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Somatic Variant Detection in FFPE Samples with Nextera™ Flex for Enrichment

Fast and flexible library prep that provides high-quality data and DNA variant detection from FFPE samples.

At a Glance

Challenge

A key step in the preparation of FFPE DNA for NGS is fragmentation into optimal molecular sizes. Mechanical shearing has been the preferred method, but requires specialized equipment and is challenging to automate.

Solution

Nextera Flex for Enrichment uses enrichment bead-linked transposomes to mediate simultaneous DNA fragmentation and tagging for preparation of sequencing-ready libraries from FFPE DNA.

Benefits

Nextera Flex for Enrichment produces high-quality sequencing data and sensitive detection of somatic variants with real-world and reference FFPE samples.

Introduction

Next-generation sequencing (NGS) confers the ability to examine a broad array of molecular variants, and enables cancer research by significantly increasing the breadth, sensitivity, and specificity of information obtainable within a single assay. As an increasing number of research and clinical laboratories embrace the power of NGS, Illumina offers simple, comprehensive solutions for producing high-quality data necessary for accurate analysis.

Formalin-fixed, paraffin-embedded (FFPE) tissues are often the main or only source of material for tumor analysis. However, the formalin fixation and paraffin embedding process impacts nucleic acid quality by fragmenting, cross-linking, and introducing damage through chemical modifications. Robust workflows are required to process FFPE samples for NGS analysis.

DNA fragmentation

A key step in the preparation of FFPE DNA for NGS is fragmentation into molecule sizes that are optimal for sequencing. Until recently, mechanical shearing using ultrasonication has been a preferred method for FFPE DNA fragmentation prior to NGS, given its robust performance and low sequence bias.[1,2] However, sonication requires specialized equipment and can be challenging to automate.

The Nextera Flex for Enrichment solution combines versatile and simple library prep and enrichment functionality for targeted enrichment and exome sequencing applications. It offers the fastest total workflow time in the Illumina portfolio, along with extraordinary flexibility for input type, input amount, and a wide range of supported enrichment sequencing applications encompassing custom panels, fixed panels, and whole-exome sequencing from Illumina or third-party vendors.[3]

This application note demonstrates the exceptional performance of Nextera Flex for Enrichment in production of high-quality sequencing data and sensitive detection of somatic variants with real-world FFPE tumor samples. Nextera Flex for Enrichment shows equivalent or better results, as compared to traditional enrichment methods that use mechanical DNA fragmentation followed by ligation-based adapter preparation.

Methods

Samples and DNA extraction

A combination of real-world human tissue-derived FFPE samples and formalin-compromised engineered cell line reference standards from Horizon Discovery (HD) were obtained for analysis (Table 1). DNA was extracted from FFPE samples using the QIAGEN AllPrep DNA/RNA FFPE Kit (QIAGEN, Cat No. 80234), quantified, and qualified as described in the Nextera Flex for Enrichment Reference Guide.[4,] As part of the Nextera Flex for Enrichment protocol, the experiment started with 50 ng of DNA input into library preparation tagmentation. After library preparation was completed, 500 ng of each pre-enriched library was pooled by mass at single plexity for enrichment. For TruSeq™ DNA Library Prep for Enrichment, DNA was sheared with a Covaris Focused-Ultrasonicator, as described in the TruSeq DNA Exome Reference Guide.[6]

Table 1: Samples for analysis with Nextera Flex for Enrichment

Sample ID	Sample type	ΔCq	DNA input
5836	FFPE human tissue	4.49	50 ng
5843	FFPE human tissue	3.32	50 ng
5845	FFPE human tissue	4.26	50 ng
5848	FFPE human tissue	4.98	50 ng
5857	FFPE human tissue	2.6	50 ng
HD 803	Formalin-treated, engineered cell line	—	50 ng
HD 799	Formalin-treated, engineered cell line	—	50 ng
HD 730	High-quality, engineered cell line	—	50 ng

Library preparation and sequencing

Sequencing libraries were prepared with the Nextera Flex for Enrichment or TruSeq DNA Library Prep for Enrichment pre-enrichment library prep reagents and one of three custom enrichment probe sets (Table 2). Libraries were sequenced on the NextSeq™ 550 System at a read length 2 × 125 bp.

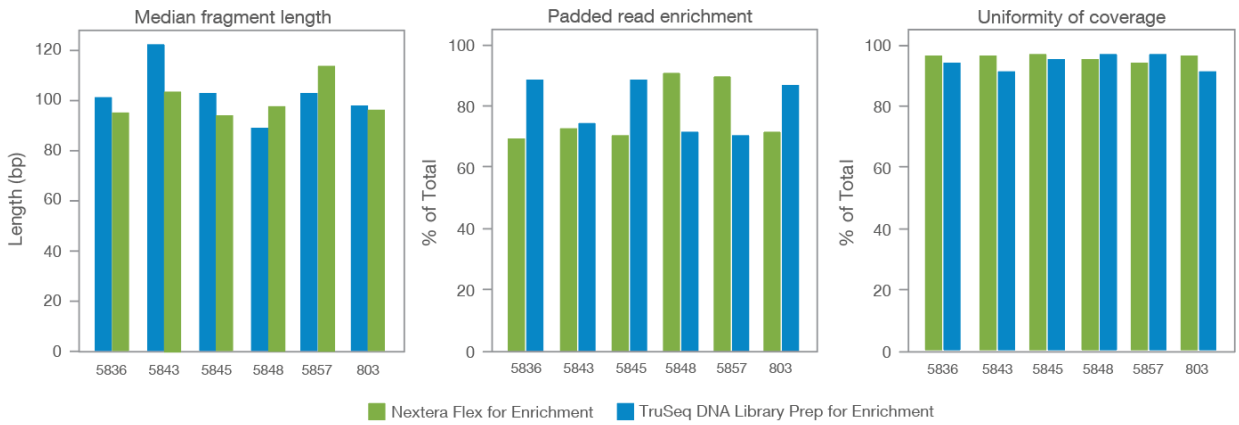


Figure 1: High-quality sequencing data—Nextera Flex for Enrichment produces consistent median fragment lengths (left) post-tagmentation, and provides high padded read enrichment (center) and coverage uniformity (right), compared to TruSeq DNA Library Prep for Enrichment with ultrasonication. Data represents example comparison data. Actual performance specifications may vary.

Table 2: Enrichment probe sets

Panel	Panel size	Reads per sample
Panel A	0.05 Mb (small)	0.5 M
Panel B	12 Mb (large)	80 M
Panel C	2 Mb (mid)	60 M

Data analysis

Sequencing runs were demultiplexed and converted to FASTQ files in BaseSpace™ Sequence Hub. Downstream analysis was performed with the Enrichment App v3.1.0 for alignment to reference sequences and variant identification.

Results

To demonstrate the exceptional performance of Nextera Flex for Enrichment in generating high-quality sequencing libraries from FFPE DNA, results were compared against TruSeq DNA Library Prep for Enrichment libraries prepared from mechanically fragmented FFPE DNA.

Equivalent fragmentation enables high-quality sequencing data

The Nextera Flex for Enrichment tagmentation reaction consistently fragmented FFPE DNA, resulting in median fragment lengths similar to that produced by ultrasonication (Figure 1). In addition, Nextera Flex for Enrichment provided high padded read enrichment and coverage uniformity for the real-world human tissue FFPE samples that were assayed, with metrics comparable to TruSeq DNA Library Prep for Enrichment (Figure 1).

Precise variant calling in real-world FFPE samples and reference standards

To demonstrate assay capabilities, real-world human tissue FFPE samples were evaluated using Nextera Flex for Enrichment or TruSeq DNA Library Prep for Enrichment and the Panel C probe set for variant calling accuracy. Results show significant correlation between observed variant frequencies with Nextera Flex for Enrichment and TruSeq DNA Library Prep for Enrichment for each sample (Figure 2).

In addition, HD formalin compromised reference standards were evaluated using Nextera Flex for Enrichment for variant calling accuracy. Results show significant correlation between observed and expected variant allele frequencies (VAF), with 100% of variants detected for all standards at ≥ 5% variant frequency (Figure 3).

Accurate identification of somatic variants

To further demonstrate the equivalent performance of Nextera Flex for Enrichment compared to TruSeq DNA Library Prep for Enrichment, somatic variant calling was evaluated by both methods. Results show that Nextera Flex for Enrichment identified the same variants as TruSeq DNA Library Prep for Enrichment (Figure 4).

To access BaseSpace Sequence Hub public data sets from this study, go to basespace.illumina.com/projects/89560471 (requires sign-in)

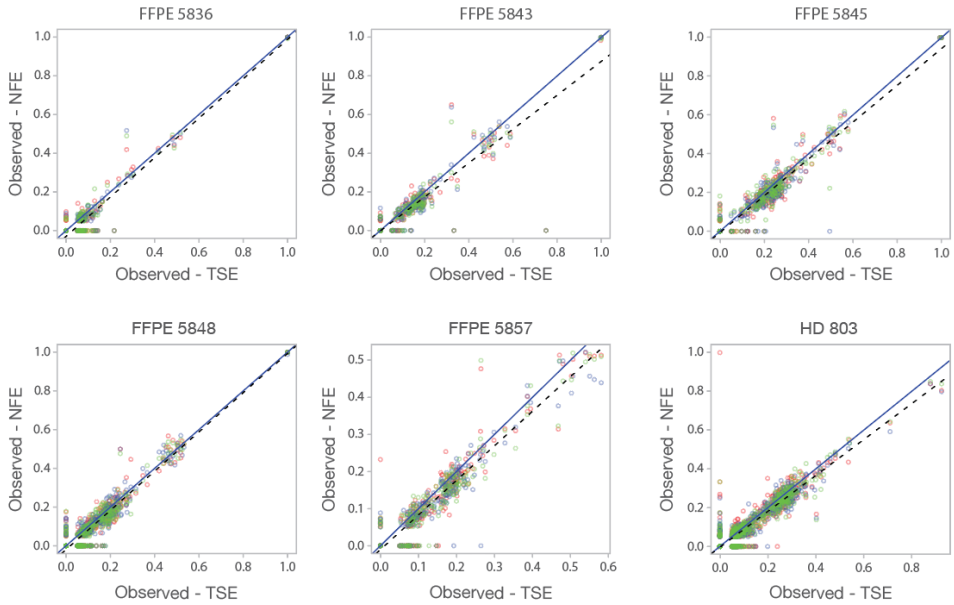


Figure 2: Somatic variant calling in FFPE samples—Observed variant frequency with Nextera Flex for Enrichment (Observed – NFE) was plotted against observed variant frequency with TruSeq Enrichment (Observed – TSE) for each sample (Panel C).

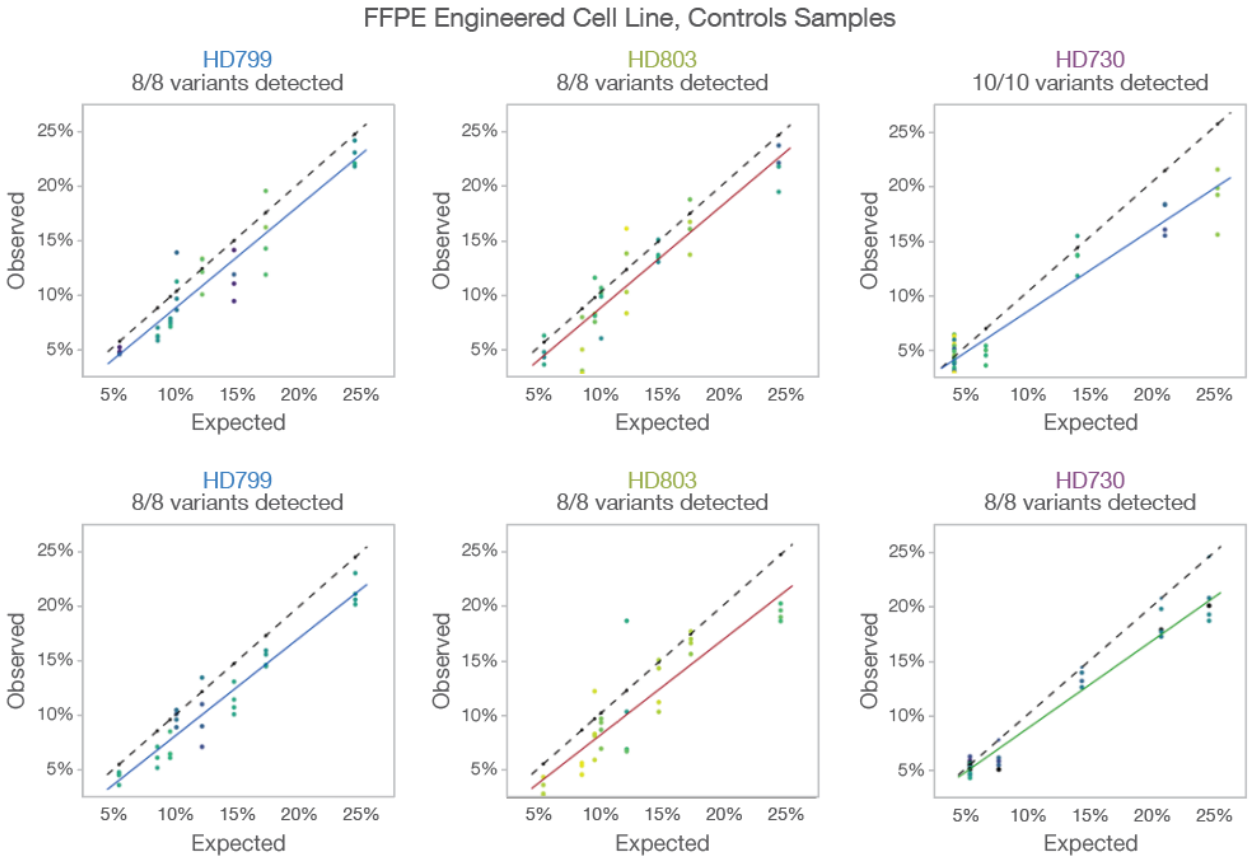
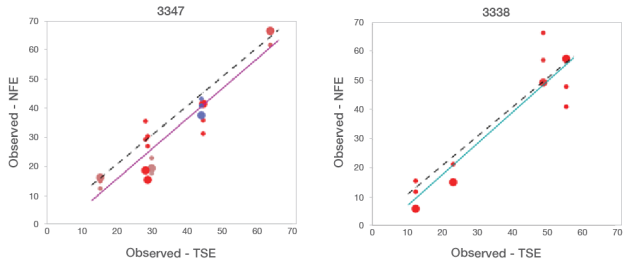


Figure 3: Somatic variant calling in reference standards—Significant correlation was seen between observed and expected variant frequency for HD reference standards, with 100% of variants detected. Libraries were prepared with Nextera Flex for Enrichment and Panel B (top) and Panel A (bottom) probe sets.



Sample ID	Gene	Type	Variant	Position
3347	*APC*	SNV	NP_000029.2:p.Gly1428Ter	112175573
3347	*APC*	SNV	NP_000029.2:p.Pro1432Leu	112175586
3347	*ESR1*	SNV	NP_001116214.1:pArg555His	152419977
3347	*GNAQ*	SNV	NP_002063.2:p.Tyr101Ter	80537095
3347	*GNAQ*	SNV	NP_002063.2:p.Tyr101Ter	80537112
3347	*RB1*	SNV	NP_000029.2:p.Thr96Ser	49027222
3347	*TP53*	SNV	NP_000537.3:p.Tyr220Cys	7578190
3338	*MSH2*	SNV	NP_000242.1:p.Ile169Val	47637371
3338	*GNAQ*	SNV	NP_002063.2:p.Tyr101Ter	80537095
3338	*GNAQ*	SNV	NP_002063.2:p.Tyr101Ter	80537112
3338	*RET*	SNV	NP_066124.1:p.Asp489Asn	43606856

Figure 4: Identification of somatic variants—Somatic variant detection was evaluated in two real-world FFPE samples with Nextera Flex for Enrichment (Observed - NFE) and TruSeq DNA Library Prep for Enrichment (Observed - TSE). The same variants were identified with both methods for both samples (table).

Summary

Preparing high-quality libraries from fragmented FFPE DNA is a key factor in the performance of any NGS-based panel. This application note demonstrates the exceptional performance of Nextera Flex for Enrichment in preparing sequencing-ready libraries from FFPE DNA that produce accurate data for downstream analyses such as somatic variant calling. When evaluated with both real-world FFPE samples and formalin compromised reference standards, Nextera Flex for Enrichment showed similar performance to, and significant correlation with, the TruSeq DNA Library Prep for Enrichment protocol. These results support Nextera Flex for Enrichment as a viable alternative to traditional mechanical fragmentation methods in the preparation of FFPE DNA libraries for NGS.

Learn More

To learn more about Nextera Flex for Enrichment, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/nextera-flex-enrichment.html

References

1. Marosy BA, Craig BD, Hetrick KN, et al. Generating exome enriched sequencing libraries from formalin-fixed, paraffin-embedded, tissue DNA for next generation sequencing. *Curr Protoc Hum Genet*. 2017;92:18.10.1-18.10.25.
2. Haile S, Pandoh P, McDonald H, et al. Automated high throughput nucleic acid purification from formail-fixed paraffin embedded tissue samples for next generation sequence analysis. *PLoS One*. 2017;12 (6):e0178706.
3. Illumina (2018) Nextera Flex for Enrichment Data Sheet. Accessed February 2019.
4. Illumina (2019) Nextera Flex for Enrichment Reference Guide. Accessed February 2019.
5. Illumina (2011) Infinium HD FFPE QC Assay Protocol. Accessed February 2019.
6. Illumina (2019) TruSeq DNA Exome Reference Guide. Accessed February 2019.

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