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TECHNICAL NOTE

Enzymatic fragmentation as an alternative to ultrasonic shearing in the TruSight™ Oncology 500 v2 DNA workflow

Evaluation of NEBNext UltraShear enzymatic fragmentation for library quality and variant detection in FFPE DNA samples

Comparable sequencing performance

NEBNext UltraShear fragmentation delivers sequencing quality comparable to ultrasonic shearing

Consistent variant detection

Variant calls show high concordance, even at low DNA input

Flexible workflow

Enzymatic fragmentation supports automation and eliminates the need for ultrasonic equipment

Introduction

In assays that enable comprehensive genomic profiling (CGP) such as TruSight Oncology 500 v2, consistent DNA fragmentation is critical for achieving uniform coverage across hundreds of genes. Fragmentation uniformity can improve detection of low-frequency variants, particularly in formalin-fixed, paraffin-embedded (FFPE) tissue samples where DNA quality can be compromised.[1,2] Focused ultrasonication is widely used for acoustic shearing of DNA in next-generation sequencing (NGS) workflows, as it produces reproducible fragment sizes with low sequence bias.[3,4] However, ultrasonication methods require dedicated capital equipment and are not easily integrated into automated workflows.[3,5]

Enzymatic fragmentation offers an alternative method for DNA shearing that uses a sequence-independent endonuclease step to generate appropriately sized DNA fragments.[6] This approach can be performed in the same plate-based format as downstream library preparation steps, enabling integration with automated liquid-handling systems while eliminating the need for dedicated ultrasonication equipment.[7] Comparative studies have shown that enzymatic fragmentation yields sequence quality and insert size distributions similar to those obtained with acoustic shearing.[8]

This technical note describes the use of the fragmentation enzyme NEBNext UltraShear within the TruSight Oncology 500 v2 DNA workflow. Performance was evaluated in terms of library quality and variant detection in FFPE DNA. The results provide practical guidance for laboratories seeking to streamline workflows, align with automation, or avoid purchasing dedicated ultrasonication equipment.

Methods

Samples and DNA input

Genomic DNA was isolated from commercially sourced FFPE samples using the QIAGEN AllPrep DNA/RNA FFPE Kit (QIAGEN, Catalog no. 80234) or the QIAamp DNA FFPE Advanced Kit (QIAGEN, Catalog no. 56604). Samples with a delta Cq (ΔCq) of approximately 1, 4–5, and 6, as determined by qPCR using the Applied Biosystems Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, Catalog no. 4368577) with primers designed for a 204 bp amplicon, were selected for the study (Table 1). Reference samples that were characterized contained 3–5 previously characterized mutations at variant allele frequencies (VAFs) of approximately 5% or 10%. DNA input amounts evaluated for each fragmentation method were 30 ng (recommended) and 10 ng (minimum). A control sample (ODC3) containing a defined set of known variants at specified variant allele frequencies was included in parallel for each fragmentation method and DNA input level.

Table 1: FFPE DNA sample characteristics

Sample no.	ΔCq	Tissue	Variant type	Gene	% VAF
1	4.334	Bladder	SNV	*PIK3CA*	10.593
				PRKDC	5.14
2	4.984	Lung	SNV	*CDH1*	5.263
				SDHA	10.795
3[a]	1.067	Lung	–	–	–
4[a]	4.088	Lung	–	–	–
5[a]	6.025	Lung	–	–	–

a. Uncharacterized sample.

FFPE, formalin-fixed, paraffin-embedded; SNV, single nucleotide variant; VAF, variant allele frequency.

Fragmentation methods

Focused ultrasonication was performed using the Covaris E220 focused-ultrasonicator (Covaris, Part no. 500239) as described in the TruSight Oncology 500 v2 Product Documentation.[1] Enzymatic fragmentation was performed with NEBNext UltraShear (New England Biolabs, Catalog no. M7634), as specified in Table 2, followed by SPRI bead cleanup (Illumina Purification Beads, Illumina, Catalog no. 20119944) at 1.8× bead-to-sample volume, according to the manufacturer's recommendations, before library preparation or fragment size analysis.

Library preparation and sequencing

Fragmented DNA was used in the TruSight Oncology 500 v2 DNA workflow as outlined in the TruSight Oncology 500 v2 Product Documentation,[1] replacing focused ultrasonication with NEBNext UltraShear enzymatic fragmentation. All subsequent steps, including end repair, adapter ligation, enrichment, and indexing, were unchanged. Libraries were sequenced using 101-bp paired-end reads on a NovaSeq™ 6000 System with an S2 flow cell.

Data analysis

DNA fragment size profiles were generated on an Agilent 4200 TapeStation system (Agilent, Catalog no. G2991BA), using Genomic DNA ScreenTape (Agilent, Catalog no. PN 5067-5365) for unfragmented DNA and High Sensitivity D5000 ScreenTape (Agilent, Catalog no. PN 5067-5592) for fragmented DNA, according to the manufacturer's instructions. DNA yields were quantified using the Qubit 1X dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific Catalog no. Q33231). Library quality metrics (coverage, on-target rate, complexity), QC pass rates, and % VAF were compared across methods and inputs. Samples were analyzed in triplicate to assess reproducibility.

Table 2: Optimized NEBNext UltraShear enzymatic fragmentation conditions

Thermal cycler			Reaction	
Step	**Temp.**	**Time**	**Reagent**	**Volume**
Lid	75°C	–	DNA sample	26 µl
Fragmentation	37°C	15 min	Buffer	14 µl
Denature	65°C	15 min	Enzyme	4 µl
Hold	4°C	∞	Total	44 µl

Results

DNA fragmentation

Fragment profiles were analyzed for 40 ng FFPE DNA samples representing three quality levels (ΔCq 1, ΔCq 4, and ΔCq 6, where lower ΔCq values indicate higher quality) (Figure 1). NEBNext UltraShear showed lower and more variable yields, ranging from approximately 21% to 41% across the quality levels tested. The small differences observed between enzymatic fragmentation and ultrasonication may be the result of enzyme sensitivity to FFPE processing.

Library and sequencing performance

Library and sequencing quality metrics, including contamination score, insert size, coverage, and assay-specific performance measures, were comparable between NEBNext UltraShear and focused ultrasonication across all FFPE DNA quality levels tested (Figure 2). For FFPE DNA of intermediate quality (ΔCq 4; Sample nos. 1 and 2, Table 1), metrics were consistent between methods when evaluated at 30 ng (recommended) and 10 ng (minimum) DNA input (Figure 3).

Variant call concordance

Variant call concordance between enzymatic fragmentation and ultrasonication was high across all FFPE DNA quality levels tested (ΔCq 1, ΔCq 4, and ΔCq 6). Only a small fraction of variants (< 1%) were unique to either fragmentation method (Figure 4).

Comparable variant detection was observed across both fragmentation methods at both 30 ng (recommended) and 10 ng (minimum) DNA input, indicating that reduced input did not affect detection concordance (Figure 5).

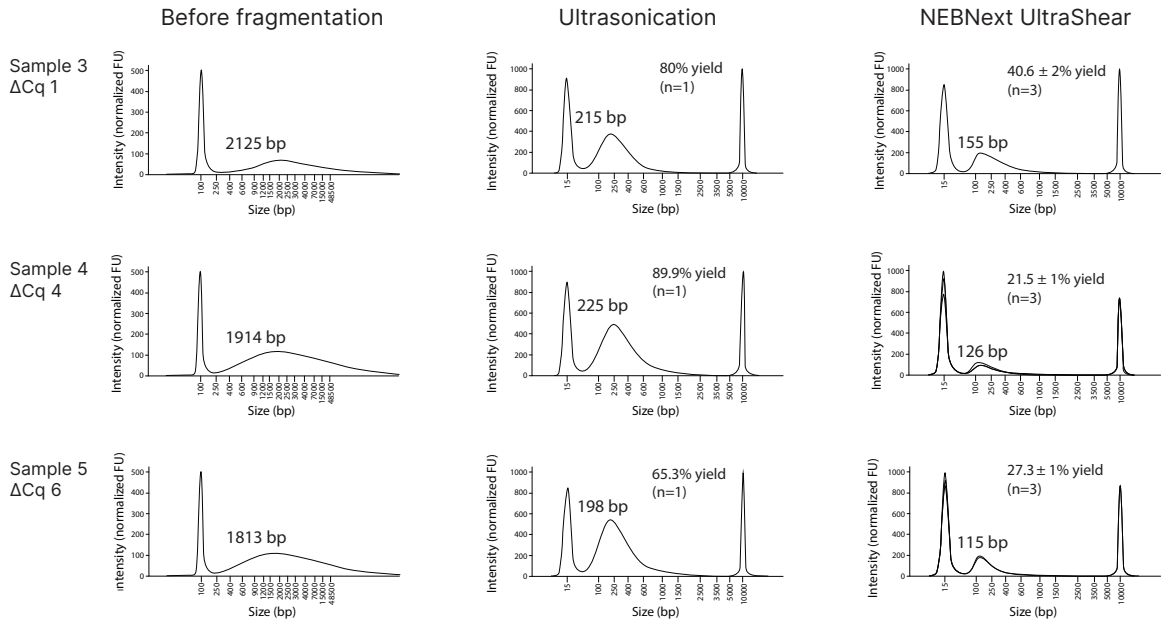

Figure 1: Fragment size profiles of FFPE DNA after ultrasonic or enzymatic fragmentation

DNA fragment profiles were obtained from FFPE DNA samples (40 ng) representing three quality levels, see Table 1); lower ΔCq values indicate higher quality). Values above peaks indicate the modal fragment size. NEBNext UltraShear generated similar fragment size profiles but with lower and more variable yields (21%–41%).

FU, fluorescence units.

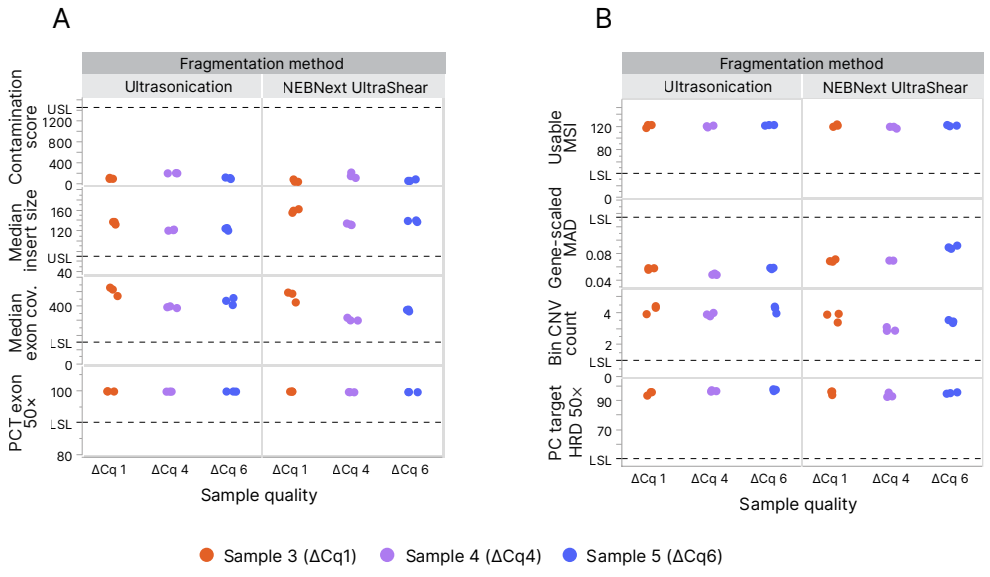

Figure 2: Library and sequencing performance metrics across FFPE DNA quality levels

Comparison of performance metrics for FFPE DNA (30 ng) at three quality levels (see Table 1), fragmented using focused ultrasonication or NEBNext UltraShear. (A) Contamination score, median insert size, median exon coverage, and percentage of exons with at least 50× coverage (PCT exon 50×). (B) Usable microsatellite instability (MSI) sites, gene-scaled median absolute deviation (MAD), median bin count for copy number variation (CNV) targets, and percentage of homologous recombination deficiency assay target regions with at least 50× coverage (PCT target HRD 50×). Each data point represents an independent library preparation. Dotted lines represent upper (USL) and lower (LSL) specification limits. Performance was comparable across both methods and quality levels.

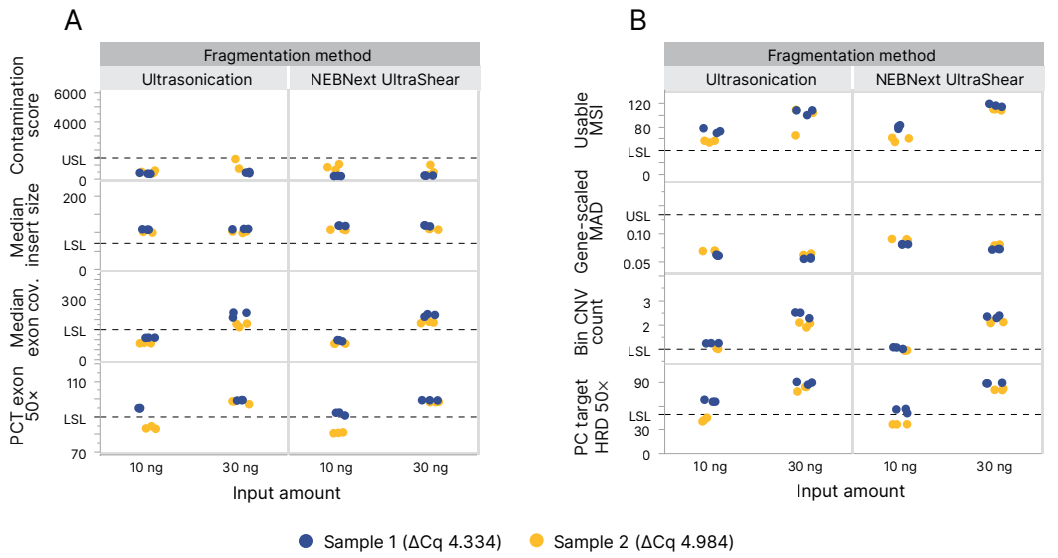

Figure 3: Library and sequencing performance metrics for ΔCq 4 FFPE DNA at two DNA input amounts

Comparison of performance metrics for FFPE DNA samples (see Table 1), fragmented using focused ultrasonication or NEBNext UltraShear for 10 ng (minimum) and 30 ng (recommended) DNA inputs. (A) Contamination score, median insert size, median exon coverage, percentage of exons with at least 50× coverage (PCT exon 50×). (B) Usable microsatellite instability (MSI) sites, gene-scaled median absolute deviation (MAD), median bin count for copy number variation (CNV) targets, and percentage of homologous recombination deficiency assay target regions with at least 50× coverage (PCT target HRD 50×). Each data point represents an independent library preparation, color-coded by sample. Dotted lines reprsent upper (USL) and lower (LSL) specification limits. Performance was consistent across methods and inputs.

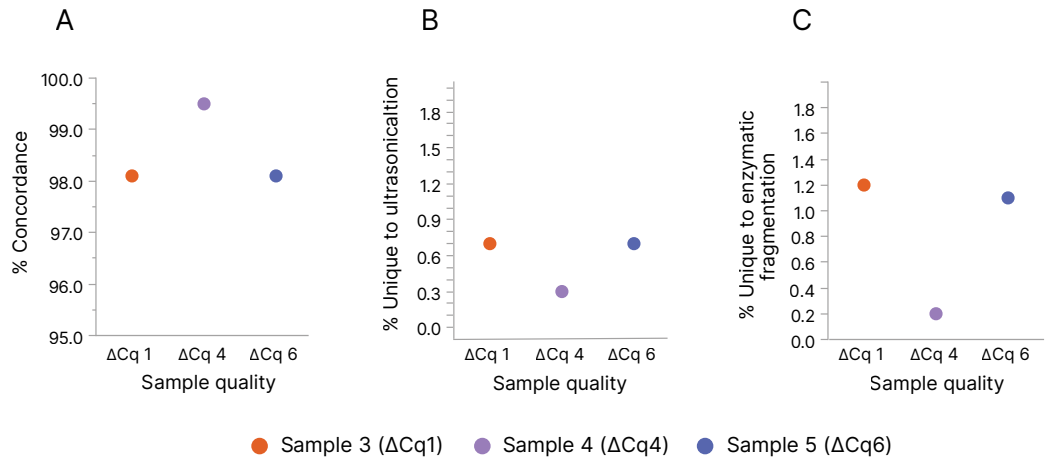

Figure 4: Variant call concordance for enzymatic fragmentation compared to focused ultrasonication

Variant call concordance between focused ultrasonication and NEBNext UltraShear for FFPE DNA samples (30 ng) of three quality levels, see Table 1; lower ΔCq indicates higher quality). (A) Percent concordance. (B) Percentage of variants unique to ultrasonication. (C) Percentage of variants unique to the enzymatic fragmentation method. Each data point represents the mean of three replicates.

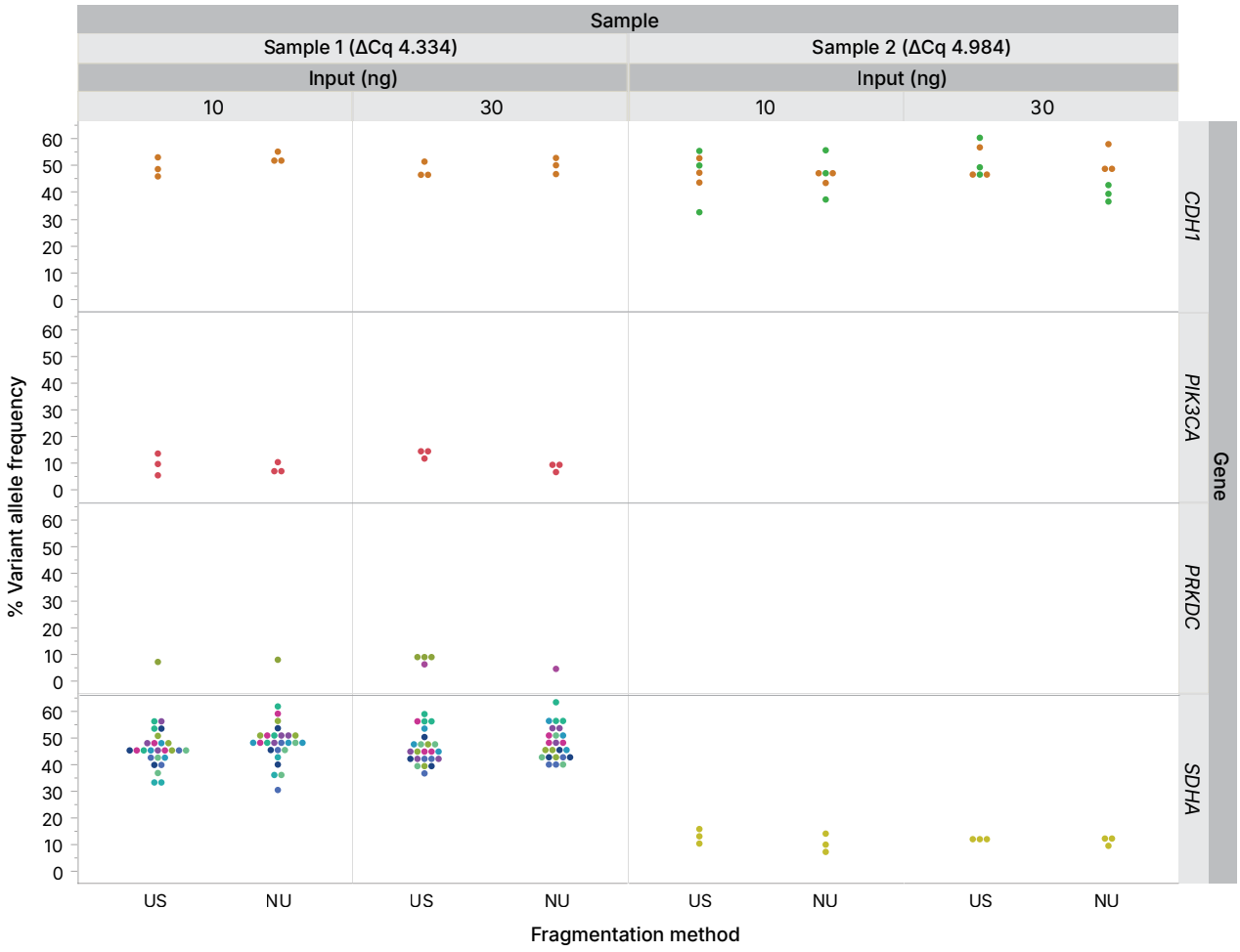

Figure 5: Comparable variant detection across fragmentation methods

Variants detected in ΔCq 4 FFPE DNA (Sample nos. 1 and 2, see Table 1) fragmented using focused ultrasonication (US) or NEBNext UltraShear (NU) at DNA inputs of 10 ng (minimum) and 30 ng (recommended). Each dot represents a detected variant, color-coded by COSMIC ID. Variant detection was consistent across fragmentation methods and DNA input amounts.

Summary

Enzymatic fragmentation using NEBNext UltraShear produced library and sequencing metrics comparable to those obtained using Covaris focused ultrasonic shearing in the TruSight Oncology 500 DNA v2 workflow. The enzymatic fragmentation method performed similarly to focused ultrasonication across FFPE DNA quality levels and DNA input amounts, with high variant call concordance and no loss in variant detection sensitivity at both the minimum and recommended input levels. These results indicate that enzymatic fragmentation can provide a viable alternative to acoustic shearing. This approach offers the potential for integration into automated workflows and reduces reliance on specialized ultrasonication equipment.*

* This document provides information for an application for Illumina technology that has been demonstrated internally and may be of interest to customers. This information is provided as-is and is not part of a commercialized Illumina workflow and is not accompanied by rights or warranties outside of those accompanying the Illumina consumables. Illumina products mentioned herein are for research use only unless marked otherwise. While customer feedback is welcomed, this application is not supported by Illumina Technical Support or Field Application Scientists.

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