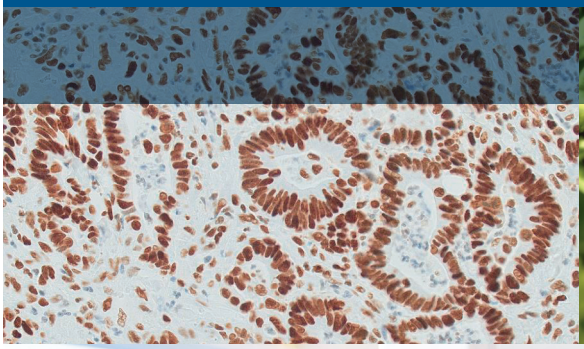
Agilent

Trusted Answers

Next-Generation Sequencing FFPE Solution

For the Pathologists



Next-Generation Sequencing: Changing the Paradigm in Molecular Pathology

Next-generation pathology

Targeted next-generation sequencing (NGS) has become a routine analytical method in biomedical research and is now also being implemented for diagnostic applications. Agilent provides the critical reagents for identifying and characterizing genes of interest, including tools for sample and library preparation, target enrichment, data analysis, and quality control.

Overcoming the challenges of FFPE samples

Formalin-fixed paraffin-embedded (FFPE) samples are the preferred method for preserving tissue biopsy samples for downstream characterization (e.g., IHC, FISH, NGS, qPCR), but the method often results in degradation of sample DNA.[1]

The process of preserving FFPE samples by formalin fixation crosslinks proteins and induces matrix effects that complicate the extraction of genetic material. In addition, formalin fixation can damage nucleic acids, resulting in DNA strand breakage and low molecular-weight fragments,[2] and induce artifacts that are indistinguishable from actionable mutations. The conditions a tissue specimen experiences before and during formalin fixation and the duration of this, also affect specimen quality and contribute to downstream sequencing artifacts[3]. As FFPE samples are often limited due to size and accessibility, DNA yields are frequently insufficient for certain sequencing workflows.

Many of these issues may be addressed through careful choice of sample preparation and target enrichment methods, as well as appropriate automation and data analysis tools.

1. Ofner, R., Ritter, C., Ugurel, S. et al. J Cancer Res Clin Oncol (2017) 143: 1199. https://doi.org/10.1007/s00432-017-2399-1
2. http://www.conversantbio.com/blog/5-problems-associated-with-ffpe-nucleic-acid-isolation
3. Do H., Dobrovic A. (2015) Clinical Chemistry, 61 (1) , pp. 64-71

The leader in target enrichment

For general sample preparation, Agilent provides reagents to assist with DNA quality through our TapeStation systems, library preparation, and target enrichment and amplification. We offer automation through our Magnis and Bravo platforms. For data interpretation the Agilent compliant, traceable/Class 1 medical device Alissa Clinical informatics software fulfills clinical-grade genomics data management by streamlining data analysis and variant assessment workflows, from raw reads to draft reports.

Agilent NGS products allow for the greatest sensitivity, providing reliable results from samples as small as 10 ng of amplifiable DNA, to samples of varying tumor content.

Users can easily, with options for automating much of the workflow, ensure operational efficiency and reproducible results in one day. Moreover, our products are compatible with various sequencing platforms.

Unlock Your Block

Agilent offers reagents and software to support the entire NGS workflow, including quality control, sample preparation, data analysis, and clinical interpretation.

Of these steps, sample preparation requires the most planning, as it necessitates the most thought due to the number of choices based on sample type.

Boosting sequencing efficiency with target enrichment

NGS workflows include a target enrichment step to prepare the sample for deep sequencing of genes of interest. Depth of sequencing is determined by tumor heterogeneity, as the variants of interest may not be present in all tumor cells. Hence, an enrichment or amplification step helps to "concentrate" or "pull" signal out of noise. An enrichment protocol employing molecular barcoding can further improve sensitivity to low allele frequency variants present in only a subset of tumor cells.

These enhancements to the target enrichment step, combined with a seamless NGS workflow (quality control, sample preparation, data analysis and interpretation), empower pathologists to focus on results, rather than the distractions of routine laboratory operations.

Agilent Portfolio in the Clinical NGS Market

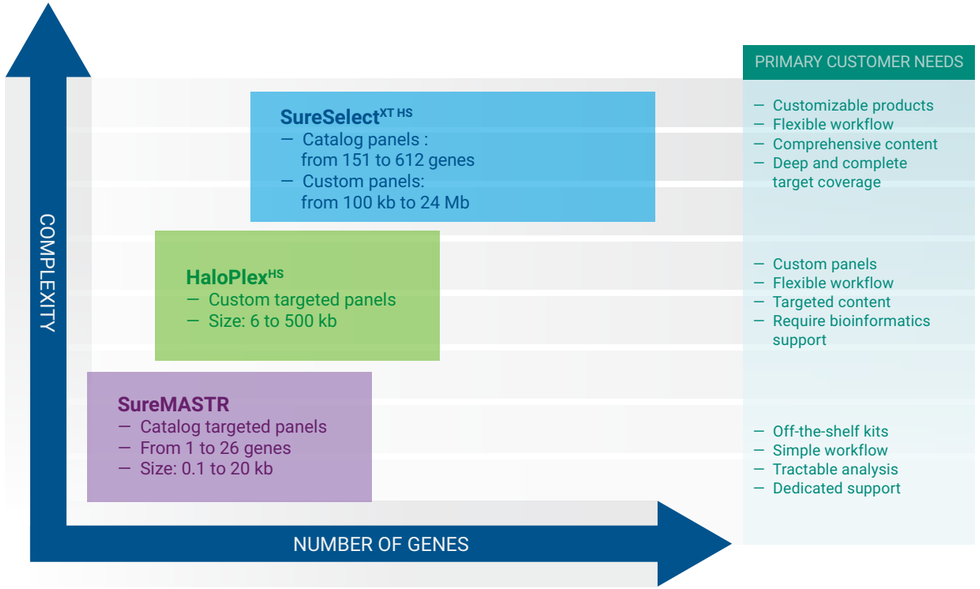


Figure 1. Relative representation of the Agilent target enrichment solution.

Three Technologies, Infinite Possibilities

Agilent SureSelect$^{XT\ HS}$ hybrid capture technology

Hybrid capture target enrichment employs hybridization probes to capture and isolate target sequences from an NGS library. Hybrid capture is sensitive and suited to detecting single nucleotide variants, translocations, structural variants, insertions and deletions, and copy number variations. Hybrid capture is the appropriate choice for FFPE tissue samples including needle biopsies, or when sample is otherwise scarce.

Features and benefits of SureSelect$^{XT\ HS}$:

- Detects low-frequency SNVs, indels, translocation, structure variants, and CNVs
- Leverages molecular barcodes, enabling down to 1 % or fewer variant allele frequency calls (ideal for residual disease monitoring)
- Customizable content for designs spanning 100 kb to 24 Mb
- Allows design of targeted panels through Agilent SureDesign
- Transforms samples into sequencing-ready libraries in 8 hours with as little as 10 ng input

Agilent HaloPlex^{HS} amplicon-based target capture technology

HaloPlex^{HS} is a high-sensitivity amplicon-based targeted sequencing method that incorporates molecular barcodes in the DNA library. Barcodes enable identification of duplicate reads, thereby significantly improving base calling accuracy even at low allelic frequencies. Combined with the flexibility of panel customization up to 500 kb, and an accelerated workflow of fewer than six hours beginning with just 50 ng gDNA, HaloPlex^{HS} provides research pathologists and technicians with unparalleled speed, accuracy, and sensitivity.

Features and benefits of HaloPlex^{HS}:

- High on-target specificity and deep coverage of target bases so key variants are not missed
- Confident detection of mutations present at below 1 % allele frequency in genetically heterogeneous samples
- Analysis of degraded DNA with sample input as low as 50 ng
- Single-day workflow, with automation protocols available
- Panel customization up to 500 kb through SureDesign

Agilent SureMASTR amplicon-based target amplification technology

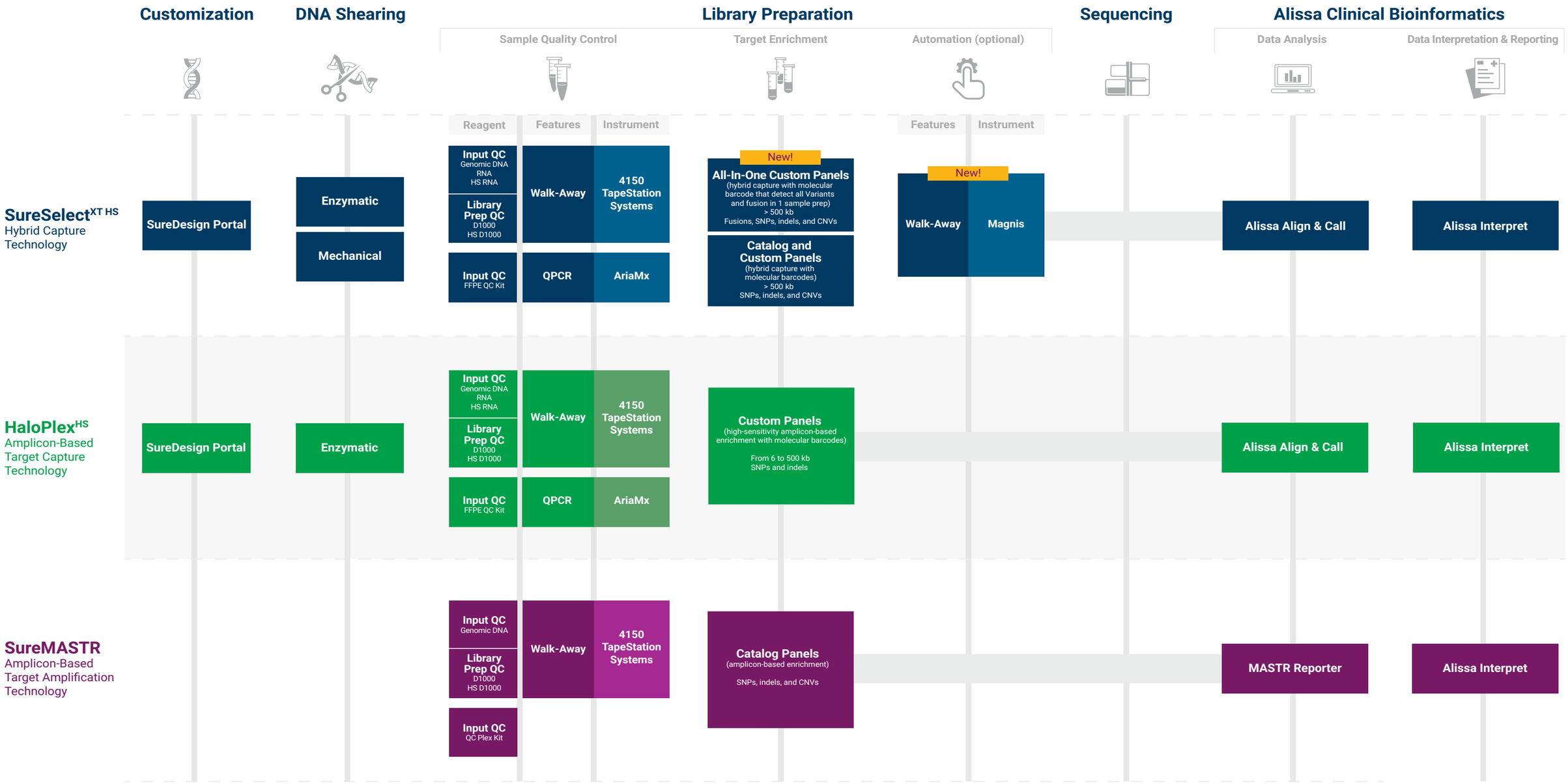
SureMASTR (multiplex amplification of specific targets for resequencing) assays are an amplicon-based target enrichment for NGS. Based on multiplex PCR, SureMASTR amplifies multiple genomic targets through PCR reactions.

Features and benefits of SureMASTR workflow:

- Rapid, straightforward workflow requiring just 3.5 hours of hands-on time and no prior NGS experience
- Pre-mixed, ready-to-use PCR primers for amplifying target DNA sequences within a standard NGS library
- Deep sequencing based on as little as 4 ng of DNA
- Intelligent primer design to reduce amplicon drop-outs from primer-interfering population SNPs
- Variant detection down to 5 % allele frequency
- Intuitive, user-friendly data analysis for all levels of NGS experience with Agilent MASTR Reporter software
- Complete workflow, from sample QC to report

When specific target panels are not commercially available, SureSelect and HaloPlex^{HS} custom oligonucleotide design service allows pathologists to create their own through SureDesign web portal. Custom design capabilities are available for DNA and RNA target enrichment, with the flexibility to capture sequences ranging in size as low as 1 kb.

Your Trusted Partner in Every Step — From Sample to Data Reporting



Seamless Comprehensive End-to-End NGS Workflow

Quality control

1 Quality Control — 2 Targeted Panels — 3 NGS Automation — 4 Bioinformatics

DNA from FFPE is often loaded with artifacts. Genetic material may be fragmented, cross-linked with protein, and contain single-stranded DNA, thus making library preparation and target enrichment challenging.

Agilent NGS FFPE quality control can be performed with PCR, qPCR, or electrophoresis. PCR kits like the Agilent QC Plex for the SureMASTR technology, or the Agilent NGS FFPE QC kit for HaloPlex/SureSelect, enable the assessment of the integrity of DNA as well as accurate quantitation of amplifiable template going into library preparation.

The Agilent 4150 TapeStation system offers an automated electrophoresis for (FFPE) sample QC. Analysis includes quantification and qualification with quality metrics for DNA integrity and RNA integrity using two different metrics.

Both methods offer rapid, reliable quality control of samples — and peace of mind results that accurately represent sequences contained in the original sample.

Prepare, Sequence, Analyze, Report

Targeted panels

1 Quality Control — 2 Targeted Panels — 3 NGS Automation — 4 Bioinformatics

SureSelect Cancer All-In-One assays

SureSelect Cancer All-In-One (AIO) assays are a revolutionary new type of NGS assay that enables the detection of single nucleotide variants (SNVs), indels, copy number variants (CNVs), and translocations in a single assay with high confidence. SureSelect Cancer AIO offers a comprehensive and flexible solution with a true single assay, delivering molecular insights from your precious cancer samples in the genes that matter to you, quickly and cost-effectively.

These assays include either catalog or custom panels (created using the SureDesign tool), the SureSelect$^{XT\ HS}$ or SureSelect^{XT} Low Input Reagent kit for NGS library preparation, and complementary SureCall software for data analysis. The customization option provides a solution that allows your NGS assay to evolve with your clinical research needs. SureSelect Cancer AIO assays provide a simple workflow with high-sensitivity genomic variant detection as part of a flexible NGS workflow that can meet the needs of many laboratories.

The SureSelect Cancer AIO workflow offers several advantages that enable you to:

- Consolidate multimodal assays to get more answers from a single DNA assay.
- Achieve sequencing-ready libraries from DNA in under 36 hours.
- Get an integrated solution from panel design to data analysis.
- Simplify your experience with catalog cancer NGS assays.

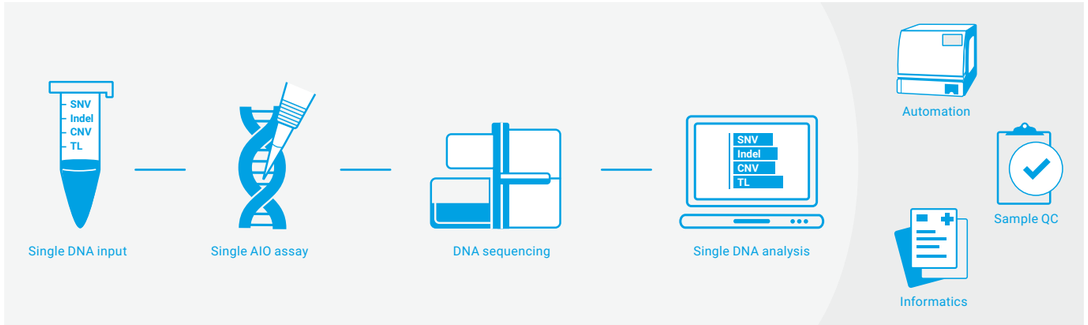


Figure 2. The SureSelect Cancer All-In-One solution consolidates the detection of all variant types into one workflow with single DNA input and single data analysis, using Agilent SureCall software or the Alissa Clinical informatics platform for alignment and variant calling (coming soon). A walkaway automation option for library preparation enabled by Magnis NGS prep system, and variant interpretation on the Alissa is available.

NGS library prep made easy

The new **SureSelect$^{XT\ HS}$ Enzymatic Fragmentation** kit removes the need for the upfront investment in an expensive pre-analytical sample preparation instrumentation required when doing hybrid-capture NGS. This kit has been optimized for a full range of DNA inputs (10-200 ng), various GC content, all different sample types (blood, fresh frozen tissue and FFPE) and qualities. A highly reproducible DNA fragmentation profile can be generated with various DNA input amounts using a single protocol. This single protocol enables an easy and streamlined NGS library preparation workflow.

Cancer NGS catalog panels

Agilent offers the **ClearSeq Comprehensive Cancer panel**, powered by SureSelect$^{XT\ HS}$. This panel targets 151 genes associated with a wide range of cancers (eg. breast, lung, colorectal, AML). It is designed for deep coverage of target bases for confident variant detection.

Agilent **SureMASTR Tumor Hotspot** assay identifies SNVs in frequently mutated cancer-associated genes, focusing on 26 relevant genes and amplifying only the hotspot target regions (exon coverage for 26 frequently mutated genes, 25.7 kb panel size). The assay is optimized to detect mutations from FFPE tissue samples, but is also compatible with FFT or blood samples. The Agilent SureMASTR Tumor Hotspot assay is supported by the MASTR Reporter software analysis research tool, that enables optimized and automated variant calling as well as integrated quality control of the entire workflow.*

Agilent **SureMASTR BRCA** solutions offer a variety of targeted, amplicon-based assays for both somatic and germline use. The assays are complete with easy to use MASTR Reporter analysis software that identifies variants in important genes related to breast- and ovarian cancers. Thanks to the amplicon-based technology, these assays are easy to implement, and give high quality and reliable results.

Custom cancer panels

For research endpoints not covered by our catalog panels, Agilent provides **SureDesign**, a web-based application for creating custom NGS target enrichment libraries. The Agilent proprietary SurePrint oligo manufacturing technology allows users to render these designs into reagent oligonucleotides.

You can now easily create your own custom cancer panel while leveraging wet lab-verified probe sets, in less than 2 weeks with no minimum order requirements. In addition, in order to ensure your success, Agilent is providing full “white-glove” support with a field team that will come to your lab to educate and train your team on NGS.

** Discover more SureMASTR assays for pathology on the Agilent website.*

NGS automation

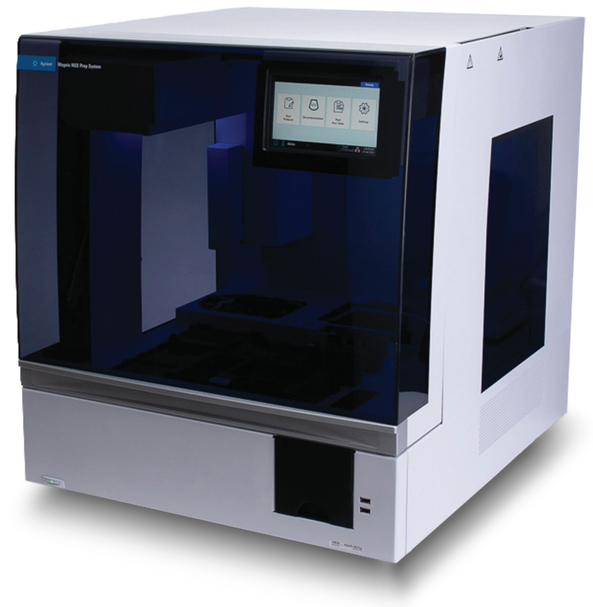
Agilent offers a complete instrument and reagent solution for automating NGS sample preparation. As a result, you can achieve excellent reliability and reproducibility with easy scale-up of your diagnostic laboratory throughput using reagents, automation, protocols, and support from a single source.

Magnis NGS prep system

The Agilent Magnis NGS Prep system was designed to run complex NGS library assays with minimal technical knowledge and hands-on time – all with the press of a button.

The Magnis system is comprised of an instrument, reagents, and protocols.

- **Complete system for NGS preparation:** This benchtop instrument self-detects and tunes; the reagents are provided pre-aliquoted, and the system comes with pre-set protocols for Agilent’s catalog and custom assays.
- **Fully automated:** This solution requires minimal staff and NGS expertise to run and the onboard wizard helps the user with reagent placement through automated barcode checking and allows assays to be set-up in less than 5 minutes.
- **Reproducible results:** the Magnis was developed to work seamlessly with SureSelect, the leader in target sequencing, delivering actionable results from NGS data that are robust, traceable, and reproducible.



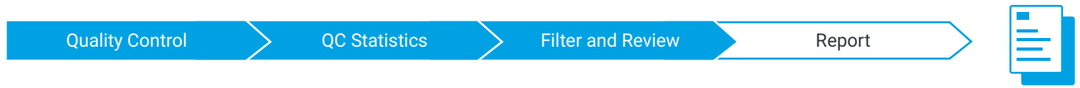
Agilent Alissa clinical informatics

Alissa Clinical informatics platform: a single sign-on workflow

Tumor profiling using NGS is rapidly gaining importance in the molecular pathology setting. Adopting this technology requires not only bioinformatics tools to analyze, interpret, and catalog the large number of variants originating from NGS assays, but also brings challenges in data management and clinical interpretation. In a high-throughput context, the delivery of actionable results from NGS data needs to be clinically robust — informed, traceable and reproducible. Moreover, in a cancer diagnostic setting, fast turnaround times are essential for patient care.

Agilent Alissa Clinical informatics delivers a unified platform for combined analysis of SNPs, indels, CNVs, and translocations.

Alissa Align and Call



- **Go from FAST Q to VCF** in seamless integration of analysis pipelines with variant assessment
- **Optimize algorithms** for Agilent assays
- **Perform comprehensive QC metrics** to ensure data quality with the clinical workflow in mind
- **Implement an automated pipeline** for somatic variant assessment

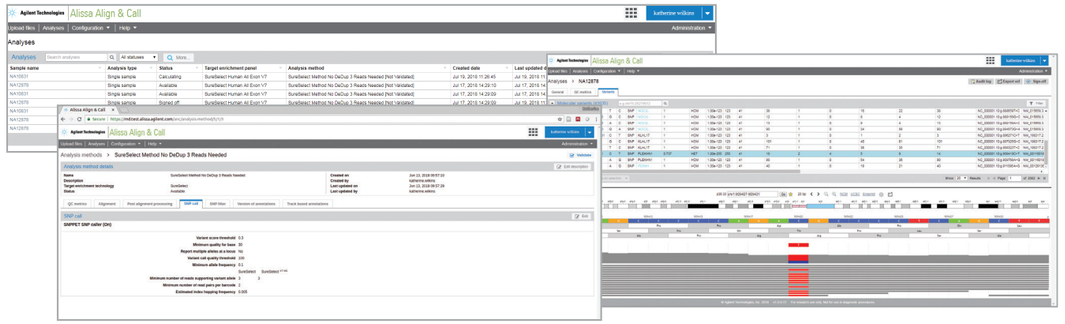


Figure 3. Alissa Align and Call: Delivering a 'white box' from raw reads to QC metrics.

Across the laboratory workflow, Alissa informatics enables efficient calling, triage in the Align and Call module and curation and reporting of sequenced cancer variants in the Interpret module. With Alissa's automatable solution, filtering and annotating variants is sped up, and identified variants of interest are efficiently and confidently curated and classified, all based on up-to-date public, premium and in-house variant knowledge. Highlighted actionable results are captured in clinical-grade draft reports.

Alissa Interpret

- **Efficiently triage and confidently classify variants** in context of the tumor tissue type and histology, tumor versus normal
- **Access public and premium knowledge databases** including N-of-One and CIVIC
- **Build a curated variant knowledge base** tailored to collect therapeutic, prognostic, and diagnostic evidence for somatic variants
- **Automate comprehensive draft reports** based on configurable templates

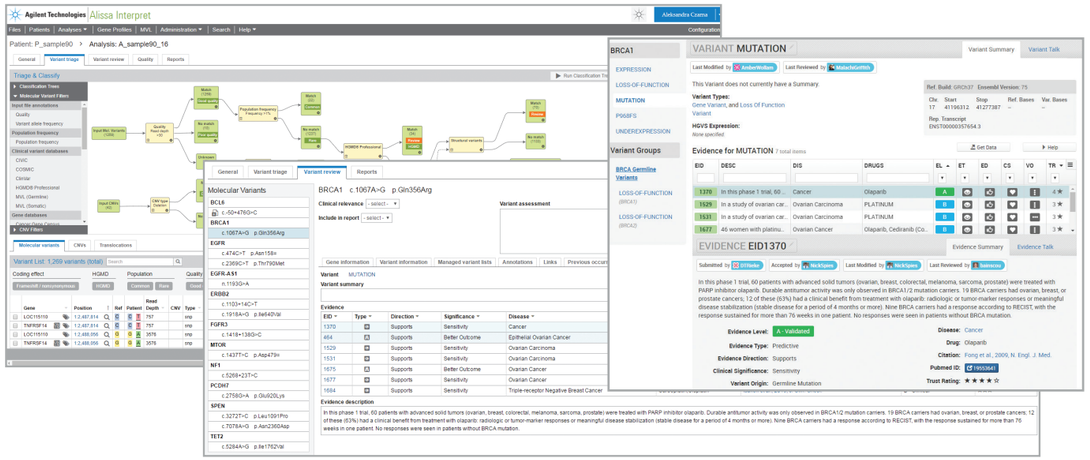


Figure 4. Alissa Interpret: Automating standard operating procedures from variant assessment to draft reports.

Learn more:
www.agilent.com/en/ngs-solutions-for-pathology

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