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APPLICATION NOTE

Targeted oncology research workflow with Pillar Biosciences and Illumina

Rapid, accurate results with oncoReveal® panels on MiSeq™ i100 Series, analyzed and reported with DRAGEN™ Amplicon and Illumina Connected Insights

Achieve accurate variant calling analysis of oncology research samples in as little as 24 hours

Detect changes with variant allele frequencies below 5%

Enable streamlined, consistent bioinformatics and reporting across the Illumina oncology research portfolio

Overview

In cancer research, targeted next-generation sequencing (NGS) allows labs to enrich reads in regions of interest with the necessary depth to detect mutations occurring at low variant allele frequencies (VAF). Targeted NGS panels also allow labs to focus their analysis on genetic variants that are most likely present based on the origin of the tumor sample, improving efficiency and throughput.

Pillar Biosciences oncoReveal panels

Pillar Biosciences oncoReveal panels are designed to target known cancer genes and are compatible with various sample types, including DNA from tissue, blood, or formalin-fixed paraffin-embedded (FFPE) tissue and RNA from tissue and FFPE tissue. These panels use a proprietary stem-loop inhibition-mediated amplification (SLIMamp®) technology that enables single-tube, targeted, amplicon-based library preparation with high sensitivity and rapid turnaround.

MiSeq i100 Series

With the MiSeq i100 Series, Illumina advances simple, accurate, and fast benchtop sequencing. Breakthrough improvements in system design, XLEAP-SBS™ chemistry, and integrated data analysis deliver enhanced usability, high data accuracy, and exceptional speed. As part of an end-to-end NGS solution, the MiSeq i100 Series is able to provide same-day results for targeted oncology research (Figure 1).

DRAGEN Amplicon pipeline

DRAGEN Amplicon software is designed for high-performance analysis of amplicon sequencing data from both DNA and RNA samples. The software offers flexible and secure deployment options via BaseSpace™ Sequence Hub, on-premises DRAGEN server, or on instrument. Secondary sequence analysis for oncoReveal panels is integrated with DRAGEN Amplicon, enabling labs to easily produce results with consistent bioinformatics across the Illumina oncology portfolio.

Illumina Connected Insights

Illumina Connected Insights is a cloud-based platform designed to streamline tertiary analysis and reporting for oncology research applications. It supports interpretation of single nucleotide variants (SNV), insertions and deletions (indels), copy number variants (CNV), RNA fusions, and biomarkers, and enables customizable reporting in multiple languages. Illumina Connected Insights integrates seamlessly with DRAGEN Amplicon pipelines, allowing ingestion of VCF files,

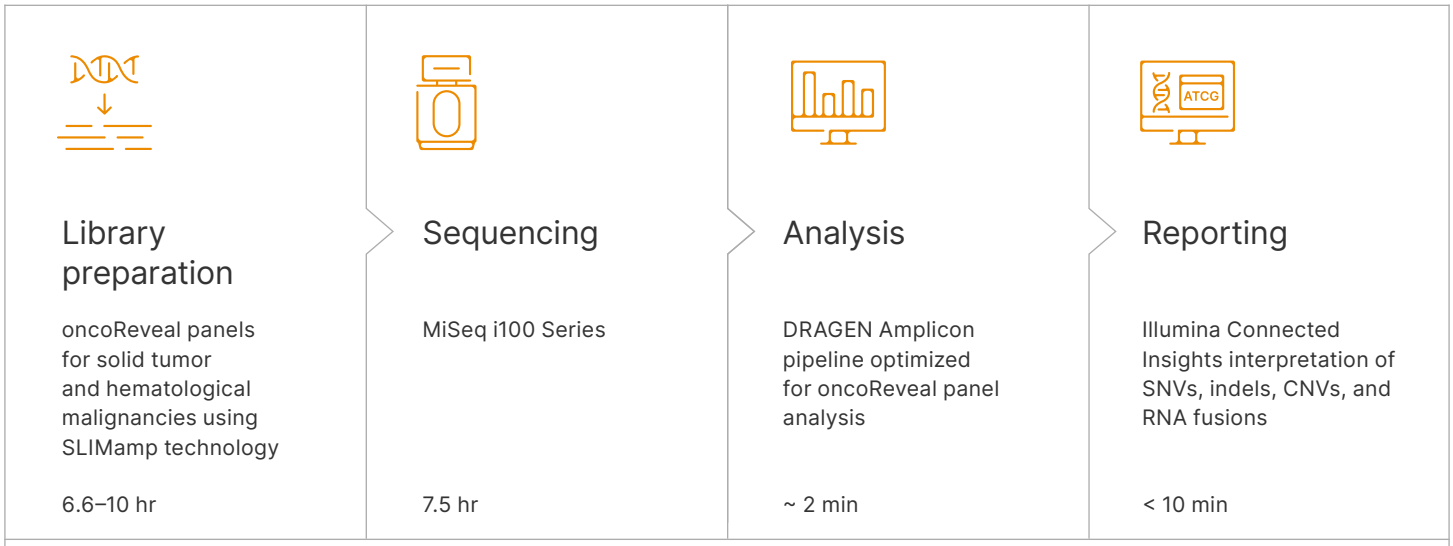

Figure 1: Pillar Biosciences OncoReveal panel workflow on Illumina MiSeq i100 Series sequencing systems
Go from samples to report in < 24 hr.

quality control (QC) metrics, and metadata. With enhancements like fusion quality scoring and integration of external knowledge bases, Illumina Connected Insights supports scalable genomics analysis workflows for a range of applications.

Integrated workflow for oncoReveal panels

Combining Pillar Biosciences oncoReveal panels, Illumina MiSeq i100 Series, DRAGEN Amplicon secondary analysis, and Illumina Connected Insights provides users with a fully integrated workflow that produces high-accuracy results in as little as 24 hours (Figure 1). The integrated workflow enables users to maintain control of samples while achieving reliable variant detection at low VAF, without the need for unique molecular identifiers (UMIs), even when working with limited DNA input or suboptimal sample quality (Table 1). In this application note, we demonstrate the performance of Pillar Biosciences oncoReveal panels when sequenced on a MiSeq i100 Series system and analyzed using DRAGEN Amplicon pipline for secondary analysis with Illumina Connected Insights for reporting.

Methods

Samples

Pillar oncoReveal panels support a wide range of applications and sample types. For this analysis, we used human research samples and commercially available and internally developed contrived samples designed to mimic clinically relevant oncology targets. These targets included SNVs, indels, representative RNA fusions, CNV events, and *FLT3*-ITDs. A total of 28 samples were used to evaluate performance across the six Pillar oncoReveal panels. Table 2 summarizes the samples used for each of the tested panels.

Library preparation

Libraries were prepared for Pillar Biosciences oncoReveal panels following the manufacturer's instructions, using 20 ng input for all assays. For RNA workflows, cDNA was generated, then for all workflows, a gene-specific PCR (GS-PCR) amplification reaction was performed with 18–26 cycles as specified for each panel. Following

Table 1: Pillar Biosciences oncoReveal panels supported by DRAGEN Amplicon pipeline

Panel	Catalog no.	Sample type	No. of genes/ amplicons	Variant types	Recommended paired-end reads per sample
oncoReveal Multi-Cancer with CNV & RNA Fusion Panel	HNA-HS-1001-48	DNA from tissue, blood, or FFPE samples	60/341	SNVs, small and medium indels, CNVs	2M
oncoReveal Multi-Cancer RNA Fusion v2 Panel	HRA-HS-1002-24	RNA from FFPE or tissue	18 genes and > 80 fusion partners, *MET* exon 14 skipping	Fusion RNA transcripts (including *MET exon* 14 skipping)	2M
oncoReveal Solid Tumor v2 Panel	HDA-CH-3003-24	DNA from tissue, blood, or FFPE samples	48/246	SNVs, small and medium indels	2M
oncoReveal BRCA1 & BRCA2 + CNV Panel	HDA-BR-1003-24	DNA from tissue, blood, or FFPE samples	2/283	Exon-level CNVs, SNVs, small and medium indels	2M
oncoReveal Myeloid Panel	HDA-MY-1001-24	DNA from whole blood, or PBMCs	58/766	SNVs, indels, ITDs	4M
oncoReveal Essential MPN Panel	HDA-MY-1002-48	DNA from whole blood, or PBMCs	3/7	SNVs, small and medium indels	30K

CNV, copy number variant; indel, insertion deletion; ITD, internal tandem duplication; PBMC, peripheral blood mononuclear cells; SNV, single nucleotide variant.

Table 2: Samples used to evaluate DRAGEN Amplicon performance with Pillar oncoReveal panels

Panel tested	Samples used
oncoReveal Multi-Cancer with CNV & RNA Fusion Panel	• ODC4 (Oncology DNA Control 4), Illumina contrived gDNA reference sample[1] • Two cell line samples containing CNVs: HCC-1954 (breast), NCI-H716 (colon) • One FFPE-derived lung cancer with known *FGFR3* amplification
oncoReveal Multi-Cancer RNA Fusion v2 Panel	• Horizon HD784 reference sample • Three normal FFPE samples from normal lung, liver, and pancreas tissue
oncoReveal Solid Tumor v2 Panel	• Mimix OncoSpan, gDNA Reference Standard (Horizon Discovery, Catalog no. HD827) • ODC4 (Oncology DNA Control 4), Illumina contrived gDNA reference sample[1] • One FFPE-derived sample from colon cancer tissue • One FFPE-derived normal sample from liver tissue
oncoReveal BRCA1 & BRCA2 + CNV Panel	• ODC4 (Oncology DNA Control 4), Illumina contrived gDNA reference sample[1] • Two FFPE-derived samples from ovarian cancer tissue • One normal sample NA12878 (HG001)
oncoReveal Myeloid Panel	• Horizon, HD829 Myeloid gDNA reference standard sample[2] • Seraseq Myeloid Mutation DNA Mix (SeraCare, Catalog no. 0710-0408) • Cell lines MV-4-11 (CRL-9591), MOLM-13, and PL-21 • FFPE with *FLT3*-ITD confirmed by PINDEL • gDNA derived from two healthy donor buffy coat samples
oncoReveal Essential MPN Panel	• Seraseq Myeloid Mutation DNA Mix (SeraCare, 0710-0408) • Myeloid gDNA reference standard (Horizon Discovery, Catalog no. HD829) • One FFPE sample derived from pancreatic tissue • gDNA derived from two healthy donor buffy coat samples

GS-PCR, the primers were digested using exonuclease followed by a bead-based clean-up step. Finally, libraries were indexed using 5–6 cycles of PCR amplification, followed by bead-based clean-up, quantification, and normalization before pooling for sequencing.

Sequencing

Libraries were sequenced using a MiSeq i100 Series system with 150-bp paired-end reads.

Secondary analysis

DRAGEN Amplicon v4.4.6 secondary analysis was used to call genotypes for each of the tested oncoReveal panels, enabling detection of SNVs and small to medium indels with VAF below 5%. In addition to SNVs and indels, the pipeline was also used to detect CNVs, ITDs, such as *FLT3*-ITD, as well as RNA fusions and splice variant events. The DRAGEN Amplicon pipeline also includes a 3′/5′ imbalance ratio metric for RNA assays. The 3′/5′ imbalance ratio is used to detect fusion oncogenes, particularly those involving driver genes such as *ALK* and *ROS1*. By evaluating the relative expression levels at the 3′ and 5′ ends of transcripts, this metric is able to improve identification of imbalances indicative of fusion events.

Panel of normals

A targeted analysis of CNVs requires a panel of normals (PON) for depth normalization. For each of the assessed oncoReveal panels in this study, a PON was constructed using 18–25 samples. These normal samples were prepared and sequenced using the same library prep and sequencing workflow as the case samples. This allowed the algorithm to subtract system-level biases that are not sample specific. DRAGEN includes a default CNV package with an accompanying PON that is used by the DRAGEN Amplicon pipeline for each panel supporting CNV detection. However, for optimal performance it is

recommended that users generate their own PON with approximately 20 normal samples to establish a baseline level for accurate CNV calling.

Tertiary analysis and reporting

Connected Insights performs tertiary analysis by transforming processed sequencing data into reportable research information. It was used to take variant calls from the DRAGEN Amplicon pipeline and, by using advanced algorithms, annotate, classify, and prioritize variants. Connected Insights cross references multiple curated databases, literature sources, and clinical guidelines to provide evidence-based interpretations. For this study, Connected Insights was used to review pathogenicity, practical implications, and biomarker associations for oncology findings.

Results

oncoReveal Multi-Cancer with CNV & RNA Fusion Panel

The oncoReveal Multi-Cancer with CNV & RNA Fusion Panel is a combined DNA and RNA panel that interrogates genes of interest across multiple solid tumor cancer types. The assay combines the DNA-based oncoReveal Multi-Cancer v4 with CNV Panel with the oncoReveal Multi-Cancer RNA Fusion v2 Panel allowing for combined sequencing. The oncoReveal Multi-Cancer v4 with CNV Panel and oncoReveal Multi-Cancer RNA Fusion v2 Panel were evaluated as components of this combined panel.

oncoReveal Multi-Cancer v4 with CNV Panel

The oncoReveal Multi-Cancer v4 with CNV Panel was evaluated using an internally-contrived sample at a mean target coverage exceeding 4000×. All 48 overlapping SNV and indel variants (with observed VAF from 8–43%) were detected using the DRAGEN Amplicon pipeline (Figure 2). Genomic base specificity was assessed to be > 99.9% using a normal FFPE sample. CNV detection, including *ERBB2*, *FGFR2*, and *FGFR3* amplifications (Table 3), was validated using FFPE and cell line samples. No false positives were detected in two additional normal FFPE samples.

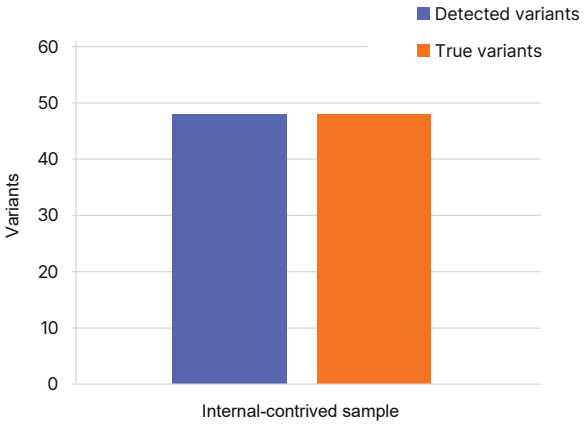

Figure 2: Variant calling performance using the DRAGEN Amplicon pipeline for oncoReveal Multi-Cancer v4 with CNV Panel analysis

All 48 variants expected in an internally-contrived sample were detected using the oncoReveal Multi-Cancer with CNV & RNA Fusion Panel and the DRAGEN Amplicon pipeline.

Table 3: Gene amplifications used in evaluating the oncoReveal Multi-Cancer v4 with CNV Panel

Gene	Variant type	Sample type	Detected?
ERBB2	Amplification	HCC1954 cell line	Yes, PASS in CNV VCF
FGFR2	Amplification	NCI-H716 cell line	Yes, PASS in CNV VCF
FGFR3	Amplification	FFPE	Yes, PASS in CNV VCF

oncoReveal Multi-Cancer RNA Fusion v2 Panel

The oncoReveal Multi-Cancer RNA Fusion v2 Panel is designed to interrogate 18 driver genes and 83 fusion partners that are relevant across multiple solid tumor types using RNA isolated from FFPE samples.

The oncoReveal Multi-Cancer RNA Fusion v2 Panel was evaluated using three normal FFPE samples (for specificity) and three sequencing replicates of the Horizon HD784 reference sample, which contains three known fusions (for sensitivity). Total reads passing filter

was > 900K. All three expected fusions were detected in the Horizon HD784 sample in all three sequencing replicates (Figure 3), and no false positives were observed in the three normal FFPE samples.

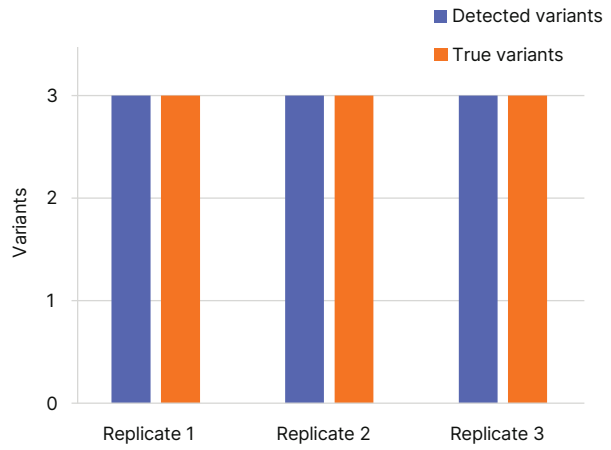

Figure 3: Variant calling performance using the DRAGEN Amplicon pipeline for oncoReveal Multi-Cancer RNA Fusion v2 Panel analysis

All three expected fusions were detected in the Horizon HD784 sample in three sequencing replicates.

oncoReveal Solid Tumor v2 Panel

The oncoReveal Solid Tumor v2 Panel is a 48-gene assay that tests for key mutations in DNA from solid tumors, including non-small cell lung, colorectal, melanoma, endometrial, pancreatic, gastrointestinal stromal tumor, bladder, thyroid, and gliomas. Additionally, genes with potential importance in immuno-oncology such as *POLD1* and *POLE* are included in the panel.

The oncoReveal Solid Tumor v2 Panel was evaluated using Mimix OncoSpan gDNA Reference Standard and internally-contrived samples with a mean target coverage of 7000×. All covered SNV and indel variants were successfully detected using the DRAGEN Amplicon pipeline (Figure 4). Specifically, 40 variants were detected in the internal contrived sample and 20 in the Mimix OncoSpan gDNA Reference Standard. Additionally, two FFPE samples were evaluated—one normal and one with a known *KRAS* variant. The *KRAS* variant was detected at 30% VAF, and no false positives were observed in the normal sample.

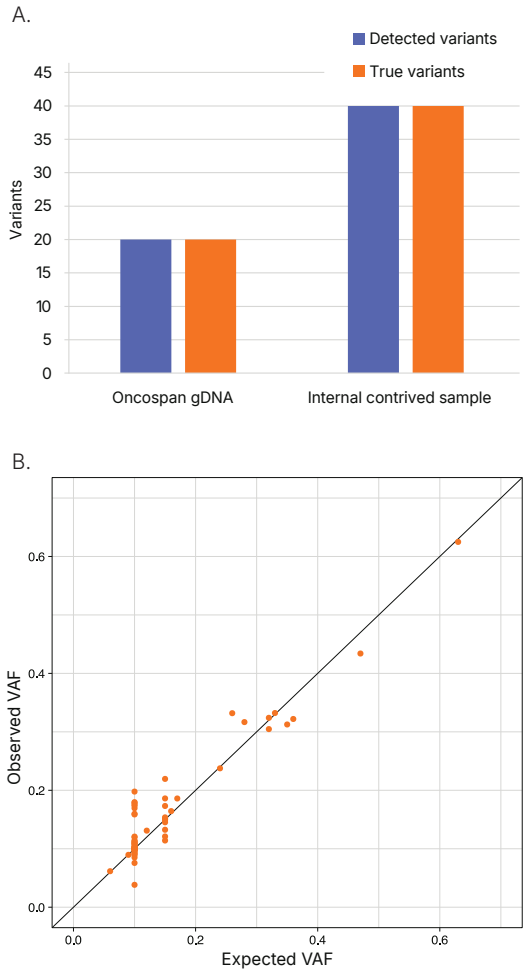

Figure 4: Variant calling performance using the DRAGEN Amplicon pipeline for oncoReveal Solid Tumor v2 Panel analysis and comparison of expected and observed VAF

All expected SNV and indel variants were detected in Mimix OncoSpan, gDNA Reference Standard and internally-contrived samples using the oncoReveal Solid Tumor v2 Panel and the DRAGEN Amplicon pipeline.

oncoReveal BRCA1 & BRCA2 + CNV Panel

The oncoReveal BRCA1 & BRCA2 + CNV Panel is a focused panel that targets exon-level CNVs, SNVs, and small and medium indels in *BRCA1* and *BRCA2*.

The oncoReveal BRCA1 & BRCA2 + CNV Panel was evaluated using an internally-contrived control (with six expected small variants) and an FFPE sample (with a known *BRCA1* variant), achieving a mean target coverage above 5000×. All six covered SNV and indel variants were detected using the DRAGEN Amplicon pipeline (Figure 5, Table 4). A known *BRCA1* deletion (*BRCA1*. c.3008_3009del with VAF at 0.48) was also successfully detected. Genomic position specificity was determined to be > 99.9%. A *BRCA1* Exon 7–9 loss CNV event was identified in the FFPE sample at a lower CNV threshold. If this threshold is increased, the number of false positives in the normal FFPE sample will increase.

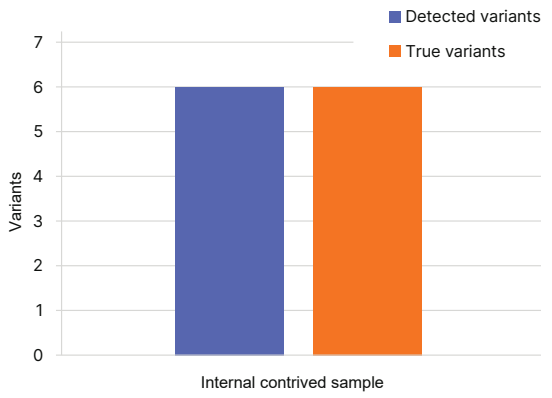

Figure 5: Variant calling performance using the DRAGEN Amplicon pipeline for oncoReveal BRCA1 & BRCA2 + CNV Panel analysis

Six expected SNV and indel variants were detected in an internally-contrived control and FFPE samples using the oncoReveal BRCA1 & BRCA2 + CNV Panel with the DRAGEN Amplicon pipeline. Variants detected and VAF reported in Table 4.

oncoReveal Myeloid Panel

The oncoReveal Myeloid Panel is designed to assess 58 genes relevant to myeloid cancers.

The oncoReveal Myeloid Panel was evaluated using a Horizon Myeloid HD829 DNA reference standard sample,[2] Seraseq Myeloid Mutation DNA Mix sample, and normal PBMC samples, achieving a mean target coverage above 5000×. All 20 covered SNV and indel variants with observed VAF from 3–69% were detected in both samples (Figure 6). Genomic base specificity was confirmed to be > 99.99% using healthy PBMC samples. *FLT3*-ITD detection was validated across FFPE samples, cell lines, and contrived samples, with no false positives observed in normal PBMC samples. Table 5 summarizes the *FLT3*-ITD variants detected across sample types, where 100% sensitivity was achieved. The table includes the specific variant notation, its length in base pairs, and the corresponding sample source.

Table 4: Variants detected using oncoReveal BRCA1 & BRCA2 + CNV Panel

Gene	HGVS nomenclature	Protein	Observed VAF	Mutation type
BRCA2	c.1114A>C	p.Asn372His	8%	SNV
BRCA2	c.7934del	p.Arg2645fs	12%	Deletion
BRCA2	c.9090dupA	p.T3030fs	22%	Insertion
BRCA1	c.5266dup	p.Gln1756fs	2%	Insertion
BRCA1	c.4327C>T	p.Arg1443Ter	11%	SNV
BRCA1	c.1961_1962del	p.Lys654fs	22%	Deletion

HGVS, Human Genome Variation Society

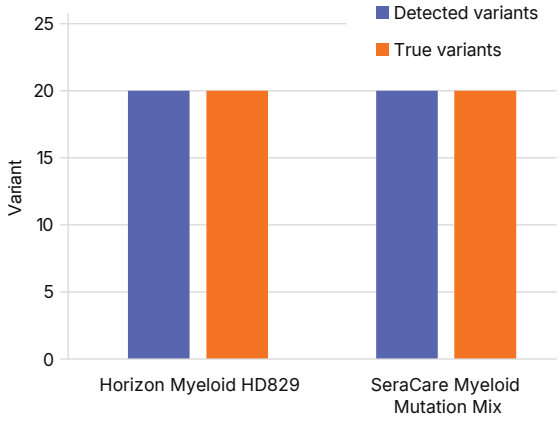

Figure 6: Variant calling performance using DRAGEN Amplicon pipeline for oncoReveal Myeloid Panel analysis

All 20 covered SNV and indel variants were detected in Horizon Myeloid HD829 DNA reference standard and Seraseq Myeloid Mutation DNA Mix samples using the oncoReveal Myeloid Panel and the DRAGEN Amplicon pipeline.

oncoReveal Essential MPN Panel

The oncoReveal Essential MPN Panel is designed to assess key mutations within the *MPL*, *JAK2*, and *CALR* genes associated with myeloproliferative neoplasms (MPN).

The oncoReveal Essential MPN Panel was evaluated using the Seraseq Myeloid Mutation DNA Mix and Horizon HD829 Myeloid gDNA reference standard, achieving a mean target coverage above 2500×. All covered SNV and indel variants were detected using DRAGEN Amplicon (Figure 7, Table 6) and no false positives were observed in healthy PBMC and normal FFPE samples.

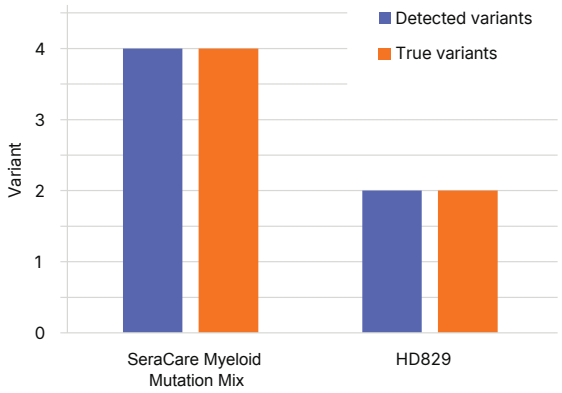

Figure 7: Variant calling performance using the DRAGEN Amplicon pipeline for oncoReveal Essential MPN Panel analysis

All four covered SNV and indel variants were detected in the Seraseq Myeloid Mutation DNA Mix and two expected variants were detected in the Horizon, HD829 Myeloid gDNA reference standard using the DRAGEN Amplicon pipeline. Table 6 shows the variants tested along with their observed VAF.

Table 5: FLT3-ITD variants detected across sample types using the oncoReveal Myeloid Panel

***FLT3* ITD variant**	**Length**	**Sample type**	**Observed VAF**
FLT3:c.1835_1836insCTCATATGATCTCAAATGGGAGTTTCCAAGAGAAAATTTAGAGTT	45 bp	FFPE tissue	45%
FLT3:c.1799_1800insATTCATATTCTCTGAAATCAA	21 bp	Cell line MOLM-13	61%
FLT3:c.1837+15_1837+16insTCAAAACGGTACAGGTGACCGGCTCCTCAGATAATGAGTACTTCTACGTTGATTTCAGAGAATATGAATATGATCTCAAATGGGAGTTTCCAAGAGAAAATTTAGAGTTTGGTAAGAATGGAATGT	126 bp	Cell line PL-21	< 1%
FLT3:c.1772_1801dup	30 bp	Cell line MV4-11	61%
FLT3:c.1806_1807insGGGGCTTTCAGAGAATATGAATATGATCTCAAA	33 bp	Seraseq Myeloid Mutation Mix	14%
FLT3:c.1759_1800dup	42 bp	Seraseq Myeloid Mutation Mix	8%

Table 6: Variants tested using oncoReveal Essential MPN Panel with observed VAF

Gene	HGVS nomenclature	Protein	Observed VAF	Mutation type	Sample
JAK2	c.1611_1616del	p.Val537_Phe539del	5%	Deletion	HD829
JAK2	c.1849G>T	p.Val617Phe	4%	SNV	HD829
MPL	c.1544G>T	p.Trp515Leu	6%	SNV	Seraseq Myeloid Mutation Mix
JAK2	c.1624_1629del	p.Asn542_Glu543del	9%	Deletion	Seraseq Myeloid Mutation Mix
JAK2	c.1849G>T	p.Val617Phe	7%	SNV	Seraseq Myeloid Mutation Mix
CALR	c.1099_1150del	p.Leufs*46	4%	Deletion	Seraseq Myeloid Mutation Mix

HGVS, Human Genome Variation Society

Summary

In partnership with Pillar Biosciences, Illumina has developed a fully integrated sequencing workflow incorporating oncoReveal panels, Illumina MiSeq i100 Series sequencing, the DRAGEN Amplicon pipeline, and Illumina Connected Insights for precision oncology research. Pillar Biosciences oncoReveal panels provide cancer researchers with targeted NGS options for a range of cancers and tissue types. oncoReveal panels use SLIMamp technology for optimal amplification specificity and accuracy. The performance evaluation of oncoReveal panels using Illumina sequencing confirmed that the assays perform as expected across a wide range of sample types and variant classes.

When analyzed with the DRAGEN Amplicon pipeline, the oncoReveal panels demonstrated high sensitivity and specificity for detecting SNVs, indels, CNVs, ITDs (such as *FLT3*-ITD), and RNA fusions in both contrived and clinical research samples, including challenging sample types such as FFPE tissue. Optimized DRAGEN Amplicon algorithms enabled SNV and indel detection at low VAF. Additional enhancements, such as CNV normalization using a PON and 3′/5′ imbalance metrics for RNA fusion detection, contribute further to the analytical power of the integrated workflow, while maintaining a turnaround time of under 24 hours.

Learn more →

DRAGEN secondary analysis

Pillar oncoReveal Multi-Cancer CNV + RNA Fusion Panel

Pillar oncoReveal Multi-Cancer RNA Fusion v2 Panel

Pillar oncoReveal Solid Tumor v2 Panel

Pillar oncoReveal BRCA1 & BRCA2 + CNV Panel

Pillar oncoReveal Myeloid Panel

Pillar oncoReveal Essential MPN Panel

Illumina Connected Insights

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2. Revvity Health Sciences Inc. Horizon Myeloid HD829, Myeloid gDNA Reference Standard. horizondiscovery.com/-/media/Files/Horizon/resources/IFUs/HD829-IVD-Mimix-Myeloid-Cancer-Panel-gDNA-Reference-Standard-Instructions-for-Use.pdf?sc_lang=en. Published, 2025. Accessed September 12, 2025.

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