Streamlined somatic analysis for IDT xGen® custom oncology research panels with DRAGEN™ software

Push-button secondary oncology analysis that produces variant calls with minimal manual intervention

Optimized for FFPE samples

Verified performance with FFPE solid tumor samples, including heavily degraded tissues

Verified modular workflow

Ready to use, modular workflow enables streamlined flexibility required for custom xGen oncology panels

Accurate variant detection

99% SNV/indel sensitivity across > 500 known variants in Horizon Discovery reference samples and 99.9998% specificity (base pair–level)

Introduction

Targeted next-generation sequencing (NGS) of custom oncology panels is a core approach in cancer research, enabling deep sequencing of genes of interest to detect somatic variants at low variant allele frequencies (VAF). Translating these sequencing data into high-quality, reproducible research results typically requires substantial bioinformatics effort—customers must assemble, tune, and maintain custom analysis pipelines for every new panel design.

DRAGEN somatic for Integrated DNA Technologies (IDT) custom panels is a preoptimized somatic secondary analysis solution. It is purpose-built for formalin-fixed paraffin-embedded (FFPE) solid-tumor samples generated with IDT xGen cfDNA and FFPE Library Prep enriched with IDT xGen custom oncology Hybrid Capture Panels.[1] DRAGEN software outputs can be loaded into Illumina Connected Insights for downstream variant interpretation and research reporting (Figure 1). This technical note describes features of DRAGEN somatic for IDT custom panels and reports performance in variant calling with reference samples.

Built for FFPE solid-tumor samples

DRAGEN somatic for IDT custom panels is verified for analyzing NGS data from FFPE solid-tumor tissue, the sample type most used in targeted somatic oncology research. A dedicated Low-Quality FFPE mode applies additional filtering for heavily degraded tissue with a DNA integrity number (DIN) ≤ 2. The pipeline features per–base pair systematic noise profiling that is designed to suppress FFPE-associated artifacts while preserving true somatic signals.

DRAGEN secondary analysis algorithms

DRAGEN somatic for IDT custom panels runs on DRAGEN v4.5.4 somatic algorithms with parameters, filters, and a systematic noise model tuned for IDT xGen custom panel data. It performs read alignment, somatic small variant calling, including single nucleotide variants (SNVs) and insertions/deletions (indels), and tumor mutational burden (TMB) estimation in a single analysis run. Tumor-only and tumor–normal modes are supported, with or without unique molecular identifiers (UMIs). Similar to existing DRAGEN pipelines, the DRAGEN somatic for IDT custom panels pipeline accepts FASTQ (.ora or .gz), Binary Alignment Map (BAM), Compressed Reference-oriented Alignment Map (CRAM), and ORA inputs.[1] The pipeline is delivered with preoptimized parameters for IDT xGen custom panel data. It executes in two stages (Figure 2):

1. **Baseline Builder (one-time setup per panel × sequencing system combination)**: Baseline Builder runs the DRAGEN somatic pipeline on a cohort of matched normal samples (typically ~30) to generate a per–base pair systematic noise file and an optional excluded regions BED. This step is recommended for both tumor-only mode and tumor–normal analysis. Alternatively, prebuilt systematic noise files may be downloaded from the DRAGEN Software Support Site, where available.
2. **DRAGEN app for IDT custom panels**: Per-sample FASTQ (or BAM/CRAM/ORA) files are aligned with the DRAGEN Map/Align module and passed to the somatic small variant caller together with the panel BED and the systematic noise file. The pipeline outputs a hard-filtered annotated VCF containing SNVs and small indels, an aligned BAM, a TMB estimate, and a run-level quality control (QC) file. Tumor-only and tumor–normal workflows, with or without UMIs, are supported from the same application. A dedicated Low-Quality FFPE mode applies additional filtering for degraded inputs with DIN ≤ 2.

Minimal-touch, autolaunched analysis

The DRAGEN somatic for IDT custom panels pipeline is built for automated operation. When planned through BaseSpace™ Sequence Hub Run Planner, tumor-only analysis autolaunches the moment the sequencing run completes, eliminating the need for manual steps or data hand-off (Figure 1). For users with existing data, tumor–normal workflows, or automation requirements, DRAGEN somatic for IDT custom panels can be launched manually from BaseSpace Sequence Hub or programmatically via the Illumina Connected Analytics command line interface (CLI) or application programming interface (API), including ORA input (Figure 1). All three entry points produce the same outputs and support UMI workflows, Low-Quality FFPE mode, and all Illumina sequencing systems.*

* Performance verified on the NextSeq 2000 System; compatibility across other Illumina sequencing systems is supported by panels and sequencing system–specific Systemic Noise file generation.

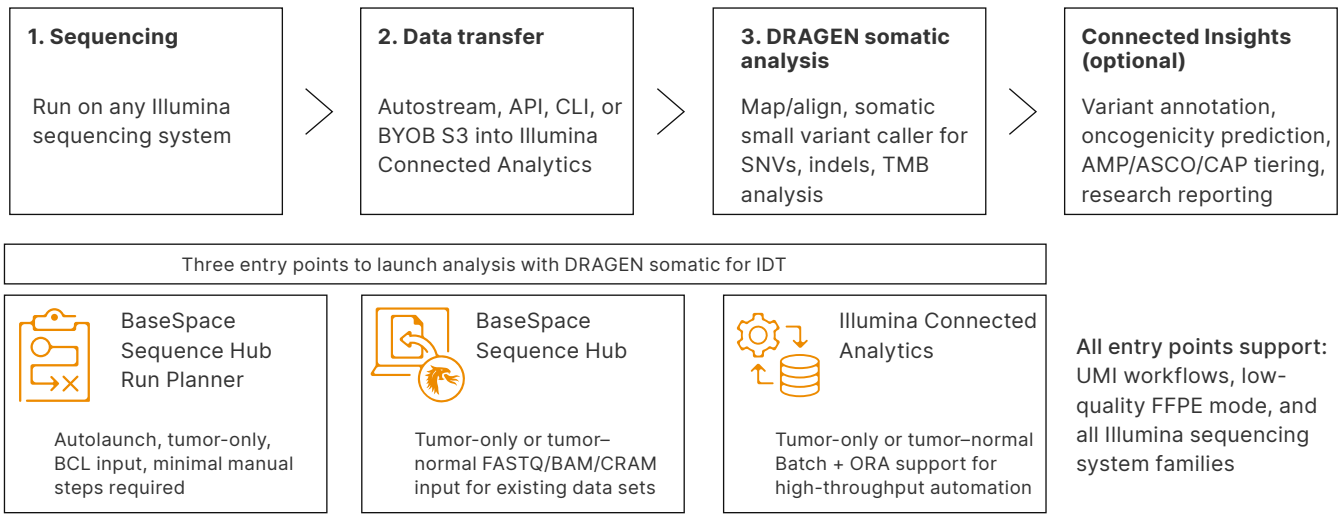


Figure 1: End-to-end workflow for DRAGEN somatic analysis on IDT xGen custom panel data

Tumor-only analysis can be autolaunched from the sequencing system via BaseSpace Sequence Hub Run Planner the moment the sequencing run completes with no manual intervention required. Two additional entry points are available for existing data sets and automation needs: via BaseSpace Sequence Hub and Illumina Connected Analytics. The annotated VCF can be loaded into Illumina Connected Insights for downstream variant annotation and research reporting. AMP, Association of Molecular Pathology; ASCO, American Society of Clinical Oncology; BAM, binary alignment map; BCL, binary base call; BYOB, bring your own bucket; CAP, College of American Pathologists; CRAM, compressed reference-oriented alignment map; FFPE, formalin-fixed paraffin-embedded; indel, insertion/deletion; SNV, single nucleotide variant; TMB, tumor mutational burden; UMI, unique molecular identifier; VCF, variant call file.

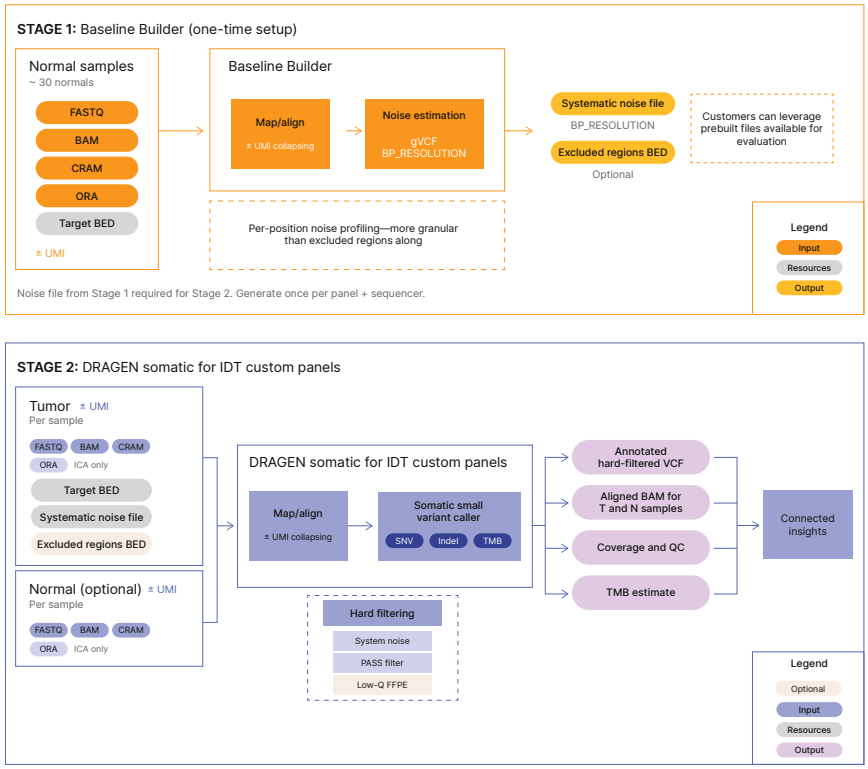


Figure 2: DRAGEN somatic for IDT custom panels pipeline setup

The DRAGEN somatic for IDT custom panels pipeline exceutes in two stages; in stage 1 (one-time setup) Baseline Builder runs the DRAGEN somatic pipeline on a cohort of matched normal samples to generate a per–base systematic noise file and excluded regions BED; in stage 2, per-sample FASTQ files are passed to the small variant caller to output an annotated VCF file containing SNVs and small indels. BAM, binary alignment map; BCL, binary base call; BED, browser extensible data; CRAM, compressed reference-oriented alignment map; indel, insertion/deletion; SNV, single nucleotide variant; TMB, tumor mutational burden; UMI, unique molecular identifier; VCF, variant call file

Quality control

Every run produces a consistent set of quality control (QC) metrics to support data review and troubleshooting. Run-level metrics include percent reads passing filter (PF) and percent bases at Q30 for each read. Per-sample metrics include average deduplicated coverage over target regions, percent of target covered at defined depth thresholds, percent duplicate-marked reads, percent bases mapped, and an estimate of cross-sample contamination. These metrics are surfaced in BaseSpace Sequence Hub and Illumina Connected Analytics alongside the VCF and BAM outputs for downstream review.

Optional downstream variant interpretation

The annotated VCF produced by DRAGEN somatic for IDT custom panels is a standard output that can be consumed by any downstream tertiary analysis tool. For customers who want a fully integrated Illumina workflow, this VCF can be loaded directly into Connected Insights for variant annotation using 55+ knowledge sources, automated guideline-based oncogenicity prediction, and a customizable research report.[2–5]

Methods

Samples and data inputs

Performance of DRAGEN somatic for IDT custom panels was evaluated on FFPE solid-tumor samples and commercially available reference standards (Table 1). Sensitivity was assessed using Horizon Discovery formalin compromised DNA (fcDNA) reference standards: HD798 (mild), HD799 (moderate), and HD803 (severe) and orthogonally characterized truth sets. FFPE specificity and interreplicate concordance were assessed using phenotypically normal and tumor–normal FFPE specimens spanning a range of DNA integrity, including heavily degraded samples (DIN ≤ 2). Sequencing was performed on the NextSeq™ 2000 Sequencing System to generate data inputs for DRAGEN somatic for IDT custom panels.

Secondary analysis

All data were analyzed with DRAGEN somatic for IDT custom panels built on DRAGEN v4.5.4 software, deployed on Illumina Connected Analytics through BaseSpace Sequence Hub.

Table 1: Samples used to evaluate DRAGEN somatic for IDT custom panel performance

Evaluation	Samples	Configuration
Sensitivity on known somatic variants	Four Horizon Discovery reference samples (HD798-mild, HD799-moderate, HD803-severe, HD827), two replicates each	Tumor-only, default settings, UMIs enabled, ~762× mean deduplicated depth
FFPE artifact mitigation	20 phenotypically normal FFPE libraries (14 high-quality, six low-quality with DIN ≤ 2)	Tumor-only, default settings
Interreplicate concordance	4 FFPE tumor-normal pairs (two with DIN ≤ 2), two independent library prep replicates sequenced separately	Tumor–normal, default settings

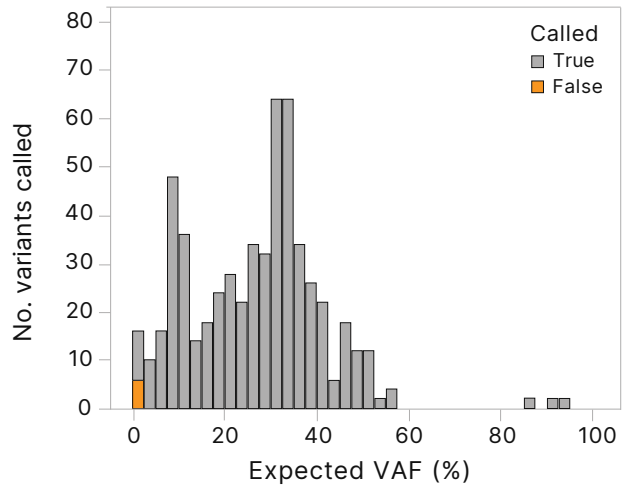
All libraries were sequenced on the NextSeq 2000 Sequencing System.
DIN, DNA integrity number; FFPE, formalin-fixed paraffin-embedded; UMI, unique molecular identifier.

Results

Sensitivity on known somatic variants

Sensitivity of DRAGEN somatic for IDT custom panels was assessed using four Horizon Discovery fcDNA reference standards with known variant truth sets across a 2.0 Mb IDT xGen custom oncology panel. Samples HD798 (mild), HD799 (moderate), and HD803 (severe) each contribute 26 expected variants per replicate, and sample HD827 contributes 214 expected variants per replicate. All four samples were sequenced in duplicate, with UMIs enabled, to a mean deduplicated depth of approximately 762×, and analyzed in tumor-only mode using default pipeline settings.

Across 582 total expected variant observations, DRAGEN somatic for IDT custom panels detected 576 variants for an overall sensitivity of 99% (Figure 3). When restricted to variants with VAF ≥ 1.5%, the range most relevant to somatic discovery in challenging samples, sensitivity was 100% (570/570).



Total sensitivity	99% (576/582)
Sensitivity ≥ 1.5% VAF	100% (570/570)

Figure 3: Variant calling sensitivity of DRAGEN somatic for IDT custom panels on reference samples
Variants called by the DRAGEN somatic for IDT custom panels (gray) and variants not called (orange) are plotted across two sequencing replicates per sample, run in tumor-only mode with UMIs at ~762× mean deduplicated depth on a 2.0 Mb IDT xGen custom panel. Aggregated across all 582 expected variant observations, sensitivity was 99% overall (576/582) and 100% at VAF ≥ 1.5% (570/570). UMI, unique molecular identifier; VAF, variant allele frequency.

FFPE artifact mitigation

Somatic calling in FFPE tissue is challenged by formalin-induced DNA damage, which can generate artifactual variant calls that resemble low-VAF somatic events. To evaluate how well DRAGEN somatic for IDT custom panels suppresses these artifacts, 20 phenotypically normal FFPE libraries (14 high-quality and 6 low-quality (DIN ≤ 2)) were analyzed in tumor-only mode with default pipeline settings. All passing somatic variants were counted as false positives, except for variants with VAF ≥ 20% or allele counts ≥ 50 in the 1000 Genomes or gnomAD databases, which were considered germline and excluded.[4,6]

The research pipeline produced a mean of 1.9 false positives per library (0.95 per Mb) in high-quality FFPE and 2.7 false positives per library (1.35 per Mb) in low-quality (DIN ≤ 2) FFPE samples (Figure 4). Importantly, no AMP/ASCO/CAP-leveled false positives were observed at VAF ≥ 5% across any of the 20 libraries. Some tumor-adjacent tissue in the cohort contained low-VAF variants that, when annotated in Illumina Connected Insights as an optional downstream step, received meaningful tier assignments for clinical research (See Appendix). Note, these either could be false positives or the result of low-level tumor-in-normal contamination.

Interreplicate concordance on clinical research samples

To evaluate call stability on representative clinical research samples, four FFPE tumor–normal pairs, two with low-quality DNA (DIN < 2), were sequenced and analyzed in duplicate using the tumor–normal mode of the DRAGEN somatic for IDT custom panels pipeline with default settings. Small variants were compared between the two replicates of each pair at two VAF thresholds.

Interreplicate concordance was 94% at VAF ≥ 5% (232 of 246 variants called in both replicates) and 97% at VAF ≥ 10% (202 of 208) (Figure 5). Most discordant calls were at VAF ≥ 5%, consistent with stochastic sampling at the lower end of the calling range, and largely resolved at VAF ≥ 10% (Figure 5). These results support the use of the default pipeline configuration across mixed-quality FFPE tumor–normal cohorts without per-sample parameter tuning.

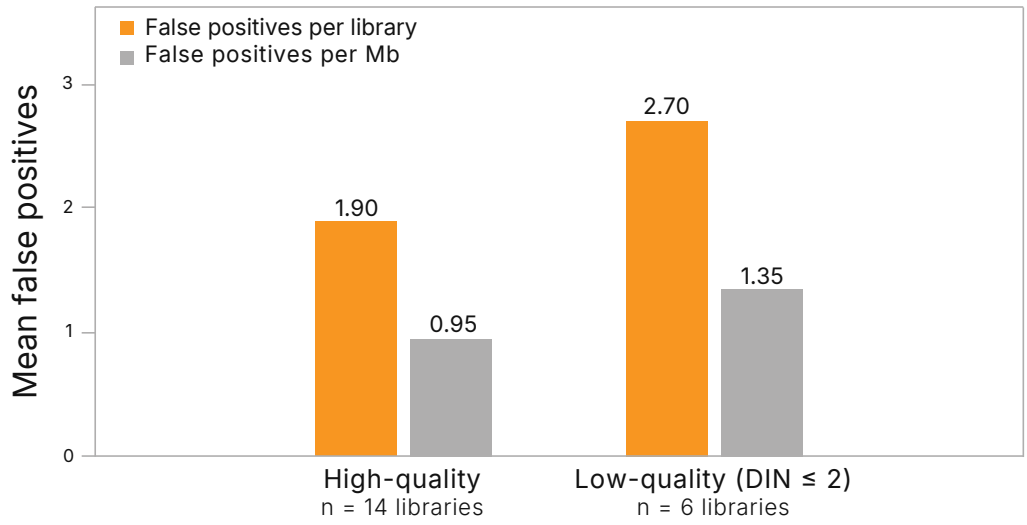


Figure 4: Mean number of false positive somatic calls

The mean number of false positive somatic calls per library and per megabase of target across 20 phenotypically normal FFPE libraries are plotted and stratified by sample quality (high-quality, n = 14; low-quality with DIN ≤ 2, n = 6). Analyses were run in tumor-only mode with default settings. No AMP/ASCO/CAP-leveled false positives were observed at VAF ≥ 5% in either group. DIN, DNA integrity number; FFPE, formalin-fixed paraffin-embedded; VAF, variant allele frequency.

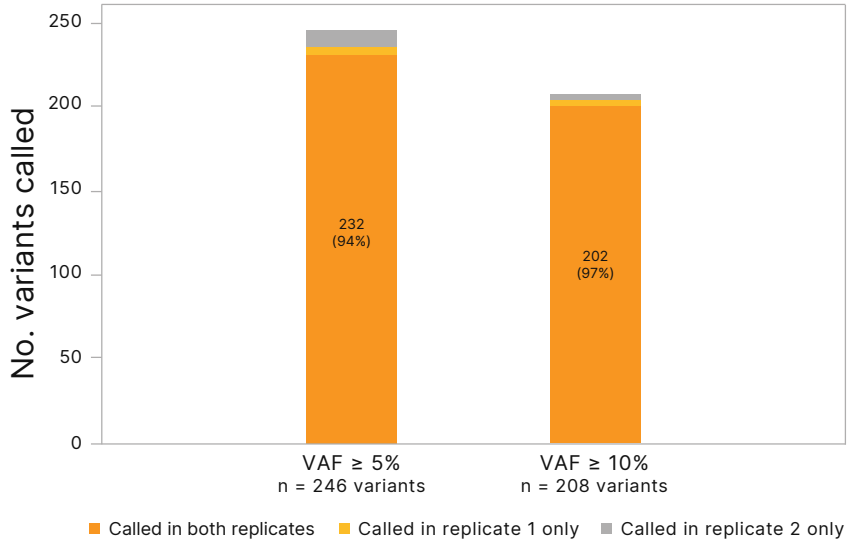


Figure 5: Interreplicate concordance for small variant calls

Plotting interreplicate concordance for small variant calls across four FFPE tumor–normal pairs (two with DIN ≤ 2), each sequenced and analyzed in duplicate with the DRAGEN app for IDT custom panels in tumor–normal mode with default settings, showed that concordance was 94% at VAF ≥ 5% (232/246) and 97% at VAF ≥ 10% (202/208). DIN, DNA integrity number; FFPE, formalin-fixed paraffin-embedded; VAF, variant allele frequency.

Summary

DRAGEN somatic for IDT custom panels is a preoptimized somatic secondary analysis solution. It is verified for use with FFPE solid tumor samples and libraries generated with IDT xGen cfDNA and FFPE Library Prep on IDT xGen custom oncology research panels. This pipeline enables a consistent, scalable path from sequencing to somatic variant calls from IDT xGen custom panel data.

Learn more →

DRAGEN secondary analysis

Illumina Connected Insights

IDT xGen Custom Hybrid Capture Panels

References

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Appendix

Low VAF variants observed in FFPE samples that received AMP/ASCO/CAP tier assignments

Variant	Connected Insights tier	Observed VAF	Gene role
PTEN p.L247*	Tier 1B	2.3%	Tumor suppressor
SDHA p.L649fs	Tier 2C	2.9%	Tumor suppressor
RB1 p.R455fs	Tier 2C	4.7%	Tumor suppressor

AMP, Association for Molecular Pathology; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; VAF, variant allele frequency.

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