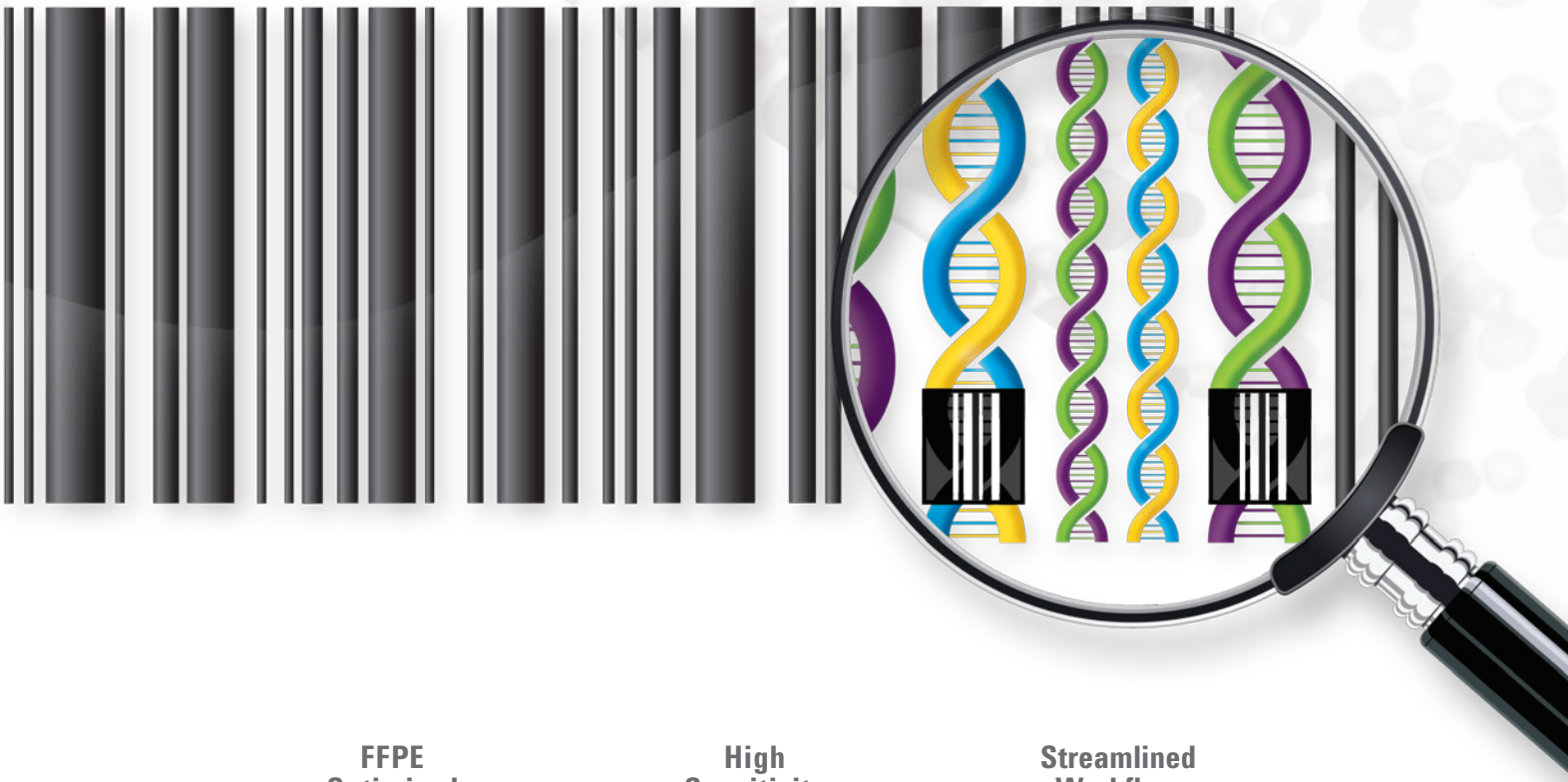
SURESELECT$^{XT\ HS}$ TARGET ENRICHMENT



FFPE Optimized

High Sensitivity

Streamlined Workflow

Agilent Technologies

What is it?

SureSelect$^{XT\ HS}$ joins the SureSelect library preparation reagent family as Agilent's highest sensitivity hybrid capture-based library prep and target enrichment solution for NGS.

KEY FEATURES

- **10 ng** of input DNA
- Optimized for high-quality intact DNA, low- and high-quality **FFPE DNA**
- **Molecular barcode (MBC)** tagged libraries increase positive predictive value (PPV)
- **Higher complexity** libraries with higher percentage reads in targeted regions
- **90-minute** hybridization and **master-mixed** reagents for faster, more efficient workflow

FFPE Optimized

The importance of an FFPE-optimized library prep

While FFPE samples provide a valuable source of diverse genetic information, one of the hurdles in isolating DNA from FFPE tissue is the purification of DNA of sufficient molecular weight and quality for amplification and detection. Typically, by the time FFPE DNA is prepared for library generation, it is too degraded for high-sensitivity NGS. It is thus crucial to optimize the library preparation and minimize the number of steps to reduce sample loss and achieve high-quality libraries from challenging sample types.

FFPE-optimized library prep from Agilent

SureSelect$^{XT\ HS}$ produces more complex libraries with a higher percentage of reads in targeted regions for a wide range of tissue types—in both fresh and FFPE samples—with only 10 - 200 ng of input material. To improve single nucleotide variant (SNV) calling and consistency across FFPE samples of varying quality, Agilent has developed a complete workflow solution that features DNA pre-qualification (FFPE QC Kit, PN G9700A and G9700B, and 4200 TapeStation System) and one-tube library preparation.

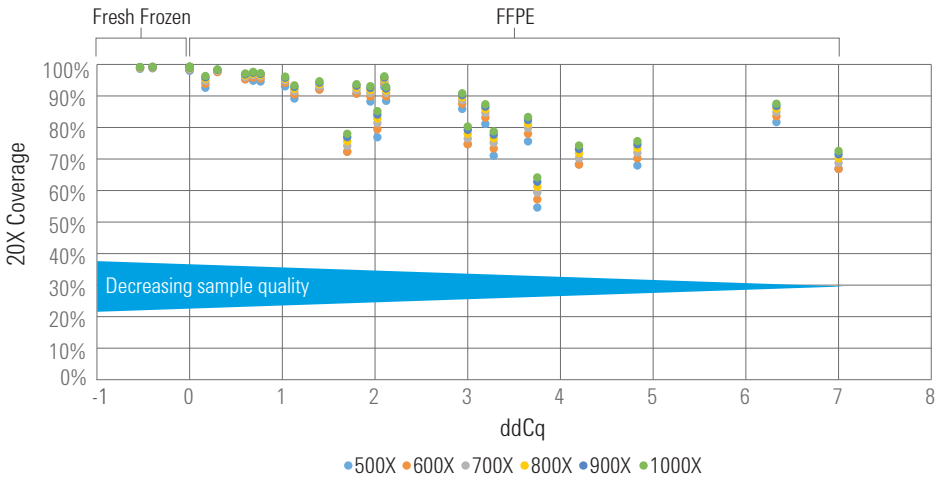


Figure 1. SureSelect$^{XT\ HS}$ optimized performance for FFPE with the majority of bases covered at 20X across decreasing sample quality. % bases covered by at least 20X read depth with increasing sequencing depth (4-8 million - 2x100 bp reads) is plotted against gDNA quality (qPCR-based gDNA quality (ddCq) and is calculated using Agilent's FFPE QC Assay kit).

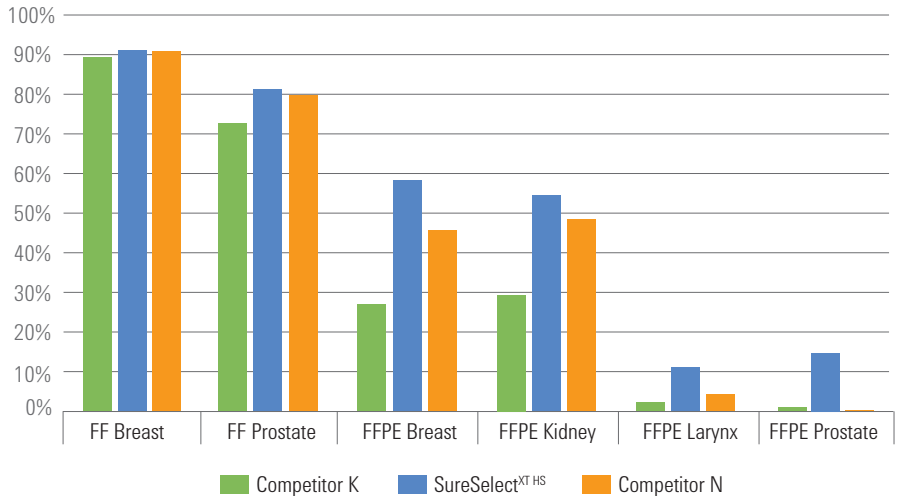


Figure 2. SureSelect$^{XT\ HS}$ outperforms other library prep solutions at 100X coverage, especially as sample quality decreases: % bases covered by at least 100X read depth is plotted against gDNA of decreasing quality (qPCR-based gDNA quality (ddCq) and is calculated using Agilent's FFPE QC Assay kit (based on 1000X sequencing depth with 8 million - 2x100bp reads).

Advantages

- Improve SNV calling from FFPE DNA
- Reduce the number of library preparation steps, preventing DNA loss
- Prepare higher complexity libraries from both low- and high-quality DNA

High Sensitivity

The significance of high sensitivity

Sensitivity refers to the percentage of true variants that are correctly identified. In most cases, sensitivity is limited by the input amount, performance of the assay, and PCR and sequencing error rates. As a result, the error level seen with traditional NGS assays typically interferes with the confidence of detecting low frequency variants due to tumor heterogeneity.

Superior sensitivity with SureSelect$^{XT\ HS}$

SureSelect$^{XT\ HS}$ incorporates molecular barcodes, which enables users to filter out artifacts produced during library preparation, target enrichment and sequencing that cause false positive variants. Molecular barcoding with SureSelect$^{XT\ HS}$ achieves industry-leading metrics, allowing the detection of rare variants down to ≤ 1%. This is significant as most users employ a confidence threshold and only call variants above 3-5% allele frequency.

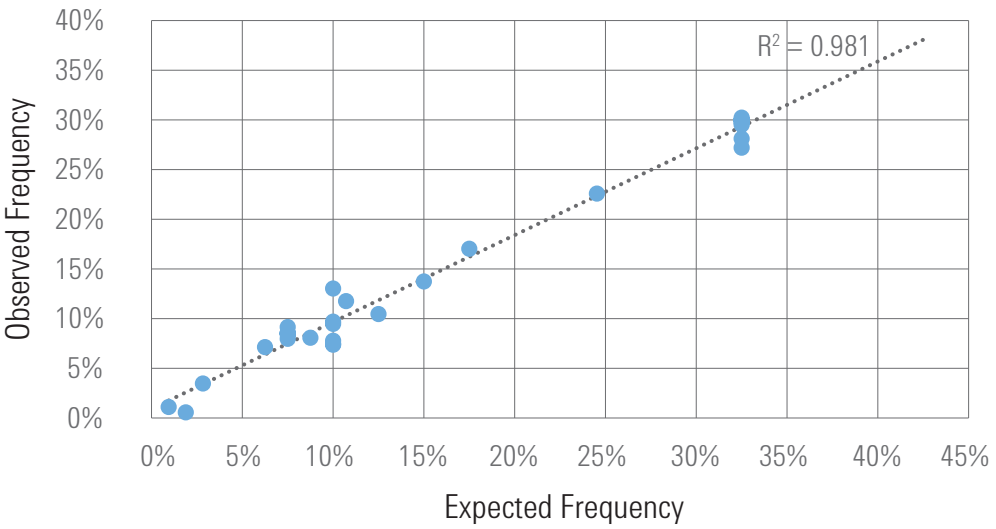


Figure 3. Detect down to 1% allele frequency. SureSelect$^{XT\ HS}$ generated library sequenced to 3000X depth produces high correlation of observed to expected frequency down to as little as 1% VAF (HD200 Quantitative Multiplex Reference Standard and ClearSeq Comprehensive Cancer Panel).

Advantages

- Detect variants at a frequency of ≤ 1%
- Eliminate amplification and sequencing artifacts that limit the sensitivity of NGS
- Increase data quality and elevate confidence in sequencing results with as little as 10 ng of starting DNA

Molecular Barcodes

What are molecular barcodes?

Molecular barcodes are unique sequences of DNA that attach to each original DNA fragment in a given sample. These unique sequences of DNA can either contain randomized nucleotides, partially degenerate nucleotides or defined nucleotides. Attachment of the molecular barcodes to a DNA fragment results in a unique identifier assigned to each input molecule.

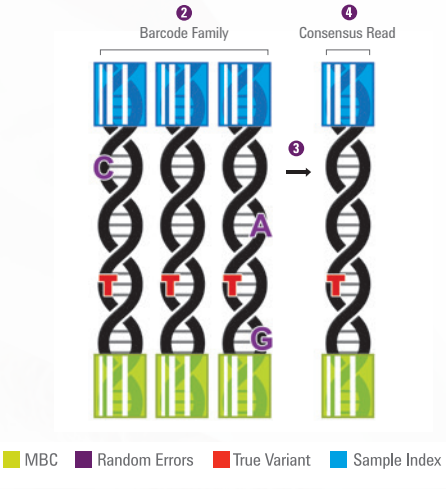
Basic molecular barcode (MBC) analysis

Step 1 Align reads

Step 2 Group read pairs to designed probes based on read start-stop position

Step 3 Group reads with an identical molecular barcode sequence for each probe

Step 4 Consolidate read information to one read per molecule (remove PCR duplicates)



Advantages

- Improve detection of low-frequency alleles by removing false positives
- Increase the ability to accurately call biologically relevant variants occurring at ≤ 1% variant allele frequency (VAF)

Why are molecular barcodes important?

Errors introduced during PCR amplification and sequencing increase the overall error rate of the original DNA template. These errors, or false positives, result in a decrease of sensitivity for real mutations that occur at low levels in a sample. This issue is especially relevant for more heterogeneous cell populations, such as certain tumor subpopulations. Molecular barcodes enable users to detect low-frequency mutations in template DNA molecules by removing false positives and providing error correction for more accurate variant calling.

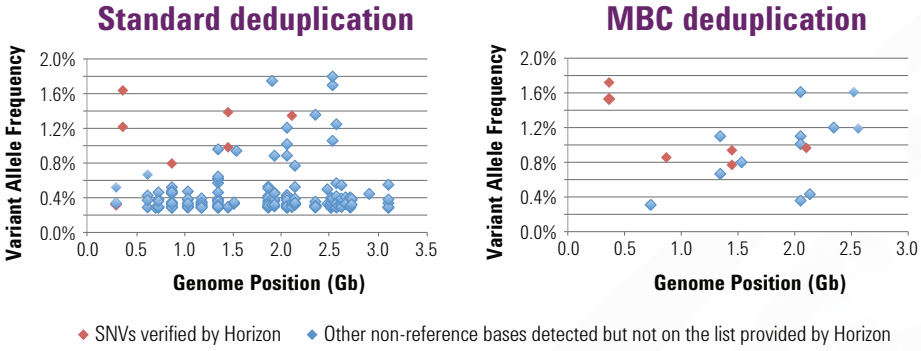
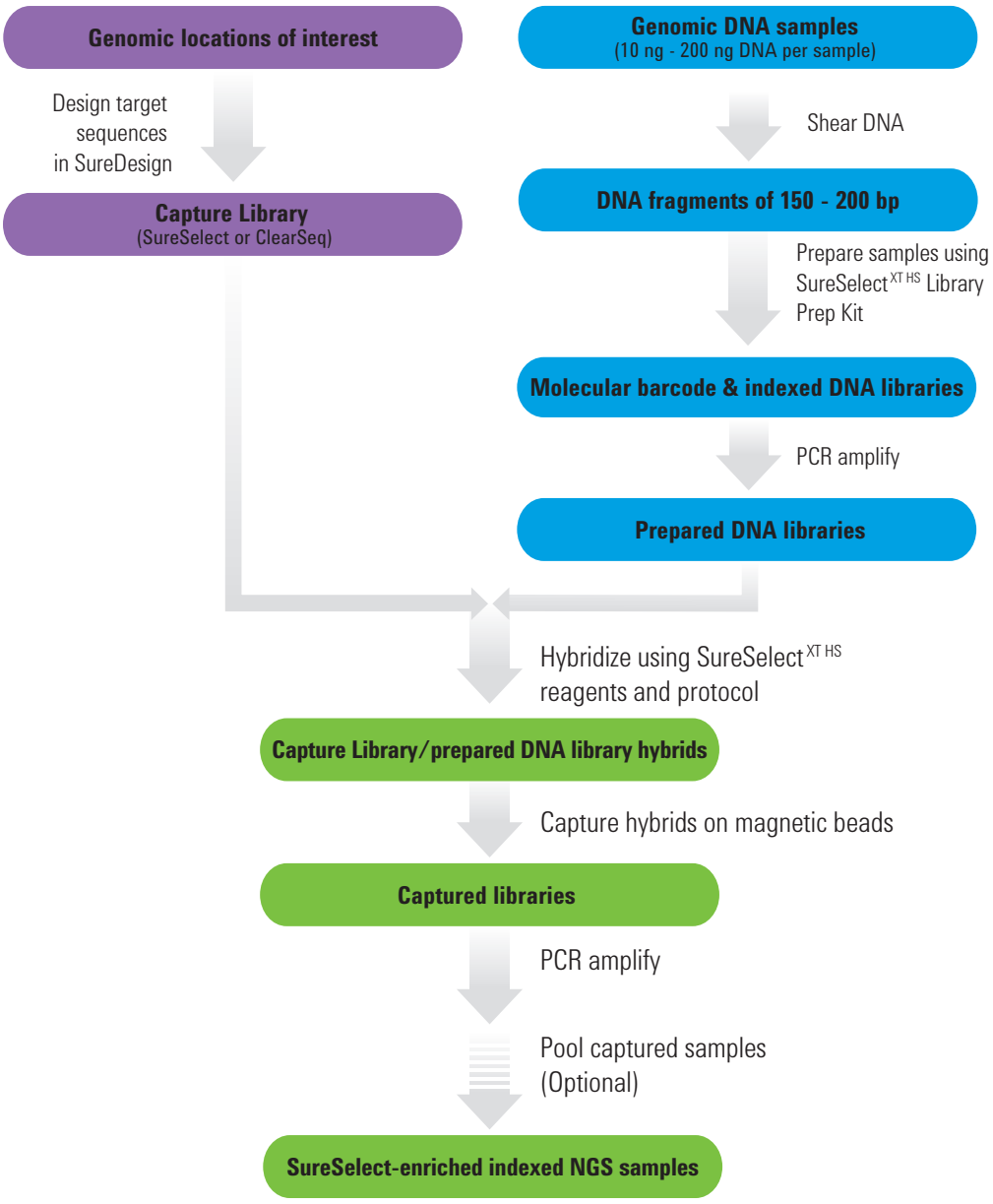


Figure 4. Improved data quality with molecular barcodes (MBC) analysis. Targeted sequencing of SureSelect$^{XT\ HS}$ libraries from Horizon cfDNA reference and a custom 164 kb SureSelect panel achieved a median coverage of 808(standard) / 906(MBC) after deduplication. 216 non-reference alleles were detected at a variant allele frequency of less than 2% with standard deduplication compared to 18 using the MBC. This represents a 92% reduction in false calls. Courtesy of Dr. L.J. Barber and Dr. M. Gerlinger, Centre for Evolution and Cancer, The Institute of Cancer Research, London, UK.

Streamlined Workflow

Expedite analysis with a streamlined workflow

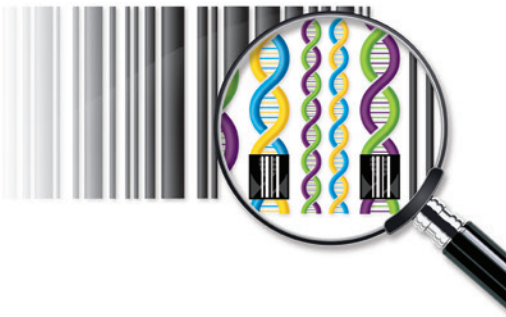
SureSelect^XT HS reduces the number of individual enzymatic steps, clean-up steps and sample transfers, allowing users to streamline and maintain library complexity, especially at lower input amounts (as little as 10 ng). Agilent delivers the fastest hybridization on the market. Our 90-minute hybridization, combined with more efficient processing with master-mixed reagents, enables users to go from samples to sequencing-ready libraries in one day. Additionally, the workflow supports deep multiplex sequencing for up to 32 samples in one pool.



Advantages

- Process samples faster and more efficiently with a 90-minute hybridization and master-mixed reagents
- Transform samples into sequencing-ready libraries in less than one day
- Streamline data analysis with the Agilent SureCall software

SureSelect Library Prep & Target Enrichment Solution



Agilent has a wide variety of library prep and target enrichment solutions that suit both cancer and constitutional applications. Starting with as little as 10 ng, or as much as 3 ug of DNA, there is a library prep and target enrichment solution that works seamlessly with a ClearSeq Panel, a SureSelect Exome or a Custom Panel.

SureSelect Library Prep Solutions for Cancer and Constitutional Applications

Product Name	SureSelect$^{XT\ HS}$	SureSelect^{XT} Low Input	SureSelect^{XT}	SureSelect^{XT2}	SureSelect^{QXT}
DNA Input	10 ng - 200 ng	10 ng - 200 ng	200 ng - 3 µg	100 ng - 1 µg	50 ng
Turnaround Time	8 hr	8 hr	1.5 day	1.5 day	7 hours
Covaris Needed	Yes	Yes	Yes	Yes	No
Library Complexity	Highest	Highest	High	Medium	Medium
Unique Features	FFPE optimized Molecular barcodes Mastermixed reagents Samples indexed prior to capture eliminating concern of cross sample contamination	FFPE optimized Molecular barcodes (optional) Mastermixed reagents Samples indexed prior to capture eliminating concern of cross sample contamination	Compatible with FFPE samples Robust variant identification	Pre-capture pooling Mastermix reagents	Transposase-based Mastermix reagents Whole genome sequencing and target enrichment compatible For intact DNA only
Key Benefits	High sensitivity for ≤1% VAF	192 sample indexes	High-complexity libraries for rare allele detection	Cost-effective	Covaris-free workflow

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