Data Sheet

Agilent
Trusted Answers

SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit

A fast, simple and reliable enzymatic fragmentation workflow for NGS library preparation

Key features

– Fragment 10-200 ng of DNA input randomly with a single workflow

– Performs robustly across various sample quality and GC content, including FFPE

– Maximize yield with high efficiency and minimum sample loss

– Works with DNA in water or standard TE buffers

Overview

The new SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit is an endonuclease-based enzymatic shearing method which generates random DNA fragments from samples of varying quality and input amounts using a single protocol. It enables the enzymatic fragmentation workflow for the SureSelect$^{XT\ HS}$ and SureSelect^{XT} Low Input Reagent Kits. Unlike mechanical shearing, there is no upfront investment in instrumentation. This module provides a fast, streamlined and highly reproducible solution for high throughput applications.

Easy single workflow

The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit is optimized for the full range of DNA inputs (10-200 ng), various GC content, 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 (Figure 1). For DNA samples with different qualities, consistent fragmentation patterns can be observed (Figure 2). It also produces normalized coverage patterns similar to mechanical shearing across a wide range of GC content (Figure 3). This single protocol enables an easy and streamlined NGS library preparation workflow.

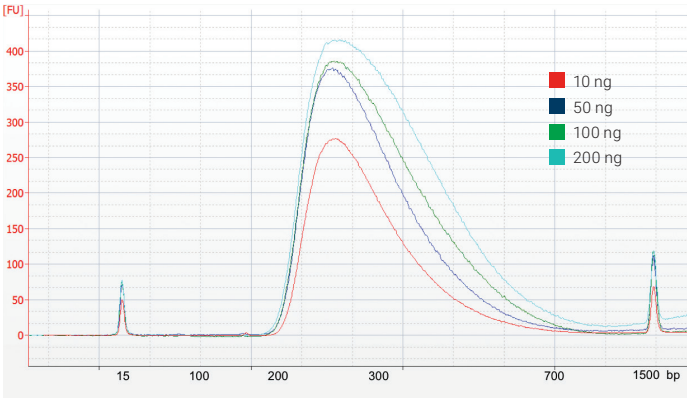


Figure 1. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit generates highly consistent DNA fragmentation profiles with various DNA input amounts. 10 to 200 ng of DNA extracted from fresh frozen lung tumor sample was sheared with the SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit for 15 minutes at 37°C. Libraries were constructed using the SureSelect$^{XT\,HS}$ Reagent Kits and analyzed with the Agilent DNA 1000 Bioanalyzer assay.

Superior performance compared to mechanical shearing

One major advantage of enzymatic shearing over mechanical shearing is higher yield and better quality of the library due to minimum sample loss. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit consistently generates higher library yield compared to mechanical shearing with 10 ng of DNA input in 3 different sample types (Figure 4). In terms of library quality, this kit shows equivalent or better coverage (Figure 5) and higher complexity (Figure 6) in fresh frozen and FFPE samples.

Compatible with DNA in water and common buffers

The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit is insensitive to different DNA buffer conditions. It eliminates the purification or neutralization steps required by other enzymatic shearing methods. Highly similar DNA fragmentation profiles can be generated with DNA stored in Tris, 0.1X TE (10 mM Tris, 0.1 mM EDTA, pH 7.5) and 1X TE (10 mM Tris, 1 mM EDTA, pH 7.5) (Figure 7).

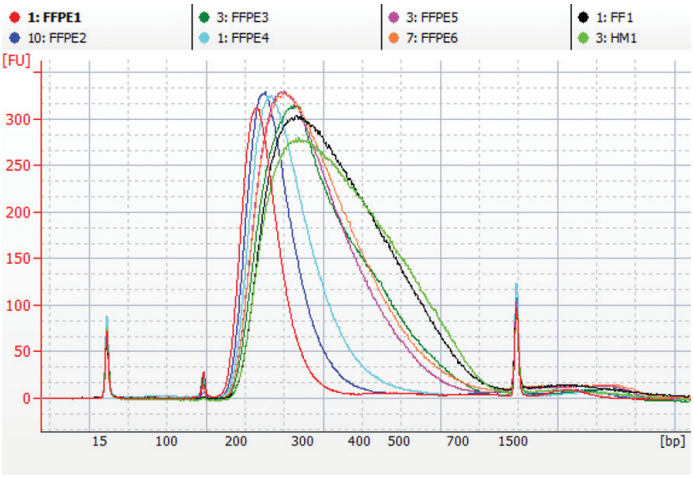


Figure 2. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit produces similar DNA fragmentation patterns from DNA samples with various quality. 10 ng of DNA extracted from HapMap, fresh frozen tissue and FFPE of varying quality (See Table I for detailed information.) was sheared with the SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit for 15 minutes at 37°C. The qualities of all samples were determined by the Agilent TapeStation (DIN) and FFPE samples were determined by the Agilent NGS FFPE QC Kit (ddCq value). Libraries were constructed using the SureSelect$^{XT\,HS}$ Reagent Kits and analyzed with the Agilent DNA 1000 Bioanalyzer assay.

Table I. Samples and corresponding DNA quality for Figure 2.

Sample	Tissue	Source	ddCq	DIN
FFPE1	Breast, normal	FFPE	7.0	2.0
FFPE2	Gastric, normal	FFPE	3.3	1.5
FFPE3	Breast, tumor	FFPE	0.6	3.4
FFPE4	Prostate, normal	FFPE	2.9	1.9
FFPE5	Liver, normal	FFPE	1.2	2.9
FFPE6	Lung, normal	FFPE	0.2	4.8
FF1	Ovary, normal	fresh frozen tissue	--	7.7
HM1	NA18997	HapMap	--	8

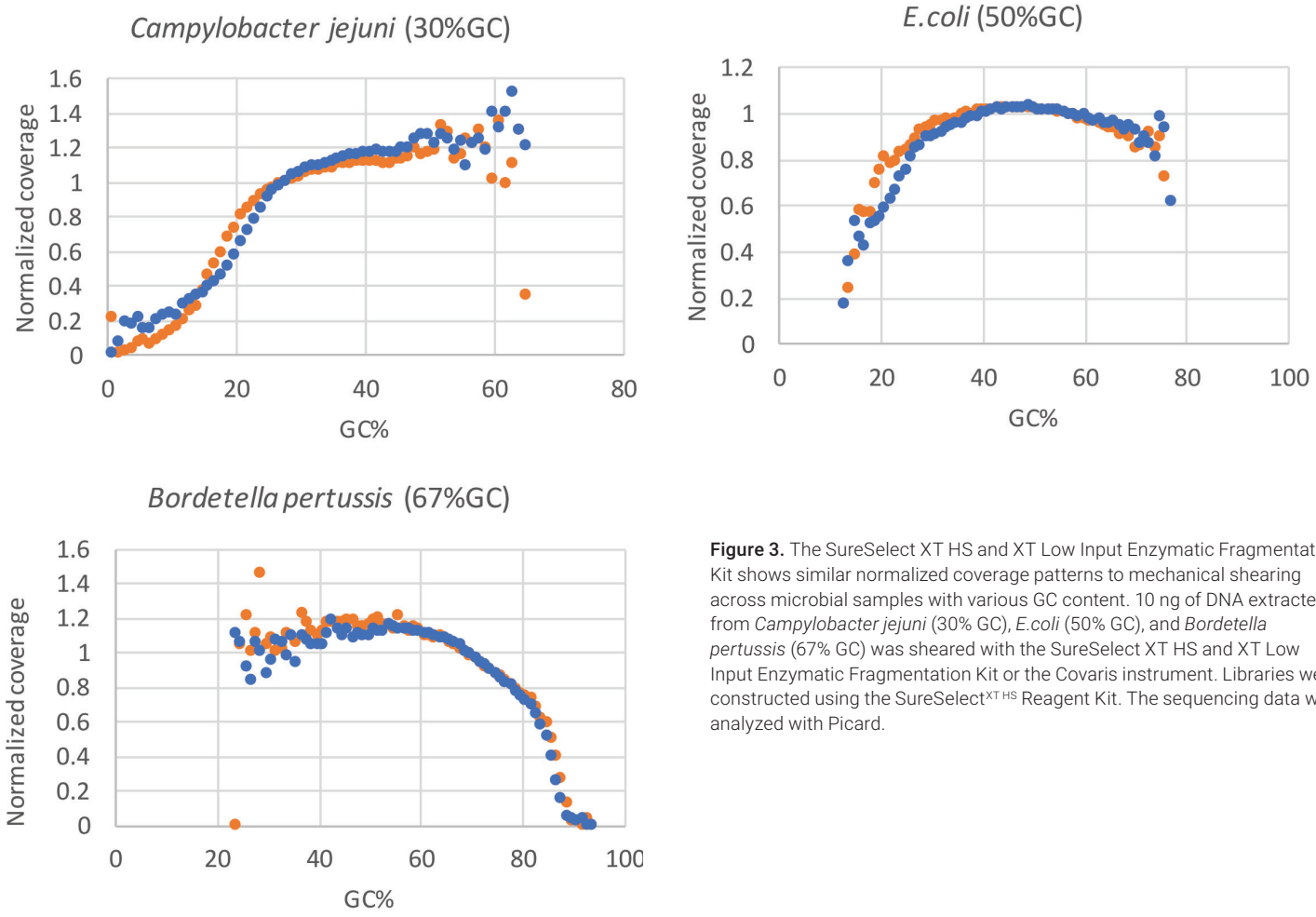


Figure 3. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit shows similar normalized coverage patterns to mechanical shearing across microbial samples with various GC content. 10 ng of DNA extracted from *Campylobacter jejuni* (30% GC), *E.coli* (50% GC), and *Bordetella pertussis* (67% GC) was sheared with the SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit or the Covaris instrument. Libraries were constructed using the SureSelect$^{XT\ HS}$ Reagent Kit. The sequencing data was analyzed with Picard.

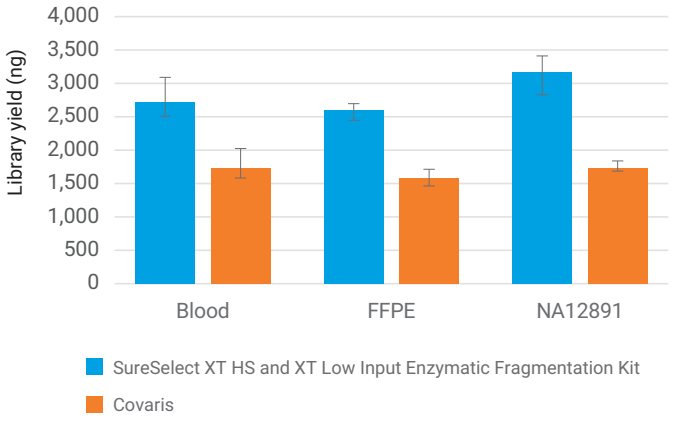


Figure 4. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit produces a higher library yield compared to a mechanical shearing workflow. 10 ng of DNA extracted from blood, skin FFPE (DIN = 3.2, ddCq = 1.8) and HapMap (NA12891) was sheared with the SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit or the Covaris instrument. The qualities of all samples were determined by the Agilent TapeStation (DIN) and FFPE samples were determined by the Agilent NGS FFPE QC Kit (ddCq value). Libraries were constructed using the SureSelect$^{XT\ HS}$ Reagent Kit. Library yield was measure by the Agilent TapeStation D1000 ScreenTape Assay.

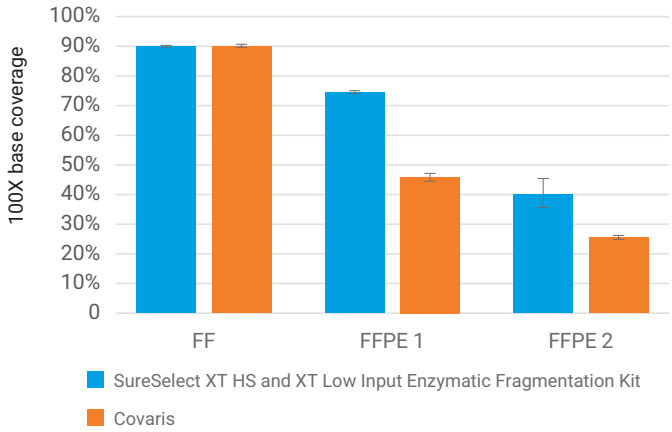


Figure 5. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit produces better 100x base coverage compared to a mechanical shearing workflow. 10 ng of DNA extracted from fresh frozen and FFPE samples was sheared with the Kit or the Covaris instrument. FF and FFPE 1 (DIN = 2.7, ddCq = 1) are uterus samples. FFPE 2 is larynx tumor sample (DIN = 2.3, ddCq = 2). The qualities of all samples were determined by the Agilent TapeStation (DIN) and FFPE samples were determined by the Agilent NGS FFPE QC Kit (ddCq value). Library constructions and target enrichments were performed using the SureSelect$^{XT\ HS}$ Reagent Kit and the ClearSeq Comprehensive Cancer Panel. Libraries were sequenced (2X100 bp) on Illumina HiSeq 2500. Reads were mapped to hg19, normalized to 1000X raw sequencing depth and 100X base coverage was then determined.

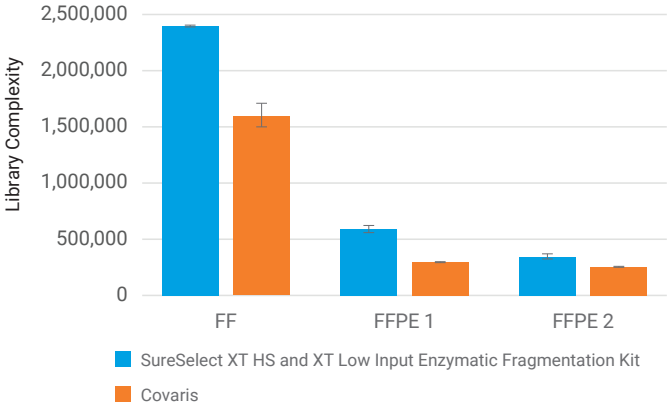
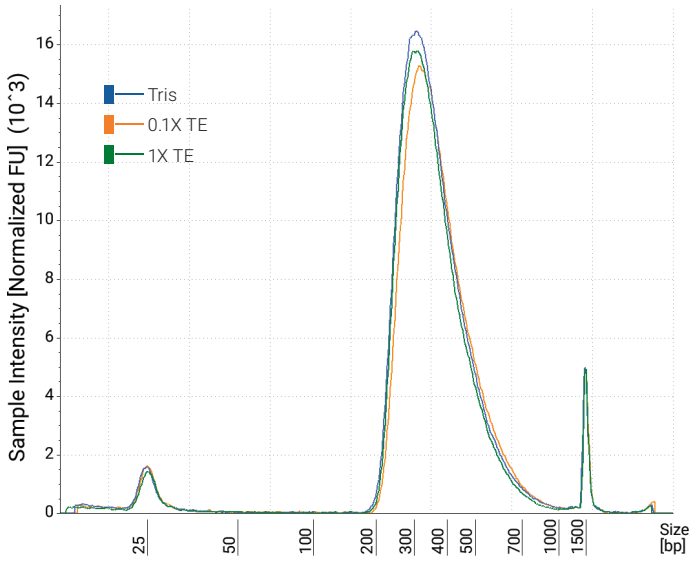


Figure 6. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit produces higher library complexity (HS-library size) compared to a mechanical shearing workflow. 10 ng of DNA extracted from fresh frozen and FFPE samples was sheared with the SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit or the Covaris instrument. FF and FFPE 1 (DIN = 2.7, ddCq = 1) are uterus samples. FFPE 2 is larynx tumor sample (DIN = 2.3, ddCq = 2). The qualities of all samples were determined by the Agilent TapeStation (DIN) and FFPE samples were determined by the Agilent NGS FFPE QC Kit (ddCq value). Library constructions and target enrichments were performed using the SureSelect$^{XT\ HS}$ Reagent Kit and the ClearSeq Comprehensive Cancer Panel. Libraries were sequenced (2X100 bp) on the Illumina HiSeq 2500. Reads were mapped to hg19, normalized to 100X raw sequencing depth and HS-library size was then determined.

A.



B.

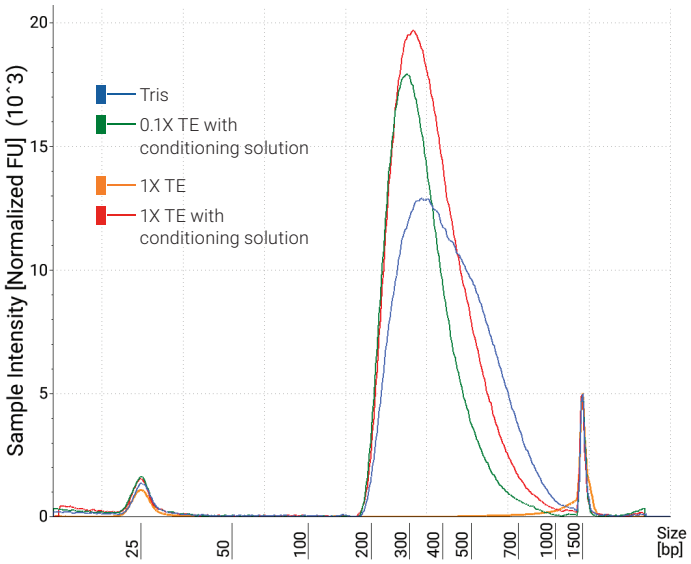


Figure 7. The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit works with different DNA buffers and shows highly similar DNA fragmentation profiles. 10 ng of DNA extracted from fresh frozen tissue was used as input. It was stored in Tris, 0.1X TE (10 mM Tris, 0.1 mM EDTA, pH 7.5) and 1X TE (10 mM Tris, 1 mM EDTA, pH 7.5). DNA samples were sheared and library-prepped with: **A.** The SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit and the SureSelect$^{XT\ HS}$ Reagent Kit. **B.** The Kapa HyperPlus Kit. Tris and one of the 1X TE samples were sheared without conditioning solution. 0.1X TE and the other 1X TE samples were sheared with conditioning solution. For DNA stored in 1X TE and sheared without conditioning solution, there was complete inhibition of fragmentation observed (orange line). Fragmented DNA was analyzed with the Agilent TapeStation D1000 ScreenTape Assay.

Ordering information

Product Name	Description
5191-4079	SureSelect XT HS Enzymatic Fragmentation Kit, 16 Reactions
5191-4080	SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit, 96 Reactions

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