PGDx elio™ tissue complete -RUO

White Paper

Amplifications and Translocations Performance

Revision: July 2020

RUO

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Introduction

PGDx elio™ tissue complete is an assay that uses next generation sequencing (NGS) to detect tumor gene alterations in genomic DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue from a variety of tumor types, using a targeted panel (>500 genes). The assay provides information on single nucleotide variants (SNVs), insertion and deletions (indels), amplifications, and translocations. It also identifies microsatellite instability (MSI) based on select mononucleotide tracts and signatures of sequence mutations and utilizes a ~1.3 Mb region of interest (ROI) to calculate tumor mutation burden (TMB).

Genomic amplifications and translocations stand as some of the more difficult somatic variants to detect but are nonetheless highly clinically significant. Therefore, the PGDx elio tissue complete – RUO assay has undergone a series of specificity, sensitivity, accuracy, and reproducibility studies to demonstrate performance across amplification and translocation genes on the panel. Results within this paper are reflective of performance in the PGDx elio tissue complete – RUO assay only. Performance data for variants reported in the FDA cleared PGDx elio tissue complete assay are not included in this paper. **Table 1** shows the studies performed for the different structural variants in the PGDx elio tissue complete – RUO assay. Specificity was assessed for all genes on the panel.

Table 1. Amplification and Translocation Performance Information Available per Gene

Variant Type	Gene	Sensitivity	Accuracy	Reproducibility
Amplification	AXL		X	
	BRCA2		X	
	CCND1	X	X	X
	CCND2		X	
	CCND3		X	
	CCNE1		X	
	CD274	X	X	
	CDK4		X	
	EGFR		X	
	FGFR1	X	X	
	FGFR2		X	
	FGFR4	X		X
	MDM2		X	
	MET	X	X	X
	MYC	X	X	X
	MYCN	X	X	X
	PDGFRA		X	
	PIK3CA	X	X	
	PIK3CB	X		

Variant Type	Gene	Sensitivity	Accuracy	Reproducibility
Translocation	BRAF		X	
	BRCA1		X	
	EGFR		X	
	EWSR1		X	
	FGFR1	X	X	
	FGFR2	X	X	
	FGFR3	X	X	
	NTRK1	X	X	
	PDGFRB		X	
	ROS1	X	X	X
	TMPRSS2		X	

Approach

The performance of PGDx elio tissue complete – RUO was assessed with studies for specificity, sensitivity, accuracy, and reproducibility using clinical FFPE samples or cell lines. The assay provides call status for 28 amplifications and 23 translocations located within the panel. For amplifications, PGDx elio tissue complete – RUO also provides a raw fold change based on sequence coverage. This fold change is derived from the ratio of normalized regions of interest to a collection of normal controls run through the assay. Amplification specificity, sensitivity, and reproducibility were assessed using raw fold change values; however, for accuracy, a tumor purity estimate was used to predict a fold change.

Results

Specificity

Translocation specificity (or false positive rate) was measured across 19 genes using 34 unique post-mortem FFPE samples across 2 unique reagent lots at 100 ng and with 1 lot of reagents at 50 ng, providing 64 replicates that passed QC metrics at 100 ng of DNA input and 26 replicates at 50 ng of DNA input (**Table 2**). Specificity was then measured by the number of replicates that were positive for the specified translocation using **Formula 1**. All translocation genes maintained 100% specificity across 50 ng and 100 ng of DNA input except for BRCA2 and FGFR2, which exhibited 98.4% specificity at 100 ng. Both false positives were identified in the same sample that upon investigation was found to have infiltrating lymphocytes that could not be confirmed to be non-cancerous. Overall, these results demonstrate the high specificity of translocations reported by PGDx elio tissue complete – RUO.

Table 2. PGDx elio tissue complete – RUO Translocations Specificity

Translocation Gene	Specificity (n/N) (2-sided 95% CI) at 100ng	Specificity (n/N) (2-sided 95% CI) at 50ng
AXL	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
BRAF	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
BRCA1	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
BRCA2	98.4% (62/63) (91.5%, 99.7%)	100% (26/26) (87.1%, 100%)
EGFR	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)

Translocation Gene	Specificity (n/N) (2-sided 95% CI) at 100ng	Specificity (n/N) (2-sided 95% CI) at 50ng
ETV4	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
ETV6	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
EWSR1	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
FGFR1	98.4% (62/63) (91.5%, 99.7%)	100% (26/26) (87.1%, 100%)
FGFR2	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
FGFR3	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
MYC	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
NTRK1	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
PAX8	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
PDGFRA	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
PDGFRB	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
RAF1	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
ROS1	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)
TMPRSS2	100% (63/63) (94.3%, 100%)	100% (26/26) (87.1%, 100%)

Specificity for copy number alterations was evaluated across 27 amplification genes using over 240 observations of post-mortem FFPE samples processed through PGDx elio tissue complete - RUO. This dataset included 88 unique samples processed in replicates at either 50 ng or 100 ng across 1 or 2 lots of reagents. As these were healthy normal samples and thought to be negative for all amplifications, the coverage across each gene was used to determine a fold change value that was then used to determine distribution of fold changes in normal samples without amplifications. Fold changes were converted to 1 if less than 25% of the amplification region had higher coverage than what was expected to prevent any potential false positive calls. The 93rd, 96th, 98th, and 99th percentiles were calculated for the fold change of each gene **(Table 3)**. Fold change distributions of FGFR3 and MET are shown in **Figure 1** as examples of distributions where the 93rd, 96th, 98th, and 99th percentiles have fold change of 1 (MET) and a distribution where fold change values are >1 for all percentiles >93% (FGFR3). The values in **Table 3** can be used to set thresholds for maintaining appropriate amplification specificity.

Table 3. Fold Change Percentiles for 27 Amplification Genes

Amplification Gene	99th Percentile	98th Percentile	96th Percentile	93rd Percentile
AXL	1.00	1.00	1.00	1.00
BRC1	1.00	1.00	1.00	1.00
BRCA2	1.52	1.00	1.00	1.00
CCND1	2.27	2.13	1.00	1.00
CCND2	1.99	1.00	1.00	1.00
CCND3	1.00	1.00	1.00	1.00
CCNE1	1.00	1.00	1.00	1.00
CD274	1.38	1.00	1.00	1.00

Amplification Gene	99th Percentile	98th Percentile	96th Percentile	93rd Percentile
CDK4	1.00	1.00	1.00	1.00
EGFR	1.00	1.00	1.00	1.00
FGFR1	1.00	1.00	1.00	1.00
FGFR2	1.00	1.00	1.00	1.00
FGFR3	2.66	2.54	2.36	1.00
FGFR4	2.20	1.97	1.00	1.00
KDR	1.36	1.00	1.00	1.00
KIT	1.00	1.00	1.00	1.00
MDM2	1.21	1.00	1.00	1.00
MET	1.00	1.00	1.00	1.00
MLH1	1.63	1.00	1.00	1.00
MSH2	1.00	1.00	1.00	1.00
MYC	1.00	1.00	1.00	1.00
MYCN	1.00	1.00	1.00	1.00
PALB2	1.00	1.00	1.00	1.00
PDGFRA	1.00	1.00	1.00	1.00
PIK3CA	2.19	2.05	1.00	1.00
PIKCB	1.51	1.00	1.00	1.00
PIK3R1	2.58	2.47	2.39	1.00

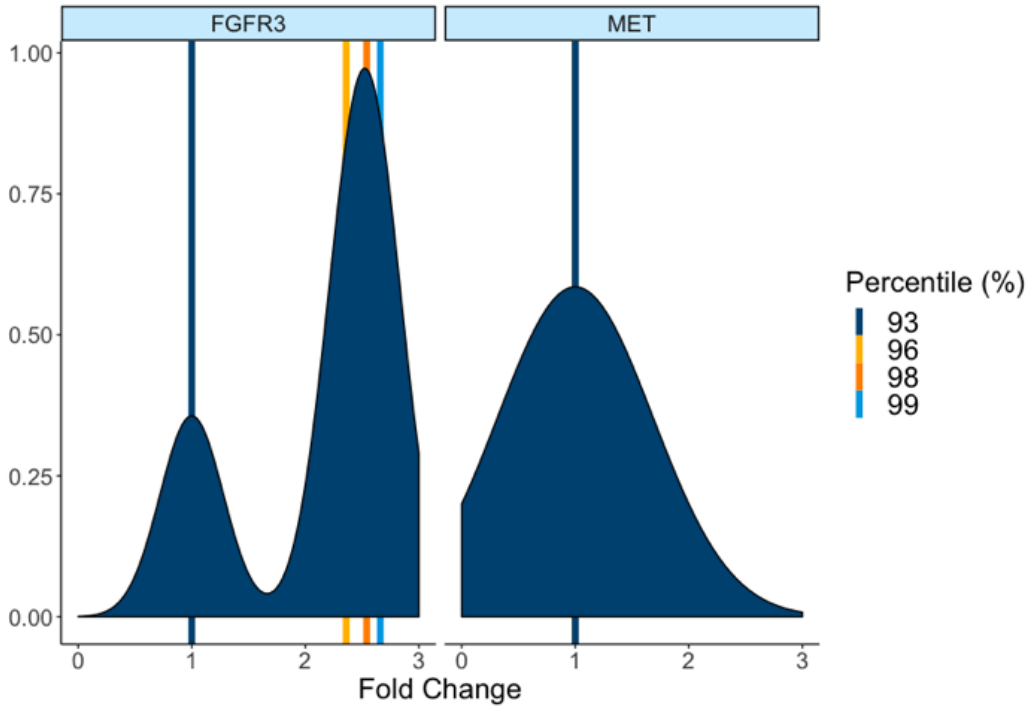

Figure 1. Fold change distribution of FGFR3 and MET in normal tissue. Across 250 observations, the fold change was measured for FGFR3 and MET. The figure depicts the range of fold changes detected with regards to the 93rd, 96th, 98th, and 99th percentiles for FGFR3, while MET exhibits a fold change of 1 at all labeled percentiles.

Sensitivity

Sensitivity across 9 amplification genes (CCND1, CD274, FGFR1, FGFR4, MET, MYC, MYCN, PIK3CA, and PIK3CB) and 1 translocation gene (ROS1) was assessed through the PGDx elio tissue complete - RUO assay in **tumor purity dilution studies.**

Cell Line Dilutions

To investigate the detection of ROS1 at low tumor purities, dilutions of a ROS1 translocation cell line into wild-type DNA were created with a targeted tumor purity of 3%-30%, across 5 tumor purity levels with 10 replicates at each level. The lowest level at which ≥ 95% of replicates were positive for ROS1 was determined to be the sensitivity limit. Tumor purity of each dilution level was measured using a MAF (mutant allele fraction)-based tumor purity calculation that is normalized to the purity of the stock sample prior to any dilutions. In this cell line, PGDx elio tissue complete – RUO reported ROS1 translocations with a 100% positive call rate at the lowest tumor purity dilution tested, 3.1% (**Table 4**). This indicates that ROS1 could likely be detected at tumor purities lower than 3.1%. Detection of translocations is dependent on the translocation partner and the number of supporting sequencing reads for the translocation, so samples with different translocation partners may be detected at different tumor purity levels. Positive call rates were calculated for each dilution using **Formula 2** in the **References** section.

Table 4. ROS1 Tumor Purity Dilution

Tumor Purity (TP) at which 95% of Replicates were Detected	TP Range for 10 replicates	Positive Call Rate (n/N) (2-sided 95% CI)
3.1%	2.5 to 3.7%	100% (10/10) (72.2%, 100%)

To investigate the detection of amplifications at low tumor purities, amplification positive cell lines were diluted to 5 tumor purity levels with 10 replicates at each level. FGFR1, PIK3CA, and PIK3CB were tested in a clinical dilution series using matched normal samples with 4 purity levels with 5 replicates at the 3 highest purity levels and 3 replicates at the lowest level. Tumor purity of each dilution level was measured using a MAF-based tumor purity calculation that is normalized to the purity of the stock sample prior to any dilutions. For the clinical sample dilutions, pathologists determined the tumor purity in the original sample and that was used to estimate the purity for each dilution.

The tumor purity dilution series, in which the diluent was DNA that was wild-type for the variant, was used to investigate the impact to amplification fold change as tumor purity decreases. Within a dilution level, reported fold change was very precise across all genes and levels, with a standard deviation under 0.4. Throughout the dilution series, signal of an amplification is present when using fold change and percentage of regions amplified in conjunction. The percentage of regions along the gene locus with a significant amplification signal are shown on the right y-axis of **Figure 2**. Those regions mostly correspond to coding exons of the gene, but also includes small segments of introns and nearby intergenic regions. The progressive decrease of signal simulated by the tumor purity dilution series correlates very well with the fold change (left y-axis in **Figure 2**) for starting values above 3-fold (FGFR1 and MET), under these conditions the percentage of amplified regions (right y-axis in **Figure 2**) is mostly unaltered and at 100%. In the case where amplified genes report a fold change below 3 in the undiluted sample, the percentage of amplified regions (**Figure 2**) is a better indicator of the decrease in the amplification signal. As the reported fold change is an average of the fold across each amplification region, in the latter condition there are still regions of the gene that are still largely amplified which keep the overall fold change relatively stable around 2-fold, despite the reduced number of amplified regions. Without the use of number of amplified regions, the possibility of mischaracterization of a gene as amplified increases due to a small fraction of a gene with high coverage. Therefore, the data presented in **Figure 2** show why both fold change and percentage of amplified regions should be taken into account when setting thresholds for amplification detection.

In addition, MET amplifications were also evaluated in a clinical sample that was diluted down to 21.5% tumor purity using a matched normal. This sample had 20 replicates at this purity level with a fold change of 2.8 ± 0.05 and on average 75% of regions were amplified, reinforcing the precision of fold change values in cell lines and clinical samples for MET amplifications.

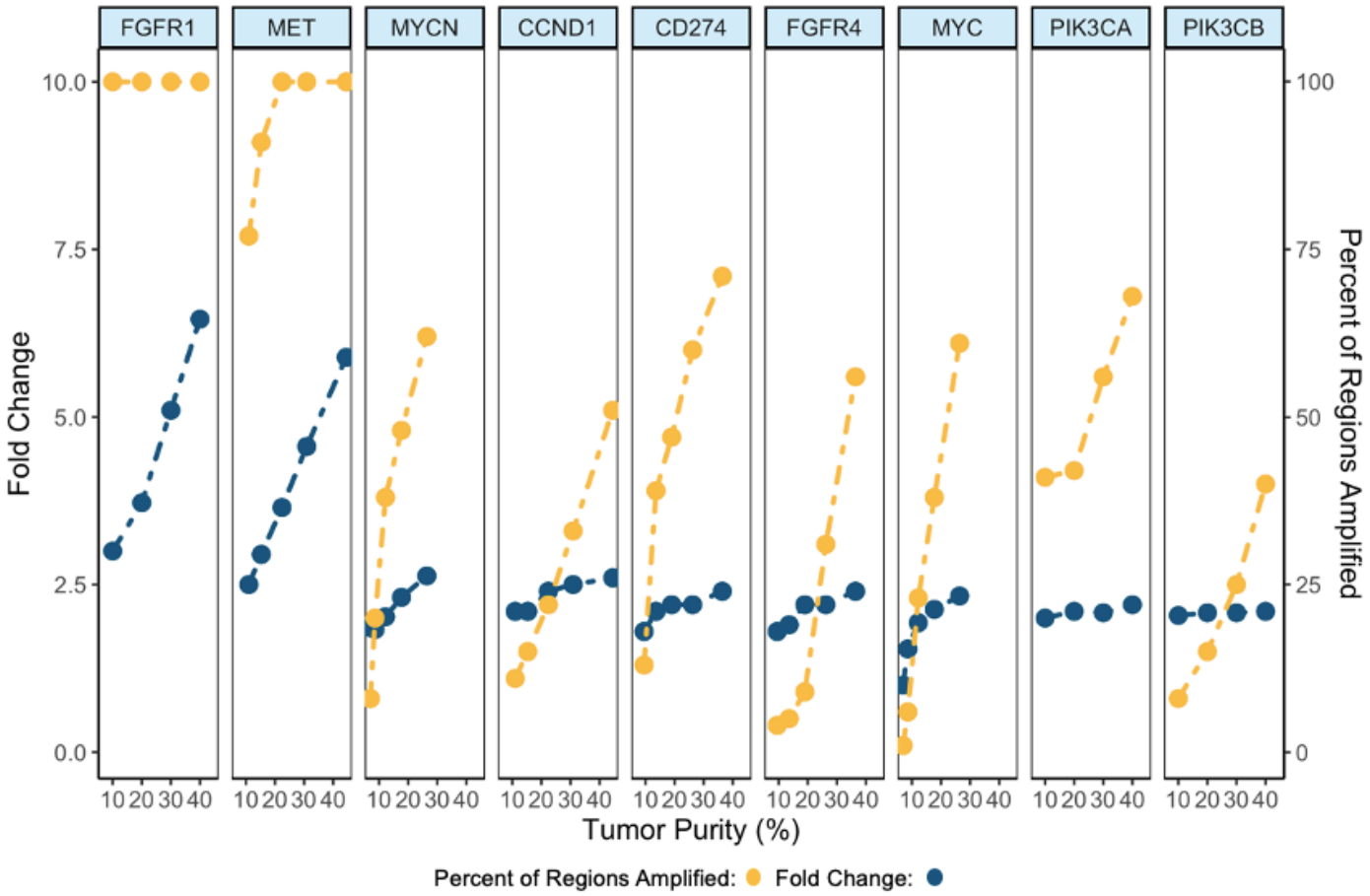

Figure 2. Tumor Purity Dilution Series for Amplifications. The average fold change (blue, left y-axis) and percent of amplified regions (yellow, right y-axis) at a given tumor purity level, across all replicates assessed is shown for all amplifications in the tumor purity dilution series. Amplification genes are shown in order of highest to lowest fold change at the highest tumor purity level, from left to right.

In silico Tumor Purity Dilution Series

To assess the performance across FGFR1, FGFR2, FGFR3 and NTRK1 translocation genes in the absence of rare clinical samples, simulations were created at a stock tumor purity of 20% and down-sampled to 15, 10, 3, and 1% in a tumor purity dilution series. For ROS1, simulations were created from a stock tumor purity of 8% and were down-sampled to 6, 5, 3, and 1% respectively, and these simulations closely resemble the levels observed in samples. Simulations were created by combining simulated mutant reads and wild-type reads from the reference genome proportionally to the target tumor purity. Twenty (20) replicates were tested in the PGDx elio tissue complete - RUO software at each level and the positive call rate was measured using **Formula 2** in the **References** section. **Figure 3** displays the performance of each translocation gene at each targeted tumor purity percentage. **Table 5** shows the positive call rates for the lowest tumor purity level with a positive call rate ≥ 95%. FGFR3 translocations were detected in 100% of samples at 1% tumor purity. FGFR2, NTRK1, and ROS1 were all detected in 100% of samples at 3% tumor purity, while FGFR1 was only detected in 95% of samples at 10% tumor purity (**Table 5, Figure 3**). This data illustrates the robust detection of translocations at tumor purity levels well below the minimum 20% requirement of the assay.

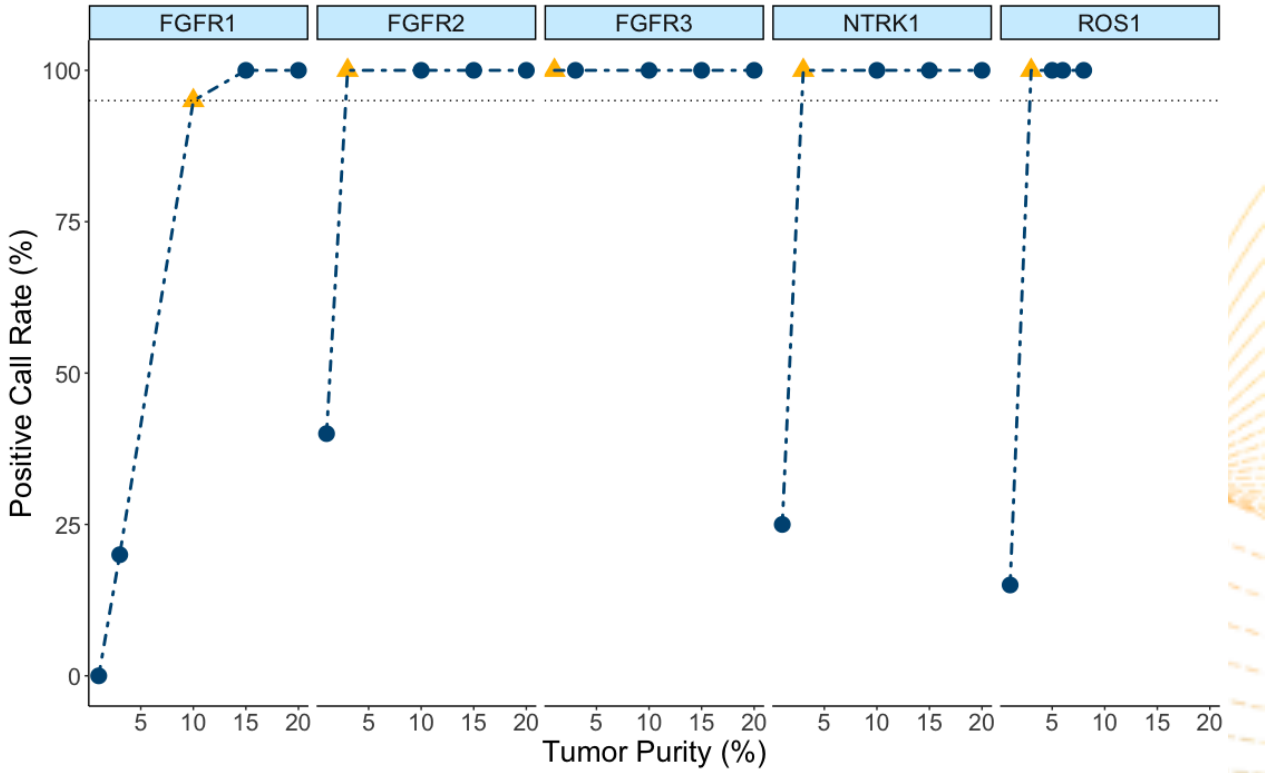

Figure 3. ***In silico*** **Tumor Purity Dilution for Translocations. At a given tumor purity level, 20 replicates were assessed for each targeted gene. A gold triangle indicates the lowest tumor purity level for which the assay exhibited a positive call rate >95%. The dashed horizontal line marks the 95% positive call rate.**

Table 5. ***In silico*** **Tumor Purity Dilution for Translocations**

Variant	Lowest TP with ≥95% Positive Call Rate	Positive Call Rate (n/N) (2-sided 95% CI)
FGFR1 Translocations	10%	95.0% (19/20) (76.4%, 99.1%)
FGFR2 Translocations	3%	100.0% (20/20) (83.9%, 100.0%)
FGFR3 Translocations	1%	100.0% (20/20) (83.9%, 100.0%)
NTRK1 Translocations	3%	100.0% (20/20) (83.9%, 100.0%)
ROS1 Translocations	3%	100.0% (20/20) (83.9%, 100.0%)

Accuracy

PGDx elio tissue complete – RUO was tested for concordance comparing to several assays across 453 samples for all amplifications and translocations. ROS1 translocation calls by PGDx elio tissue complete – RUO were compared to calls by 3 different assays (FISH, an RNA-based NGS assay, and a DNA-based NGS assay) across 288 samples in 23 tumor types. **Table 6** highlights 17 samples which were called ROS1 translocation-positive by at least one of the assays assessed. PGDx elio tissue complete – RUO was concordant to the RNA-based assay in all samples evaluated (1 positive and 36 negative). Due to the challenging breakpoint locations in ROS1 and the differences in orthogonal technologies, it is to be expected that not all methods will agree. The RNA-based assay will capture the intronic ROS1 regions differently than a DNA-based assay like PGDx elio tissue complete – RUO. Additionally, FISH assays are not specific to the translocation breakpoint or partner, showing positivity for any breakpoint the probes

can detect, whereas a DNA-based assay assess a specific region. As a break-apart assay, FISH may also report positivity in more cases than DNA-based assays, where positivity is only reported when the coding strand is maintained. Finally, there is inherent subjectivity with FISH assays due to the interpretation of the number and color of signals present in a certain area of tissue. Due to these challenges, designing a study to test the accuracy of ROS1 translocations should be done by carefully choosing an orthogonal method and understanding the limitations and differences of each assay. Ideally, determining clinical significance of any assay would be assessed using clinical outcome data.

In addition to the samples in Table 6 that were positive by at least one method, samples were concordantly negative for ROS1 translocation by the PGDx elio tissue complete – RUO assay and FISH (n=89), RNA-based assay (n=36), and a DNA-based NGS assay (n=153) for a total of 271 samples with a Negative Percent Agreement (NPA) of 100%.

Table 6. Accuracy of ROS1 Translocation Detection Across NGS and FISH Orthogonal Methods

Sample	PGDx elio tissue complete – RUO	RNA-based Assay	FISH	DNA-based Assay
1	Negative	-	Positive	-
2	Negative	Negative	Positive	-
3	Negative	Negative	Positive	-
4	Negative	-	Positive	-
5	Negative	-	Positive	-
6	Negative	Negative	Negative	-
7	Negative	Negative	Negative	-
8	Negative	Negative	Negative	-
9	Positive	Positive	Negative	-
10	Negative	Negative	Negative	-
11	Negative	Negative	Negative	-
12	Negative	-	Positive	-
13	Negative	-	Positive	-
14	Negative	-	Positive	-
15	Negative	-	Positive	-
16	Negative	-	Positive	-
17	Negative	-	-	Positive

PGDx elio tissue complete – RUO was tested for concordance to FISH and NGS assays for accuracy of amplifications. For amplifications, a tumor purity adjusted fold change was calculated and used for comparisons. Adjusted fold change values were evaluated using a cutoff of 3-fold and >25% of gene regions amplified to classify a gene as amplified. The tumor purity adjusted fold change is not a device output of the PGDx elio tissue complete – RUO product. A calculation for the tumor purity adjusted fold change can be found in the **References** section below.

MET amplification concordance was assessed against FISH. As **Table 7** indicates, there were several cases that were positive by PGDx elio tissue complete - RUO and negative by FISH. This was determined

to be due to a difference in methodology for accounting for polysomy. FISH uses a ratio of a region of the MET gene compared to the centromere of Chromosome 7 to confer positivity, whereas the PGDx elio tissue complete – RUO pipeline only assesses regions of the MET gene. Therefore, discordance should be expected in cases that are amplified in the MET gene as well as the centromere.

Table 7. MET Amplification Accuracy Comparison to FISH

		FISH Status	
		Positive	Negative
PGDx elio tissue complete – RUO	Positive	4	8[1]
	Negative	0	54

[1]7/8 of these cases were polysomy for chromosome 7.

Finally, the PGDx elio tissue complete – RUO assay was compared across 100 unique FFPE samples to another DNA-based NGS assay to assess concordance for amplifications and translocations (Tables 8 and 9). The threshold used for amplifications in this comparison was a tumor purity-adjusted fold change of 3-fold amplified regions by the PGDx elio tissue complete – RUO assay; the fold change was unknown for the orthogonal assay. The overall Positive Percentage Agreement (PPA) for amplifications was 83.0% and for translocations was 75.0%. Limited observations were available for amplifications in KDR, KIT, and AXL and translocations in FGFR1, PDGFRB, and ROS1, where concordance was 0 for 1 for these variants. Several factors affect concordance when comparing accuracy between 2 assays. Intra-tumor heterogeneity plays a role in variant positivity. If different regions of a tumor are used for DNA extraction and variant assessment, this could lead to discrepancies. While 2 assays might assess the same gene for structural variants, they might assess different regions of those genes, so while 1 assay may find a translocation or amplification at a specific genomic locus, the other assay might not assess that locus. NGS assays also use different thresholds for positivity, so one assay might be more sensitive or specific than another. Finally, different assays use varying quality control metrics that could cause variants that otherwise would have been called positive to be filtered from reporting due to a low quality of signal.

Table 8. Amplification Concordance Between PGDx elio tissue complete – RUO and DNA-based NGS Assay

Gene	Concordance	Gene	Concordance
MYC	16/21	CCND2	3/4
CCNE1	13/14	BRCA2	2/3
FGFR1	11/11	CDK4	2/3
CCND1	8/11	CD274	2/2
EGFR	7/7	PIK3CA	2/2
MDM2	5/6	CCND3	1/2
MET	5/5	FGFR2	1/1
MYCN	4/4	PDGFRA	1/1

Table 9. Translocation Concordance Between PGDx elio tissue complete – RUO and DNA-based NGS Assay

Gene	Concordance	Gene	Concordance
TMPRSS2	10/11	BRCA1	1/2
NTRK1	3/4	EGFR	1/2
BRAF	2/2	FGFR1	1/1
EWSR1	2/2	FGFR3	1/1

Reproducibility

Reproducibility was assessed across 7 samples (6 cell line blends and 1 FFPE samples) over 3 sites, 2 replicates, 6 operators, and 6 sequencing instruments for a total of 252 case-level observations. The study took place over the course of at least 20 days. For structural variants, ROS1 translocations and CCND1, FGFR4, MET, MYC, and MYCN amplifications were assessed. Each sample was assigned a reference status for ROS1 translocations, which were used to calculate PPA and NPA values. The status of ROS1 was 100% concordant in all samples across all 3 sites, 6 operators, and 6 instruments (**Table 10**). No false positives or false negatives were observed across all 252 observations. Reproducibility of amplification fold change was evaluated and found to be highly reproducible (**Table 11** and **Figure 4**).

Table 10. ROS1 Reproducibility Performance

Metric	Performance (%), n/N, 95% CI (%)
PPA	100.0% (72/72) (94.9, 100.0)
NPA	100.0% (180/180) (97.9, 100.0)

Five amplifications (CCND1, FGFR4, MET, MYC, and MYCN) were evaluated for reproducibility in a total of 4 samples known to be positive when enrolled into the study. The fold change reproducibility was measured for all 5 amplifications. Across 36 replicates for each positive sample, the fold change standard deviation was <0.5 for each amplification, demonstrating the reproducibility of fold change values in the study as shown in **Table 11**. **Figure 4** shows the high reproducibility of fold change of CCND1, FGFR4, MET, MYC, and MYCN across all 36 replicates. CCND1 and MYCN appear in two different samples, each sample demonstrating low variation in fold change.

Table 11. Fold Change Reproducibility in Amplification Positive Samples

Amplification	Mean and Standard Deviation Fold Change	Number of Positive Samples
CCND1 (Sample 3)	2.9 ± 0.2	36
CCND1 (Sample 4)	2.6 ± 0.1	36
FGFR4	3.1 ± 0.5	36
MET	5.7 ± 0.4	36
MYC	3.4 ± 0.1	36
MYCN (Sample 1)	3.3 ± 0.1	36
MYCN (Sample 2)	14.8 ± 0.5	36

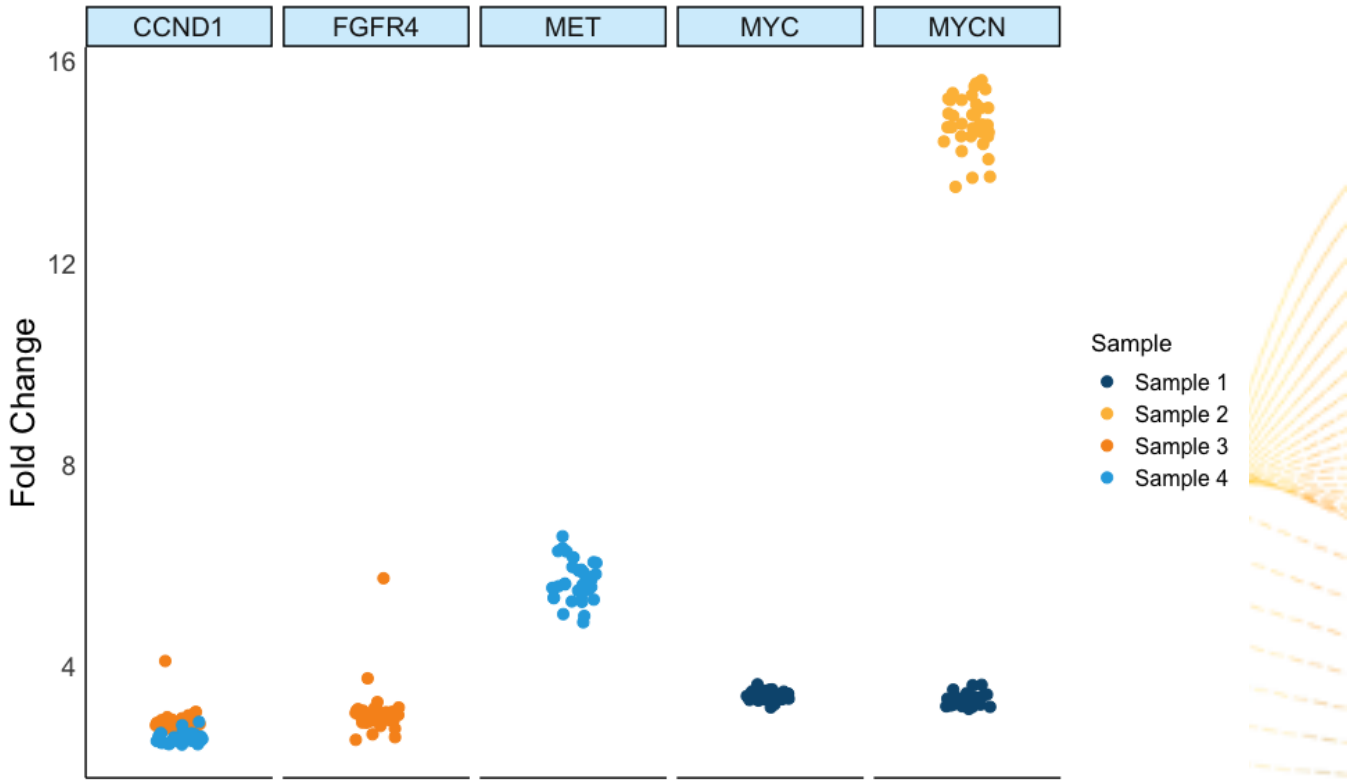

Figure 4. Overall Reproducibility of Amplification Fold Change by Sample.

Conclusion

Through the usage of laboratory experiments and *in silico* modeling, the PGDx elio tissue complete – RUO assay has exhibited excellent performance across amplifications and translocations when assessing the specificity, sensitivity, accuracy, and reproducibility of the assay. All translocations were ≥ 98.4% specific when tested at both 50 ng and 100 ng of DNA input. Percentiles of fold change values were determined for non-cancerous samples to show the specificity of fold change for each amplification gene. ROS1 was detected in 100% of replicates at 3.1% tumor purity in a cell line dilution series. An *in silico* study showed that FGFR1 translocations can be detected down to 10% tumor purity, FGFR2 and NTRK1 translocations were detected down to 3% tumor purity and FGFR3 translocations were detected down to 1% tumor purity. Amplification sensitivity was measured by performing tumor purity dilution series in both cell lines and clinical samples. All amplifications showed a reduction in signal at lower tumor purities, but the fold change in the undiluted sample dictated if fold change or percentage of amplified regions was most impacted by the decrease in tumor purity. ROS1 was most concordant with the RNA-based assay tested in the accuracy study and less concordant with FISH, as expected due to the differences and limitations between the assays. Discordance occurred when comparing PGDx elio tissue complete – RUO to FISH for MET amplifications, however this was largely attributed to the differences between tests when accounting for polysomy. Samples positive for translocations and amplifications were compared a DNA-based assay to determine accuracy, which ranged from 50% - 100% concordance between methods. ROS1 was 100% concordant across all conditions in the multi-site reproducibility study, which amplifications showed high precision of fold change values across sites, operators, and runs. Overall, the data suggest that the PGDx elio tissue complete – RUO assay has excellent performance in detecting amplifications and translocations.

References

Tumor Purity Adjusted Fold Change Calculation

The tumor purity adjusted fold change can be calculated using the raw fold change output and the tumor purity designated for a given FFPE tumor sample.

$$\text{Tumor Adjusted Fold Change} = \frac{(\text{Raw Fold Change} + \text{Tumor Purity} - 1)}{\text{Tumor Purity}}$$

Formulas

Specificity

Formula 1: $\dfrac{\textit{Number of Acceptable Observations}}{\textit{Total number of Observations}}$ *x 100%*

Positive Call Rate

Formula 2: $\dfrac{\textit{Variant(s) Detected}}{\textit{Variant(s) Detected and Variant(s) Not Detected}}$ *x*100% = Positive Call Rate

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA)

		Case Level Reference Status	
		Positive	Negative
Observed	Positive	X_{11}	X_{10}
	Negative	X_{01}	X_{00}

$$\text{NPA} = \frac{X_{00}}{X_{00} + X_{10}} \qquad \text{PPA} = \frac{X_{11}}{X_{11} + X_{01}}$$