Sequencing NEBNext® DNA Libraries with the Element Cloudbreak Freestyle™ Workflow

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INTRODUCTION

Advances in sequencing chemistries have resulted in the release of new instrumentation with a focus on lowering costs and reducing run times while also improving the quality of sequencing data. The Element Biosciences AVITI™ benchtop sequencer utilizes a novel Avidite Base Chemistry™ (ABC™) coupled with rolling circle amplification (RCA) to achieve cost-effective, high-accuracy, massively parallel sequencing.

What is Cloudbreak Freestyle?

The Element Cloudbreak Freestyle workflow expands the compatibility of the Element Biosciences AVITI sequencer to a breadth of applications by enabling the sequencing of third-party native libraries, without the need for a conversion step. Accordingly, existing workflows produce libraries that can be directly sequenced on the AVITI, spanning the range of NEBNext DNA Library Preparation product workflows.

Here, we demonstrate the compatibility and performance of libraries generated using a range of NEBNext DNA Library Prep Kits and Multiplex Oligos with the Element Cloudbreak Freestyle workflow— including the NEBNext UltraExpress® DNA and NEBNext UltraExpress FS DNA, Ultra™ II DNA and the Ultra II FS DNA, as well as the NEBNext UltraShear® FFPE DNA and FFPE DNA library prep kits.

NEBNEXT ULTRAEXPRESS DNA & NEBNEXT ULTRAEXPRESS FS DNA

The NEBNext UltraExpress products were developed to streamline library preparation, reducing hands-on and overall workflow time and improving sustainability by reducing plastic consumable usage. NEBNext UltraExpress DNA and FS DNA library prep workflows make quick work of DNA library prep from either fragmented or intact DNA, taking you from input DNA to library in under two hours. NEBNext UltraExpress workflows enable greater scalability, providing uniform conditions across the input material range, including a single adaptor dilution and PCR cycle number. When combined with the Element Cloudbreak Freestyle workflows, NEBNext UltraExpress library preparation kits represent fast, streamlined solutions to address a wide range of sequencing applications.

NEBNEXT ULTRA II DNA & NEBNEXT ULTRA II FS DNA

For samples of increasingly lower quantity, the NEBNext Ultra II DNA and Ultra II FS workflows represent the most sensitive kits available, enabling high yield preparation of high-quality libraries with DNA input mass ranging as low as 100 picograms. Ultra II kits use a fast, streamlined, automatable workflow and require fewer PCR cycles while also improving GC coverage.

NEBNEXT ULTRASHEAR FFPE DNA & NEBNEXT FFPE DNA

DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissues present unique challenges for library preparation. These samples often have low input mass, degraded DNA molecules, and artifactual sequence errors introduced during fixation, embedding, storage, and extraction.

MATERIALS

- NEBNext UltraExpress DNA Library Prep Kit (NEB #E3325)
- NEBNext UltraExpress FS DNA Library Prep Kit (NEB #E3440)
- NEBNext Ultra II FS Library Prep Kit (NEB #E7805/E6177)
- NEBNext Ultra II DNA Library Prep Kit (NEB #E7645/E7103)
- NEBNext UltraShear FFPE DNA Library Prep Kit (NEB #E6655)
- NEBNext FFPE DNA Library Prep Kit (NEB #E6650)
- NEBNext Multiplex Oligos for Illumina® (Unique Dual Index UMI Adaptors DNA Set 1) (NEB #E7395)
- genomic DNA (Coriell Institute for Medical Research, NA12878)
- tumor and normal FFPE DNA (Biochain, colon & rectum)
- SPRIselect® Reagent Kit (Beckman Coulter, Inc. #B23317) or AMPure® XP Beads (Beckman Coulter, Inc. #A63881)
- AVITI 2x75 Sequencing Kit Cloudbreak FS High Output (Element Biosciences #860-00015)

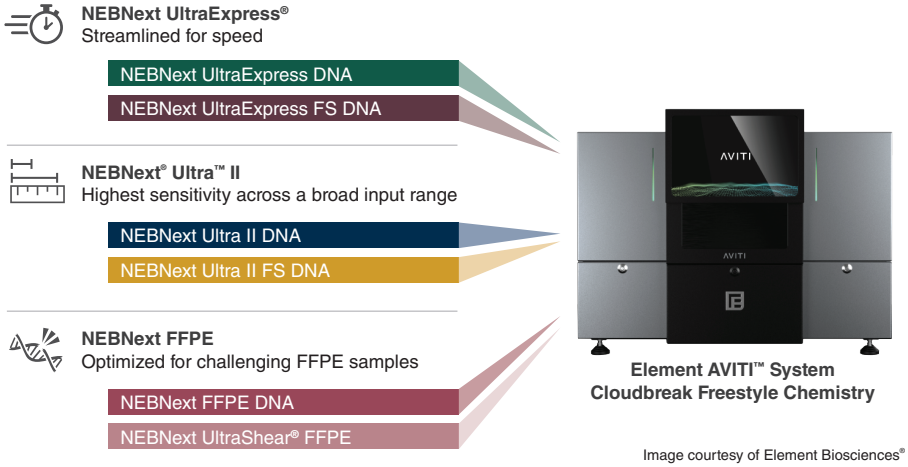


Image courtesy of Element Biosciences®

The NEBNext UltraShear FFPE DNA Library Prep Kit was developed to overcome these challenges. Included in this workflow are the NEBNext FFPE Repair Module v2, which enzymatically scans and corrects a range of typical error modes, as well as NEBNext UltraShear, which was engineered to enzymatically fragment DNA without generation of artifactual errors.

METHODS & RESULTS

To demonstrate compatibility between NEBNext DNA Library Prep Kits and the Element Cloudbreak Freestyle workflow, libraries were prepared using NEBNext UltraExpress DNA, NEBNext UltraExpress FS DNA, Ultra II DNA, Ultra II FS DNA, NEBNext UltraShear FFPE DNA and FFPE DNA kits and sequenced with the Element Cloudbreak Freestyle chemistry.

NEBNext UltraExpress DNA, NEBNext UltraExpress FS DNA

Libraries were prepared in duplicate from 10, 50, 100, and 200 ng of Human NA12878 genomic DNA (Coriell Institute for Medical Research) using the NEBNext UltraExpress DNA Library Prep Kit (NEB #E3325) and the NEBNext UltraExpress FS DNA Library Prep Kit (NEB #E3440). A single protocol workflow was applied, using the same adaptor dilution and PCR conditions for all input amounts. NEBNext UltraExpress DNA libraries were amplified with 8 PCR cycles, and NEBNext UltraExpress FS DNA libraries with 6 PCR cycles.

NEBNext UltraExpress DNA libraries were prepared using DNA sheared to 175 bp using the Covaris ML230 instrument. NEBNext UltraExpress FS DNA libraries were prepared using intact DNA enzymatically fragmented for 20 minutes. Libraries were sequenced using 2x75 bp on the Element AVITI instrument using the Element Cloudbreak Freestyle chemistry (Aviti 2x75 Sequencing Kit Cloudbreak FS High Output; Element Biosciences #860-00015) and downsampled to 2 million read pairs. Reads were trimmed using SeqPrep (v0.1), aligned using Bowtie2 (v2.5.0) to the T2T reference genome, duplicates were marked using MarkDuplicates (v1.56.0), and quality metrics assessed using Picard Alignment Summary Metrics (v1.56.0). Results shown in Figure 1.

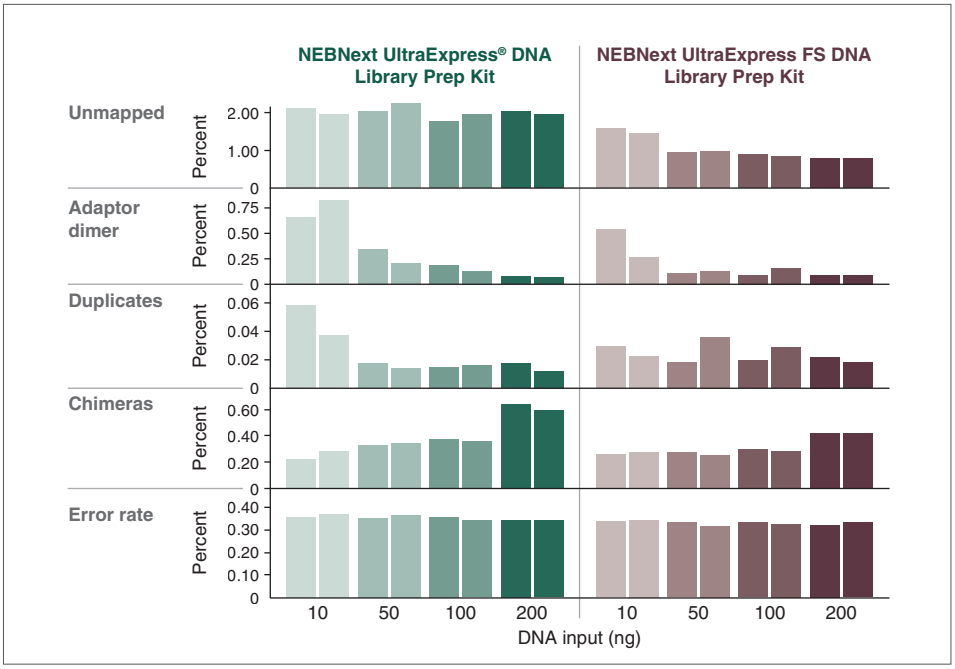
NEBNext Ultra II DNA, NEBNext Ultra II FS DNA

Libraries were prepared in duplicate from 0.1, 5, 100, and 500 ng of intact human NA12878 genomic DNA (Coriell Institute for Medical Research) using the NEBNext Ultra II FS Library Prep Kit (NEB #E7805/E6177) and 13, 8, 4, and 4 PCR cycles for each input amount, respectively. Libraries were prepared using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645) in duplicate from 0.5, 5, 100, and 1000 ng of human NA12878 genomic DNA (Coriell Institute for Medical Research). DNA was sheared to 350 bp using the Covaris ML230 instrument. Libraries were sequenced (2x75 bp) on the Element AVITI instrument using the Element Cloudbreak Freestyle chemistry (Aviti 2x75 Sequencing Kit Cloudbreak FS High Output; Element Biosciences #860-00015) and downsampled to 2 million read pairs. Reads were trimmed using SeqPrep (v0.1), aligned using Bowtie2 (v2.5.0) to the T2T reference, duplicates were marked using MarkDuplicates (v1.56.0), and GC bias assessed using SAM/BAM GC Bias Metrics (v1.56.0). Results shown in Figure 2.

NEBNext UltraShear FFPE DNA, NEBNext FFPE DNA

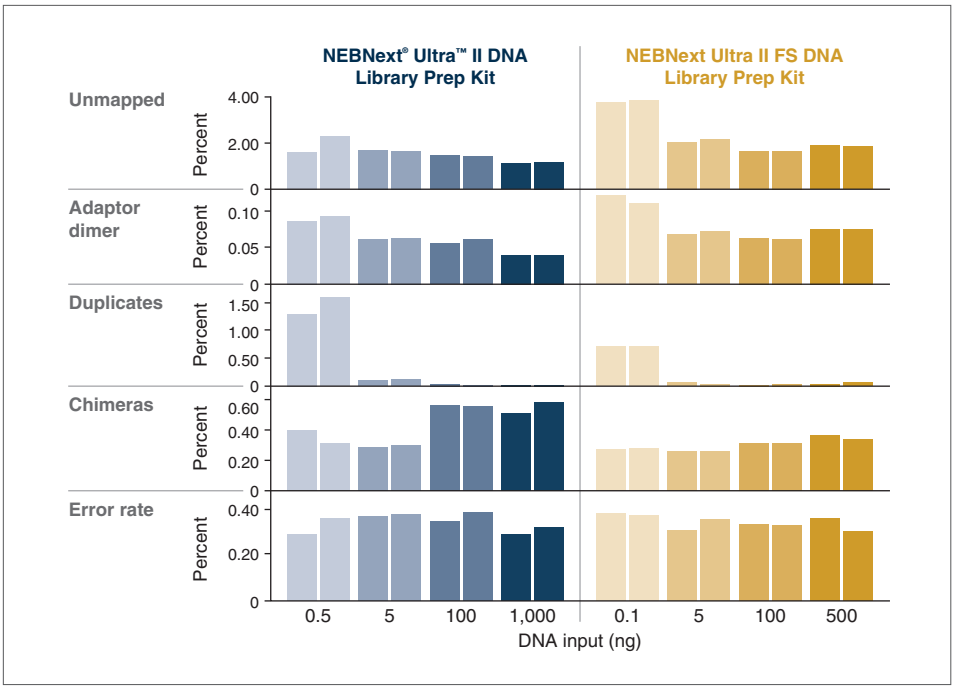
Libraries were prepared in duplicate from 100 ng of tumor and normal DNA (Biochain Institute, Inc.) extracted from FFPE colon (DIN 6) or rectum (DIN 2) using either the NEBNext UltraShear FFPE DNA Library Prep Kit (NEB #E6655), the NEBNext FFPE DNA Library Prep Kit (NEB #E6650), or the NEBNext Ultra II DNA Library Prep Kit (NEB# E7645/E7103). Covaris shearing using a 350 bp program on the ML230 instrument was used for the NEBNext FFPE DNA Library Prep Kit and NEBNext Ultra II DNA Library Prep Kit. The NEBNext Unique Dual Index UMI adaptors and 7 PCR cycles were used for all samples. Libraries were sequenced (2x75 bp) on the Element AVITI instrument (Aviti 2x75 Sequencing Kit Cloudbreak FS High Output; Element Biosciences #860-00015) using the Element Cloudbreak Freestyle chemistry and downsampled to 2 million read pairs. Reads were trimmed using seqprep (v0.1), aligned using bowtie2 (v2.5.0) to the GRCh38 reference, duplicates were marked using MarkDuplicates (v1.56.0), and quality metrics assessed using Picard Alignment Summary Metrics (v1.56.0). Results shown in Figure 3. The average frequency of C→T mutations at each cytosine position was calculated using Tasmanian (v1.0.7). Results shown in Figure 4.

FIGURE 1: **NEBNext UltraExpress DNA and NEBNext UltraExpress FS DNA libraries sequenced using the Element Cloudbreak Freestyle chemistry produce high quality sequencing data**



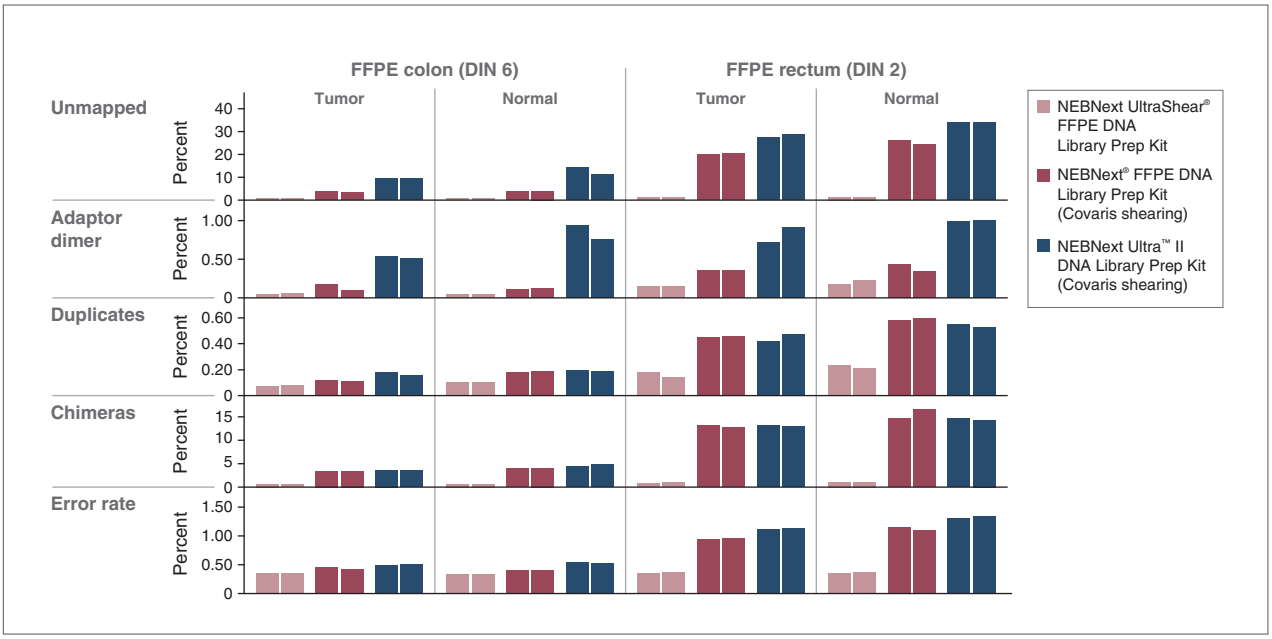
NEBNext UltraExpress DNA and NEBNext UltraExpress FS DNA libraries were prepared in duplicate from 10, 50, 100, and 200 ng of human genomic DNA using the single protocol workflow. Libraries were sequenced (2 × 75 bp) on the Element AVITI instrument with Cloudbreak Freestyle chemistry and downsampled to 2 million read pairs for analysis.

FIGURE 2: **NEBNext Ultra II DNA and NEBNext Ultra II FS DNA libraries sequenced using the Element Cloudbreak Freestyle chemistry produce high quality sequencing data**



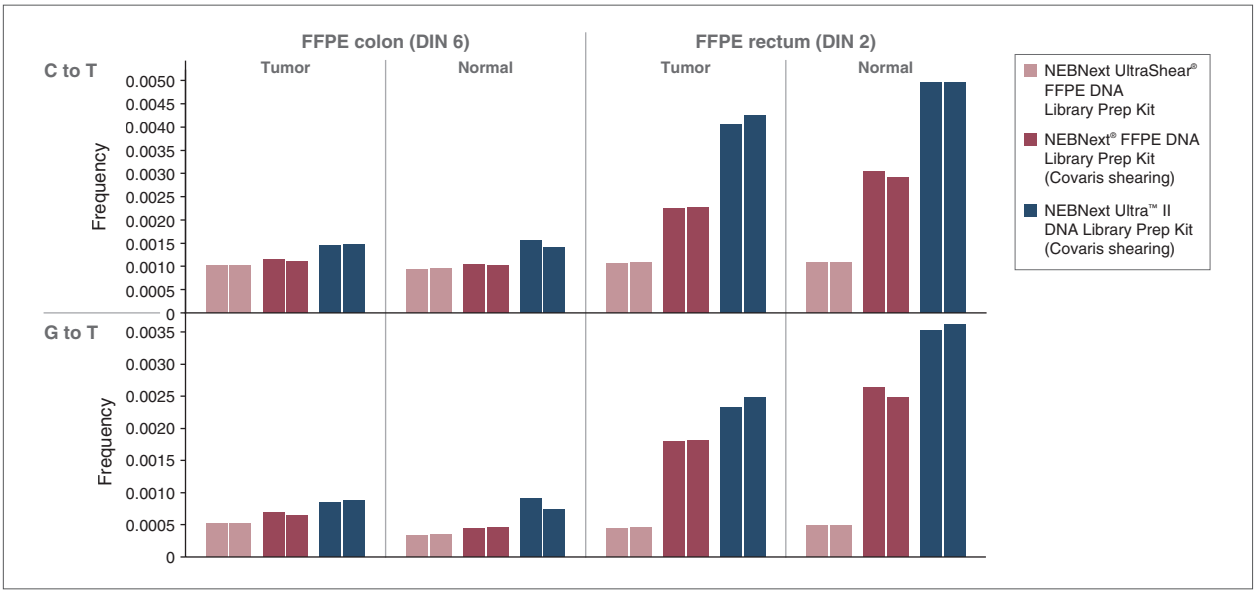
NEBNext Ultra II and Ultra II FS libraries were prepared from Covaris-sheared (0.5–1000 ng; 350 bp) and intact (0.1–500 ng) human genomic DNA, respectively. All libraries were prepared in duplicate, sequenced (2 × 75 bp) on Element AVITI using Cloudbreak Freestyle chemistry, and downsampled to 2 million read pairs for analysis.

FIGURE 3: **NEBNext FFPE DNA libraries sequenced using the Element Cloudbreak Freestyle chemistry produce high quality sequencing data**



NEBNext UltraShear FFPE, FFPE, and Ultra II DNA libraries were prepared in duplicate from 100 ng of FFPE-derived tumor and normal human DNA from colon and rectum. Covaris shearing (350 bp) was used for FFPE and Ultra II kits. Libraries were sequenced (2 × 75 bp) on the Element AVITI instrument with Cloudbreak Freestyle chemistry and downsampled to 2 million read pairs for analysis.

FIGURE 4: **NEBNext UltraShear FFPE DNA libraries sequenced using the Element Cloudbreak freestyle chemistry result in minimal damage-derived base substitution errors, with increased benefits on highly degraded samples**



The average frequency of C→T mutations at each cytosine position (top panel) and G→T mutations at each guanine position (bottom panel) is shown for NEBNext UltraShear FFPE, FFPE, and Ultra II DNA libraries. C→T mutations, which result from cytosine deamination, and G→T mutations, which result from oxidative damage, are effectively repaired in the NEBNext UltraShear FFPE DNA libraries.

TABLE 1: **NEBNext DNA Products with Demonstrated Element Cloudbreak Freestyle™ Workflow Compatibility**

DNA LIBRARY PREP PRODUCTS	CATALOG #	INPUT RANGE	WORKFLOW TIME	CLOUDBREAK FREESTYLE COMPATIBLE?
NEBNext UltraExpress DNA Library Prep Kit	E3325	10 ng – 200 ng	1.8 hrs*	Yes
NEBNext UltraExpress FS DNA Library Prep Kit	E3440	10 ng – 200 ng	1.8 hrs**	Yes
NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645/E7103	500 pg – 1 ug	1.7 hrs – 3.2 hrs*	Yes
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805/E6177	100 pg – 1 ug	1.7 hrs – 3.2 hrs**	Yes
NEBNext UltraShear FFPE DNA Library Prep Kit	E6655	5 ng – 250 ng	3 hrs – 4 hrs**	Yes
NEBNext FFPE DNA Library Prep Kit	E6650	5 ng – 250 ng	2.8 hrs – 3.8 hrs*	Yes

* does not include fragmentation
** includes fragmentation

CONCLUSION

NEBNext DNA library preparation kits are fully compatible with sequencing on the Element AVITI platform using Cloudbreak Freestyle chemistry, requiring no workflow adjustments. Sequencing results deliver high-quality metrics aligned with expected performance standards.