NEBNext® Ultra™II FS DNA: A Robust Enzyme-based DNA Library Preparation Method Compatible with Plant and Animal Samples

Karen R. McKay[1], Lynne Apone[1], Vaishnavi Panchapakesa[1], Pingfang Liu[1], Brittany Sexton[1], Yvonne Helbert[2], Lei Zhang[2], Bradley W. Langhorst[1], Timur Shtatland, Maggie Heider[1], Chen Song[1], Christine Sumner[1], Christine Rozzi[1], Melissa Arn[1], Daniela Munafo[1], Fiona Stewart[1], Kevin J. McKernan[2], Eileen T. Dimalanta[1] and Theodore B. Davis[1]

[1]New England Biolabs, Inc. 240 County Road, Ipswich, MA 01938, USA. [2]Medicinal Genomics Corp., Woburn, MA 01801, USA

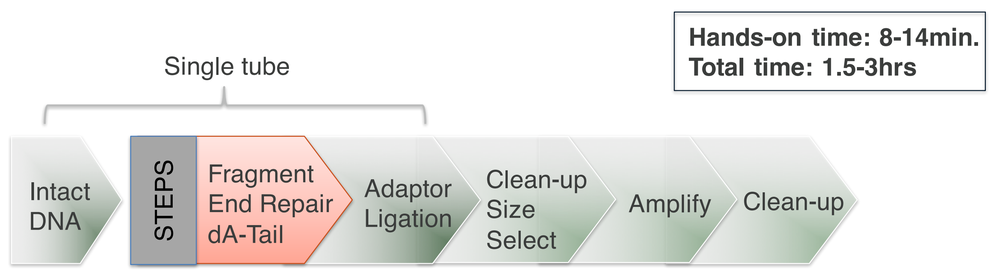
Introduction

Next generation sequencing (NGS) is currently an important tool used in many fields to answer biological questions. DNA fragmentation is the critical initial step in the construction of high quality NGS libraries, however, current fragmentation methods create a bottleneck in library preparation throughput. To meet this challenge, we have developed a robust library construction method (NEBNext Ultra II FS) that integrates enzyme-based DNA fragmentation with end-repair and dA-tailing in a single step, followed by adaptor ligation in the same tube. This method eliminates the need for expensive equipment to fragment DNA; moreover, the optimized workflow reduces the numerous cleanup and liquid transfer steps, reducing the time, cost, and errors associated with library construction.

The robustness of the Ultra II FS DNA library preparation workflow was tested using genomic DNA from a variety of sources including the model organism *Arabidopsis thaliana*, the less-documented genome of *Cannabis sativa*, and *Sus scrofa* (pig). Libraries were prepared from a range of DNA inputs to achieve different insert sizes with or without PCR amplification. All libraries were sequenced, reads aligned to the appropriate reference genome, and quality metrics generated using Picard tools. Compared with the traditional, mechanical shearing based library preparation method, Ultra II FS is significantly easier to automate, has higher library conversion rate and similar or superior sequencing quality. We further discuss several applications of Ultra II FS in plant and animal research, including genome assembly and sample quality control.

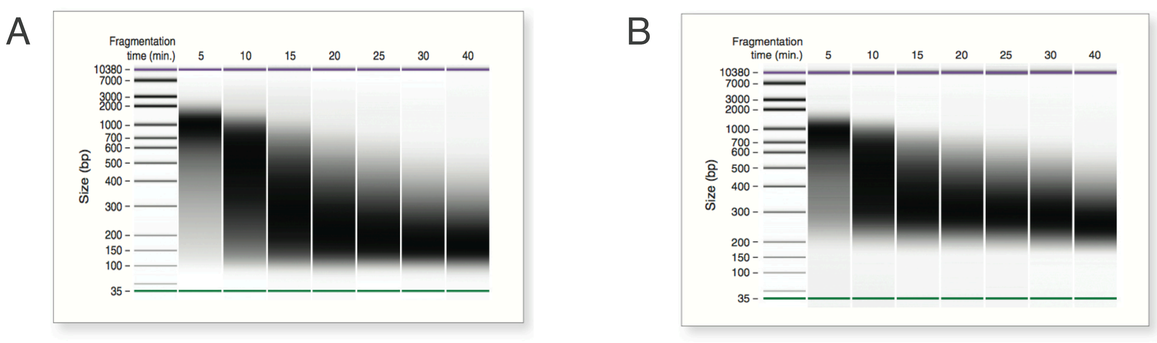
Streamlined workflow

Enzyme based fragmentation combined with NEBNext® Ultra™ II DNA Library Preparation for Illumina®



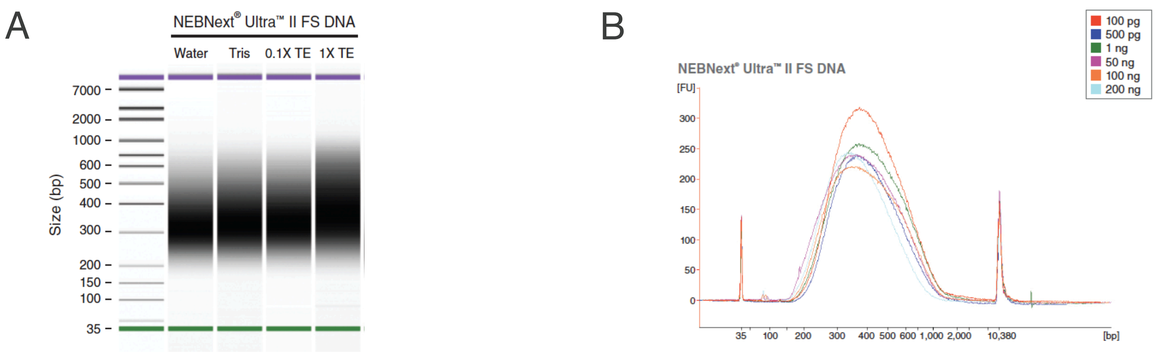
Genomic DNA isolated from a variety of sources was used to construct Illumina libraries. DNA (100pg - 500ng) was fragmented, end repaired and dA-tailed in a single step followed by adaptor ligation in the same tube. PCR amplified and PCR-free libraries were sequenced, reads aligned to the appropriate reference genome, and quality metrics generated using Picard tools.

Ultra II FS enables time-dependent fragmentation



A) 100 ng of input genomic DNA was incubated with the NEBNext Ultra II FS Enzyme Mix and Reaction Buffer for 5, 10, 15, 20, 25, 30 and 40 minutes at 37˚C, followed by 65˚C for 30 minutes. After clean-up using NEBNext Sample Purification Beads, size was assessed using the Agilent Bioanalyzer. B) Libraries were constructed using the NEBNext Ultra II FS kit and 100 ng of input, with fragmentation times of 5, 10, 15, 20, 25, 30 and 40 minutes, and 4 PCR cycles. Size selection was not performed. After clean-up using NEBNext Sample Purification Beads, size was assessed using the Agilent Bioanalyzer. NEBNext Ultra II FS shows expected final library sizes consistent with the fragmentation sizes seen in A). Human NA19240 genomic DNA was used to generate the figures above.

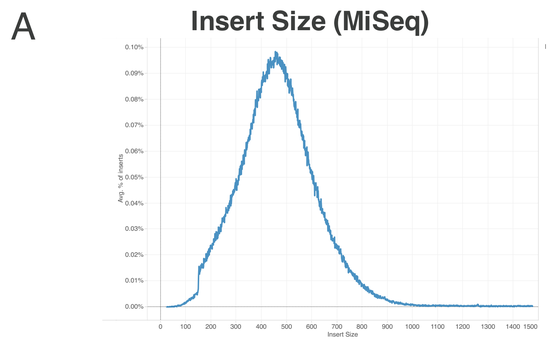
Robust fragmentation independent of DNA input and buffer

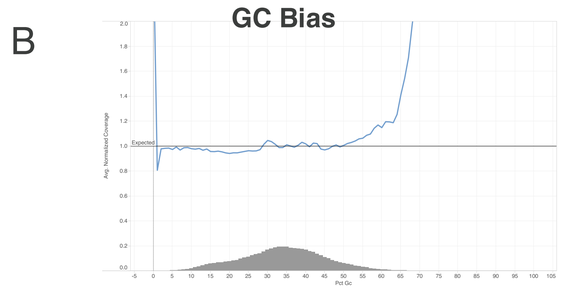


A) Libraries were made using 100 ng input genomic DNA using the NEBNext® Ultra™ II FS kit. Libraries were fragmented to generate 200 bp inserts (320 bp libraries) from DNA resuspended in H_2O, 10mM Tris, 0.1X TE or 1X TE . Library size distribution was assessed using the Agilent® Bioanalyzer®. B) Libraries were prepared from input genomic DNA using the input amounts shown. NEBNext® Ultra™ II FS libraries were prepared using a 20-min fragmentation time. Library size was assessed using the Agilent® Bioanalyzer®. Low input (1 ng and below) libraries were loaded on the Bioanalyzer® without dilution. High input libraries were loaded with a 1:5 dilution in 0.1X TE. Human NA19240 genomic DNA was used to generate the figures above.

Results

Large insert size with *Cannabis sativa* female leaf

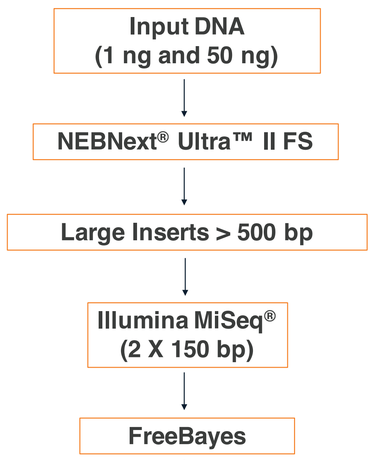


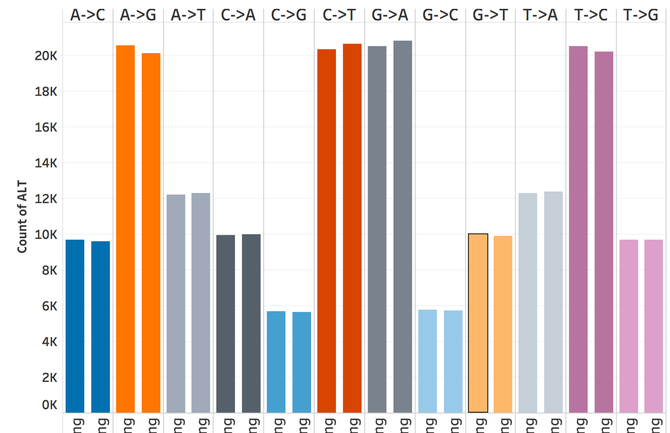


Large insert size libraries can be generated with the NEBNext® Ultra™ II FS kit. 250 ng of Jamaican Lion Female leaf DNA was fragmented for 5 minutes. After adaptor ligation, a bead based size selection was performed for large fragment size. The library was amplified for 5 cycles using the NEBNext Ultra II Q5 master mix and sequenced on an Illumina MiSeq® (2 X 150 bp). Reads were aligned to Jamaican Lion reference genome (August 2018 assembly) using Bowtie 2.2.4.

A) Insert size was calculated using Picard Metrics B) GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1. 117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored line represents the normalized coverage for the library.

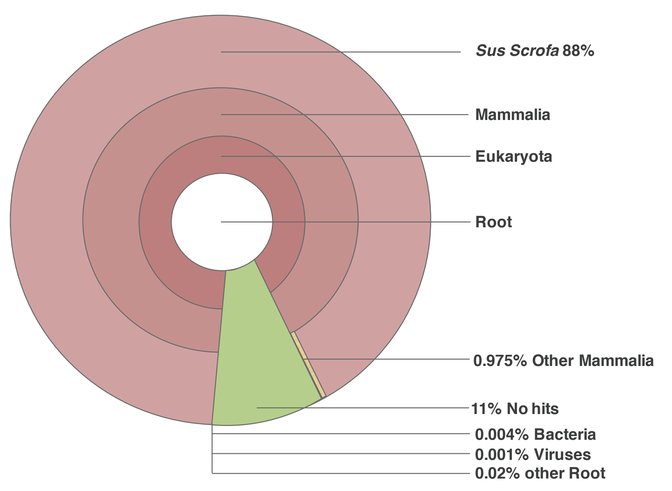
Reliable SNP detection with 1ng Arabidopsis DNA

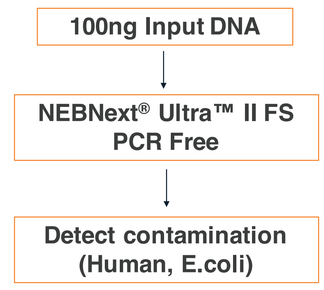




This germ line variant summary plot shows that the SNPs are represented comparably regardless of input amount, demonstrating that NEBNext Ultra II FS can be used for accurate SNP detection with minimal sample input.

Quality control of reagents using *Sus scrofa*





An uncommon source of DNA was used as a quality control measure for detecting contaminants such as human and bacterial DNA in our final libraries, which indicates the purity of our library preparation reagents.

Conclusions

- Ultra™ II FS enables high-quality library construction from a broad range of DNA quantities and qualities and is compatible with plant and animal samples.
- Ultra™ II FS maintains reliable SNP detection with minimal sample input.
- Large insert sizes can be generated with Ultra™ II FS.
- Ultra™ II FS provides a robust, time-dependent fragmentation amenable for manual or automated library preparation.

Acknowledgements

Authors would like to acknowledge the technical assistance provided by Laurie Mazzola, Danielle Fuchs, and Aine Quimby at the New England Biolabs' Sequencing Core Facility.