG00000067369	11999
TP63	chr3	ENSG00000073282	15979	TRAF7	chr16	ENSG00000131653	20456
TSC1	chr9	ENSG00000165699	12362	TSC2	chr16	ENSG00000103197	12363
TSHR	chr14	ENSG00000165409	12373	TYRO3	chr15	ENSG00000092445	12446
U2AF1	chr21	ENSG00000160201	12453	VEGFA	chr6	ENSG00000112715	12680
VHL	chr3	ENSG00000134086	12687	VTCN1	chr1	ENSG00000134258	28873
WAS	chrX	ENSG00000015285	12731	WEE1	chr11	ENSG00000166483	12761
WHSC1	chr4	ENSG00000109685	12766	WHSC1L1	chr8	ENSG00000147548	12767
WISP3	chr6	ENSG00000112761	12771	WRN	chr8	ENSG00000165392	12791
WT1	chr11	ENSG00000184937	12796	XIAP	chrX	ENSG00000101966	592
XPA	chr9	ENSG00000136936	12814	XPC	chr3	ENSG00000154767	12816
XPO1	chr2	ENSG00000082898	12825	XRCC1	chr19	ENSG00000073050	12828
XRCC2	chr7	ENSG00000196584	12829	XRCC3	chr14	ENSG00000126215	12830
YAP1	chr11	ENSG00000137693	16262	YES1	chr18	ENSG00000176105	12841
ZBTB2	chr6	ENSG00000181472	20868	ZNF217	chr20	ENSG00000171940	13009
ZNF703	chr8	ENSG00000183779	25883				

Table A3.2 Regions of The PGDx elio tissue complete CDx Panel that are Excluded from Variant Analysis Due to Consistent Below-Target Coverage

Gene	Transcript	Exon	Gene	Transcript	Exon
ABL2	CCDS30947.1	2	MRE11A	CCDS8299.1	18, 19
APC	CCDS4107.1	4, 13	MSH2	CCDS1834.1	4, 16
ARID2	CCDS31783.1	7	MYB	CCDS47481.1	1
ATM	CCDS31669.1	1, 15, 28, 60	NBN	CCDS6249.1	6, 12, 15, 16
ATR	CCDS3124.1	37	NCOR1	CCDS11175.1	8, 14
ATRX	CCDS14434.1	2, 3, 5, 8, 10, 12, 13, 14, 15, 20, 22, 33	NPM1	CCDS4376.1	7
AURKA	CCDS13451.1	3	NT5C2	CCDS7544.1	2, 5, 8, 10, 14
BIRC2	CCDS58169.1	7	NTRK3	CCDS10340.1	9
BMPR1A	CCDS7378.1	1	PAK1	CCDS44687.1	14
BRIP1	CCDS11631.1	9, 12	PAK3	CCDS14554.1	4, 7, 9
BTB	CCDS14482.1	3, 8	PBRM1	CCDS43099.1	8
BUB1B	CCDS10053.1	13	PIK3C2G	CCDS44839.1	6, 9
CD274	CCDS6464.1	5	PIK3C3	CCDS11920.1	25
CD79A	CCDS12589.1	4	PIK3CB	CCDS3104.1	6
CDK8	CCDS9317.1	2, 8, 9	PMS1	CCDS2302.1	7
CHD2	CCDS10374.2	32	PMS2	CCDS5343.1	13
CHEK2	CCDS13843.1	4, 6, 7	POT1	CCDS5793.1	1
CREBBP	CCDS10509.1	21	PRKCI	CCDS3212.2	15
CSF1	CCDS30797.1	1	PRKDC	NM_006904	2, 4, 5, 14, 19, 75
CYLD	CCDS42164.1	13	PRSS8	CCDS45469.1	2
DCUN1D1	CCDS3240.1	2	PTEN	CCDS31238.1	3, 8
DNMT1	CCDS12228.1	7, 11, 13	PTK2	CCDS56557.1	3, 28
DOT1L	CCDS42460.1	12	PTPN11	CCDS9163.1	1
EIF1AX	CCDS14196.1	4, 5, 7	PTPRD	CCDS43786.1	5, 6, 15
EP300	CCDS14010.1	23	PTPRO	CCDS44837.1	5
EPCAM	CCDS1833.1	4, 8, 9	RAD50	CCDS34233.1	9, 16, 17, 18, 19, 20
ERCC3	CCDS2144.1	4	RAD51C	CCDS11611.1	6
ERCC5	CCDS32004.1	5	RAF1	CCDS2612.1	12
ERCC8	CCDS3978.1	2, 5, 12	RANBP2	CCDS2079.1	2, 8
ETV1	CCDS55088.1	4	RASA1	CCDS34200.1	6, 15, 19
FAM175A	CCDS3605.2	3, 4, 7	RB1	CCDS31973.1	6, 9, 11, 14, 15, 16, 17
FANCA	CCDS32515.1	9	REL	CCDS1864.1	8, 9, 10
FANCB	CCDS14161.1	3, 4, 5	RFWD2	CCDS30944.1	6, 20
FANCC	CCDS35071.1	3	RICTOR	CCDS34148.1	9, 12, 19, 22
FANCD2	CCDS2595.1	13, 19	ROS1	CCDS5116.1	3

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Gene	Transcript	Exon	Gene	Transcript	Exon
FANCI	CCDS10349.2	10	SETD2	CCDS2749.2	2
FANCL	CCDS1860.1	14	SF3B1	CCDS33356.1	11
FANCM	CCDS32070.1	8, 18, 19	SH2D1A	CCDS14608.1	4
FAS	CCDS7394.1	6, 7	SLIT2	CCDS3426.1	7, 12
FUBP1	CCDS683.1	4, 5	SPTA1	CCDS41423.1	1, 46, 49
GNA13	CCDS11661.1	3	STAG2	CCDS43990.1	1, 2, 4, 5, 6, 8, 10, 11, 13, 18, 19, 20, 22, 25, 33
GPC3	CCDS14638.1	4	STAT3	CCDS32656.1	10
HDAC2	CCDS43493.2	13	STAT4	CCDS2310.1	5, 10, 11, 13, 23
HGF	CCDS47626.1	12	SUZ12	CCDS11270.1	4, 5, 6, 11, 15
INPP4B	CCDS3757.1	2	TAF1	CCDS14412.1	23, 26, 31, 33
IRS2	CCDS9510.1	2	TET1	CCDS7281.1	8
JAK1	CCDS41346.1	1	TMPRSS2	CCDS33564.1	13
JAK2	CCDS6457.1	13	TOP2A	CCDS45672.1	32
JAK3	CCDS12366.1	12	TP53	CCDS11118.1	2
KDM6A	CCDS14265.1	4, 5, 7, 8, 9, 14, 21	TSHR	CCDS9872.1	8
KMT2C	CCDS5931.1	9, 22, 30	TYRO3	CCDS10080.1	1
KRAS	NM_033360	6	WEE1	CCDS44536.1	2
LRP1B	CCDS2182.1	6, 70	WRN	CCDS6082.1	6, 13, 26
MAGI2	CCDS5594.1	7, 11	XIAP	CCDS14606.1	2, 3, 4, 5
MAX	CCDS9771.1	2	XPO1	CCDS33205.1	5, 7, 21
MED12	CCDS43970.1	43	XRCC2	CCDS5933.1	2
MERTK	CCDS2094.1	12	YES1	CCDS11824.1	2
MLH1	CCDS2663.1	15			

Table A3.3 Low Complexity and Repeat Genomic Regions Excluded from Reporting of Non-hotspot SNVs and Indels Classified as Variants with Potential Significance

Gene	Transcript	Exons	Masked region coordinates
ABL2	CCDS44283.1	10	chr1:179079938-179079982
ACVR1B	NM_004302	1	chr12:52345518-52345523
AMER1	CCDS14377.2	1	chrX:63411959-63412030
APC	CCDS4107.1	15	chr5:112177303-112177356
AR	CCDS14387.1	1	chrX:66765159-66765262, chrX:66766339-66766409
ARID1A	CCDS285.1	1; 16	chr1:27022978-27023029, chr1:27023138-27023173, chr1:27023257-27023297, chr1:27023908-27023938; chr1:27100182-27100206
ARID1B	CCDS5251.2	1	chr6:157099075-157099100, chr6:157099166-157099187, chr6:157099303-157099376, chr6:157099403-157099459, chr6:157099483-157099507, chr6:157099699-157099743, chr6:157099785-157099807, chr6:157099872-157099898, chr6:157099975-157100144, chr6:157100203-157100228, chr6:157100239-157100267, chr6:157100431-157100457
ASXL2	NM_018263	6	chr2:25991700-25991731
ATM	CCDS31669.1	5; 30	chr11:108114678-108114679; chr11:108164038-108164039
ATRX	CCDS14434.1	9	chrX:76938248-76938317
AURKB	NM_004217	2	chr17:8113547-8113552
BAP1	CCDS2853.1	12	chr3:52438494-52438531
BBC3	CCDS12697.1	1	chr19:47731476-47731557
BCL2	CCDS11981.1	1	chr18:60985649-60985680, chr18:60985766-60985796
BCL2L11	CCDS2092.1	3	chr2:111886202-111886257, chr2:111886264-111886330
BCL6	CCDS3289.1	6	chr3:187443285-187443290
BCORL1	CCDS14616.1	3; 6	chrX:129147643-129147991; chrX:129159222-129159250
BRAF	CCDS5863.1	1	chr7:140624399-140624427
BRD4	CCDS12328.1	8; 12; 13; 18	chr19:15366894-15367032; chr19:15355275-15355365; chr19:15353892-15354030; chr19:15349669-15349728
BTG1	CCDS9043.1	1	chr12:92539312-92539313
C12orf5	NM_020375	6	chr12:4462895-4462986
CALR	CCDS12288.1	9	chr19:13054605-13054699
CASP8	NM_001228	10	chr2:202151326-202151327
CBL	CCDS8418.1	1	chr11:119077233-119077255
CCND1	CCDS8191.1	5	chr11:69465976-69466008
CCND3	CCDS4863.1	1	chr6:41909327-41909356
CDK12	CCDS11337.1	5	chr17:37650872-37650941
CDKN1C	CCDS7738.1	1	chr11:2906071-2906255
CEBPA	CCDS54243.1	1	chr19:33792575-33792645, chr19:33792755-33792776, chr19:33792966-33792998, chr19:33793008-33793046, chr19:33793200-33793222
CECR5	CCDS33595.1	1	chr22:17640043-17640090
CHD4	CCDS8552.1	3	chr12:6711145-6711167
CIC	CCDS12601.1	20	chr19:42799128-42799253

Gene	Transcript	Exons	Masked region coordinates
CPM	NM_001874	9	chr12:69246532-69246641
CREBBP	CCDS10509.1	29; 31	chr16:3781847-3781928; chr16:3778401-3778464
CSF1R	CCDS4302.1	10; 21	chr5:149447797-149447822; chr5:149433732-149433770
CUL4A	CCDS41908.1	1	chr13:113864010-113864059
DAXX	CCDS4776.1	4	chr6:33287797-33287870, chr6:33287881-33287921
DCTN5	NM_032486	6	chr16:23681264-23681290
DHFR	NM_000791	1	chr5:79950700-79950733
DICER1	CCDS9931.1	22	chr14:95562982-95563006
DNMT1	CCDS45958.1	5	chr19:10290861-10290912
DNMT3B	CCDS13205.1	9	chr20:31381340-31381403
E2F3	CCDS4545.1	1	chr6:20402595-20402623, chr6:20402825-20402851
EGFR	CCDS5514.1	28	chr7:55272947-55272950
EPCAM	CCDS1833.1	7	chr2:47607046-47607105
EPHA5	CCDS3513.1	1	chr4:66535417-66535459
EPM2AIP1	NM_014805	1	chr3:37027991-37028191
ERCC1	CCDS12663.1	8	chr19:45916804-45916834
ERCC2	CCDS46112.1	12	chr19:45862116-45862172
ERCC6	CCDS7229.1	4	chr10:50732295-50732345
ERCC6-PGBD3	NM_001277058	5	chr10:50732295-50732345
ETV4	NM_001986	1	chr17:41623303-41623307
EWSR1	NM_005243	1	chr22:29664316-29664323
EXT1	CCDS6324.1	1	chr8:119122697-119122718
EZH2	CCDS5891.1	5	chr7:148525889-148525916
FANCB	CCDS14161.1	8	chrX:14861687-14861699
FANCI	CCDS10349.2	20; 28	chr15:89835986-89836016; chr15:89848570-89848576
FANCM	CCDS32070.1	15	chr14:45650631-45650632
FAT1	CCDS47177.1	2	chr4:187584500-187584573
FBXW7	CCDS3777.1	1	chr4:153332605-153332629
FGF12	NM_004113	6	chr3:191861788-191861791
FGF3	CCDS8195.1	1	chr11:69633564-69633585
FGFR1	CCDS6107.2	3	chr8:38285914-38285934
FGFR3	CCDS3353.1	1; 12	chr4:1795660-1795662; chr4:1807532-1807555
FH	CCDS1617.1	7	chr1:241667340-241667373
FLCN	CCDS32580.1	5	chr17:17124691-17124698
FOXA1	CCDS9665.1	2	chr14:38060999-38061027, chr14:38061130-38061175, chr14:38061517-38061546
FOXL2	CCDS3105.1	1	chr3:138664555-138664728, chr3:138664862-138664906, chr3:138665015-138665065
FUBP1	CCDS683.1	1	chr1:78444615-78444645
GATA4	CCDS5983.1	1	chr8:11566172-11566204

Gene	Transcript	Exons	Masked region coordinates
GATA6	CCDS11872.1	1	chr18:19751622-19751654, chr18:19751750-19751778, chr18:19751820-19751861, chr18:19752073-19752104
GID4	CCDS11190.1	1	chr17:17942777-17942786
GNA11	CCDS12103.1	1	chr19:3094648-3094650
GNA13	CCDS11661.1	1	chr17:63052427-63052430
GNAS	CCDS13472.1	1	chr20:57466780-57466782
GPR124	CCDS6097.2	1; 19	chr8:37654829-37654868; chr8:37699121-37699165, chr8:37699300-37699323, chr8:37699468-37699491
HDAC2	CCDS43493.2	12	chr6:114264518-114264579
HSP90AA1	CCDS9967.1	4; 8	chr14:102551150-102551210, chr14:102551247-102551294; chr14:102549393-102549481
HSP90AB1	CCDS4909.1	5	chr6:44218030-44218204
IGF2R	CCDS5273.1	1	chr6:160390277-160390337
IKZF3	NM_012481	8	chr17:37916698-37916877
IL10	CCDS1467.1	2	chr1:206944739-206944762
IL7R	NM_002185	1	chr5:35857070-35857077
INHBA	CCDS5464.1	2	chr7:41729668-41729754
INSR	CCDS12176.1	1; 13	chr19:7293863-7293896; chr19:7141825-7141829
IRS1	CCDS2463.1	1	chr2:227660808-227660829, chr2:227661396-227661419
IRS2	CCDS9510.1	1	chr13:110434567-110434613, chr13:110435242-110435359, chr13:110436297-110436322, chr13:110437266-110437303, chr13:110437860-110437967, chr13:110438196-110438245, chr13:110438313-110438342, chr13:110438362-110438402
KAT6A	CCDS6124.1	16	chr8:41790634-41790788, chr8:41791830-41791934, chr8:41792010-41792078
KCNMB3	NM_171830	1	chr3:178969433-178969647
KDM6A	CCDS14265.1	1	chrX:44732821-44732848
KLHL6	CCDS3245.2	3	chr3:183226057-183226155
KMT2A	CCDS31686.1	1; 3	chr11:118307274-118307393, chr11:118307400-118307430; chr11:118344478-118344563
KMT2C	CCDS5931.1	36; 43	chr7:151879585-151879610; chr7:151859821-151859869
KMT2D	CCDS44873.1	34; 39	chr12:49431291-49431320, chr12:49432661-49432709; chr12:49426230-49426255, chr12:49426566-49426788, chr12:49426888-49426923, chr12:49427251-49427287, chr12:49427650-49427696
LATS2	CCDS9294.1	3	chr13:21562480-21562521
LRP1B	CCDS2182.1	90	chr2:140992353-140992455
LTK	CCDS10077.1	7	chr15:41803369-41803433
LZTR1	CCDS33606.1	7	chr22:21343964-21343973
MAF	CCDS10928.1	1	chr16:79633069-79633128, chr16:79633134-79633214, chr16:79633217-79633263, chr16:79633356-79633387
MAGI2	CCDS5594.1	22	chr7:77648702-77649011
MAML1	CCDS34315.1	1	chr5:179160112-179160114, chr5:179160341-179160388

Gene	Transcript	Exons	Masked region coordinates
MAP2K4	CCDS11162.1	1	chr17:11924223-11924261
MAP3K1	CCDS43318.1	14	chr5:56177849-56177875
MAPK1	CCDS13795.1	1	chr22:22221709-22221732
MAX	CCDS9774.1	4	chr14:65550975-65551019
MED12	CCDS43970.1	42	chrX:70360589-70360699
MEF2B	CCDS12394.1	7	chr19:19256719-19256741
MEF2BNB	NM_005919	10	chr19:19256719-19256741
MEF2BNB-MEF2B	CCDS12394.1	7	chr19:19256719-19256741
MEN1	CCDS31600.1	3	chr11:64575022-64575037
MKNK1	CCDS538.1	7	chr1:47037087-47037213
MSH2	CCDS1834.1	3	chr2:47637248-47637290
MSH3	CCDS34195.1	1; 5; 7	chr5:79950700-79950737; chr5:79968061-79968062; chr5:79970800-79970804
MSH6	CCDS1836.1	10	chr2:48033916-48033917
MTOR	CCDS127.1	38	chr1:11190667-11190732
MYCN	CCDS1687.1	1; 2	chr2:16082632-16082719, chr2:16082862-16082914; chr2:16085613-16085653
MYOD1	CCDS7826.1	1; 2	chr11:17741867-17741901; chr11:17742459-17742488
NCOA3	CCDS13406.1	17; 18	chr20:46277842-46277843; chr20:46279815-46279902
NCOR1	CCDS11175.1	11; 34	chr17:16042320-16042321; chr17:15967423-15967467
NKX2-1	CCDS9659.1	2	chr14:36986714-36986744, chr14:36986759-36986780, chr14:36986815-36986931
NKX3-1	CCDS6042.1	1	chr8:23540150-23540171
NOTCH3	CCDS12326.1	1; 18; 24; 33	chr19:15311649-15311698; chr19:15291906-15291975; chr19:15288608-15288691; chr19:15272196-15272233
NOTCH4	CCDS34420.1	1; 24	chr6:32191659-32191691; chr6:32166912-32166924
NPM1	CCDS4376.1	6	chr5:170819938-170819980
NT5C2	CCDS7544.1	17	chr10:104849427-104849471
NUTM1	NM_175741	1	chr15:34635841-34635896
OS9	CCDS31843.1	11	chr12:58112064-58112081
PAK1	CCDS44687.1	5	chr11:77069990-77070015
PAK3	CCDS14554.1	5	chrX:110406187-110406227
PALB2	CCDS32406.1	10	chr16:23632681-23632687
PDK1	CCDS2250.1	1	chr2:173420914-173420948
PGBD3	NM_170753	1	chr10:50732295-50732329
PHOX2B	CCDS3463.1	3	chr4:41747990-41748055, chr4:41748071-41748133
PIK3CB	CCDS3104.1	2	chr3:138474594-138474595
PIK3CG	CCDS5739.1	1	chr7:106509944-106509996
PIK3R2	CCDS12371.1	1; 5; 13; 15	chr19:18266923-18266974; chr19:18272243-18272288; chr19:18279283-18279358; chr19:18280065-18280102

Gene	Transcript	Exons	Masked region coordinates
PIM1	NM_002648	1	chr6:37138209-37138267
PLCG2	CCDS42204.1	29	chr16:81973495-81973521
POLD1	CCDS12795.1	12	chr19:50910238-50910239
POLE	CCDS9278.1	1; 43	chr12:133263868-133263889; chr12:133210870-133210938
PPP2R1A	CCDS12849.1	7	chr19:52719061-52719092
PPP2R2A	CCDS34867.1	9	chr8:26223904-26223924
PTK2	NM_005607	2	chr8:141994175-141994220
PTPN11	CCDS9163.1	7	chr12:112910806-112910836
PTPRD	CCDS43786.1	7	chr9:8524923-8524958
PTPRS	CCDS12140.1	11	chr19:5229532-5229560
RAD21	CCDS6321.1	11	chr8:117862876-117862908
RAD51B	CCDS9789.1	10	chr14:69061200-69061322
RAD51D	CCDS45646.1	3	chr17:33443876-33443926
RAD52	CCDS8507.2	8	chr12:1025547-1025614
RARA	CCDS11366.1	8	chr17:38512422-38512447
RASA1	CCDS34200.1	1; 14	chr5:86564538-86564594; chr5:86669978-86669979
RB1	CCDS31973.1	1	chr13:48878076-48878129
RBM10	CCDS14274.1	3	chrX:47030561-47030605
RECQL4	NM_004260	1	chr8:145743169-145743178
RET	CCDS7200.1	1	chr10:43572743-43572772
RPS6KA4	CCDS8073.1	1	chr11:64126706-64126708
RPS6KB2	CCDS41677.1	1; 15	chr11:67196015-67196017; chr11:67202527-67202593
SDHD	NM_001276506	4	chr11:111963802-111963931
SGK1	NM_001143678	1	chr6:134496805-134496809
SLC7A8	NM_012244	1	chr14:23652347-23652368
SMAD3	NM_005902	1	chr15:67358483-67358484
SMARCA4	CCDS12253.1	3; 9; 26; 32	chr19:11097197-11097241; chr19:11107027-11107057; chr19:11144441-11144543; chr19:11170486-11170547
SMO	CCDS5811.1	1; 12	chr7:128829040-128829062; chr7:128851941-128851972
SOCs1	CCDS10546.1	1	chr16:11349182-11349242
SOX10	CCDS13964.1	1	chr22:38379782-38379793
SOX17	CCDS6159.1	2	chr8:55372215-55372241, chr8:55372259-55372288
SOX9	CCDS11689.1	3	chr17:70120020-70120137
SPEN	CCDS164.1	10; 11	chr1:16248775-16248823; chr1:16262459-16262524
SPTA1	CCDS41423.1	2	chr1:158654963-158655029
SUFU	CCDS7537.1	1; 2	chr10:104263929-104263985; chr10:104268969-104269058
SUZ12	CCDS11270.1	1	chr17:30264348-30264373
TAF1	CCDS14412.1	38	chrX:70683756-70683789
TERT	CCDS3861.2	1; 2	chr5:1294898-1294919; chr5:1294664-1294687
TET2	CCDS47120.1	9	chr4:106196233-106196313

Gene	Transcript	Exons	Masked region coordinates
TGFBR1	CCDS6738.1	1	chr9:101867538-101867566
TGFBR2	CCDS33727.1	2	chr3:30664689-30664734
TIPARP	CCDS3177.1	5	chr3:156422774-156422854
TMPRSS2	NM_005656	1	chr21:42880030-42880086
TOP1	CCDS13312.1	3; 4	chr20:39690040-39690066; chr20:39704828-39704926
TP53BP1	CCDS10096.1	4	chr15:43773131-43773185
TSC1	CCDS6956.1	21	chr9:135771988-135772008
TSPAN31	NM_005981	6	chr12:58141528-58141727
U2AF1	CCDS13694.1	8	chr21:44513267-44513310
UBFD1	NM_019116	7	chr16:23582262-23582278
VEGFA	CCDS34457.1	1	chr6:43738518-43738568, chr6:43738719-43738756
WAS	CCDS14303.1	10	chrX:48547297-48547329
WEE1	CCDS7800.1	1	chr11:9595504-9595535, chr11:9595580-9595611, chr11:9595666-9595743, chr11:9595941-9595986
WHSC1L1	CCDS43729.1	23	chr8:38133374-38133402
WRN	CCDS6082.1	11; 28	chr8:30945377-30945432; chr8:31004567-31004568
WT1	CCDS7878.2	1	chr11:32456485-32456525
XRCC1	CCDS12624.1	9	chr19:44056379-44056429
ZNF2	NM_021088	5	chr2:95848977-95849096, chr2:95849362-95849668
ZNF703	CCDS6094.1	1; 2	chr8:37553541-37553569; chr8:37554932-37554977, chr8:37555934-37555962, chr8:37555979-37556010