EPICENTRE® Biotechnologies

Rapid, high-throughput library preparation for next-generation sequencing

Advances in next-generation sequencing have led to new library preparation methods compatible with multiple sequencing platforms. Current methods (both mechanical and enzymatic) face limitations: multi-step protocols, sample loss, lack of automation and labor costs. With the continued decline of sequencing costs and increase in sample throughput, there is greater demand for more cost-effective, streamlined library preparation methods. Here we describe Epicentre's patented Nextera™ technology, which addresses current issues in sample preparation and provides a simplified procedure amenable to high-throughput workflows.

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Most second-generation sequencers use technologies for sequencing that differ only slightly from one another, and most adhere to a common library preparation procedure with minor modifications. This procedure includes DNA fragmentation (sonication, nebulization or shearing) followed by DNA repair and end-polishing (blunt-end or A-overhang) and finally platform-specific adaptor ligation. Typically, this results in significant sample loss and limited throughput. To streamline the workflow, increase throughput and reduce sample loss, Epicentre has developed Nextera technology, a single-tube, 5-min reaction capable of simultaneously fragmenting and tagging DNA using only 50 ng of starting DNA. This flexible, scalable, simple and efficient method creates libraries for multiple sequencing platforms, whole-genome amplification and other applications in under 2 h.

Method overview

Nextera technology uses *in vitro* transposition to prepare sequencer-ready libraries. During *in vitro* transposition with the Nextera Enzyme Mix (transposase and transposon complex, also referred to as a Transposome™ complex), strand transfer occurs via random, staggered double-strand DNA breaks in the target DNA and covalent attachment of the 3′ end of the transferred transposon strand to the 5′ end of the target DNA. When free transposon ends are used in the reaction, the target DNA is fragmented and the transferred strand of the transposon-end oligonucleotide is covalently attached to the 5′ end of the fragment. Independent tags can also be added to the fragmented DNA by appending the transposon end sequence with an engineered adaptor sequence. After extension, the sequencing adaptors enable amplification by emulsion PCR (emPCR), bridge PCR (bPCR) and other methods. The amplified library can then be sequenced. Deep sequencing of Nextera-fragmented libraries produces accuracy, coverage and bias comparable to those of control libraries created by physical shearing and other enzyme-based methods (unpublished data).

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Molecular weight distribution of Nextera-generated libraries

The molecular weight distributions of libraries prepared using Nextera technology can be controlled for the read-length requirements of different sequencers. To show that molecular weight distributions are consistent and reproducible across different sample types, we prepared genomic DNA libraries from phage λ, *Escherichia coli* and human DNA using both Roche 454–Compatible and Illumina-Compatible Nextera Enzyme Mixes according to the standard protocol (**Fig. 1**). The reaction conditions were optimized to yield fragment sizes appropriate for the respective sequencers. Each final fragment size includes approximately 100 base pairs (bp) of adaptor sequence; therefore, the actual genomic DNA sequenced is smaller than the apparent molecular weight of the library. It should also be noted that not all sample types will result in similar molecular weight distributions, as many factors (for example, complexity of the sample type and quantification method) can affect the molecular weight distribution.

Deep sequencing of Nextera Roche 454–compatible libraries

Libraries were prepared from multiple sample types and then sequenced to validate Nextera technology for Roche 454. *E. coli*, plasmid, cosmid and soy genomic DNA was fragmented and 5′ end–tagged with Nextera Enzyme Mix (Roche-Compatible). Roche

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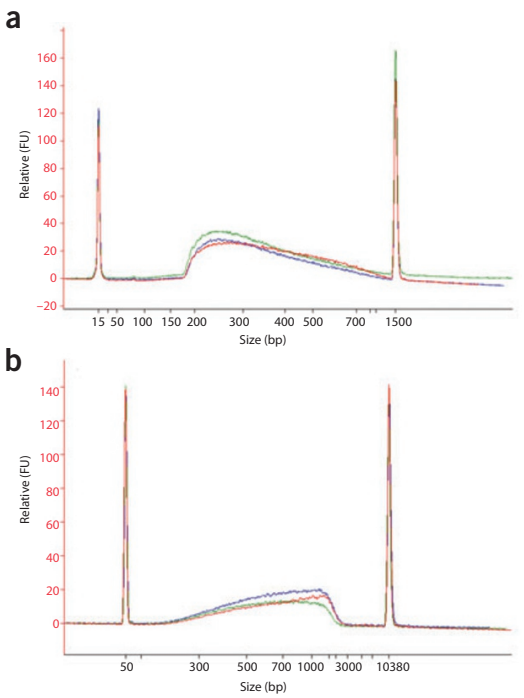

Figure 1 | Molecular weight distribution of libraries prepared using Nextera Illumina-Compatible Enzyme Mix (**a**) and Roche-Compatible Enzyme Mix (**b**). Libraries prepared from bacteriophage λ (red), human (blue) and *E. coli* (green) DNA were examined using a Bioanalyzer (Agilent); traces show relative fluorescence units (FU) plotted against DNA fragment size.

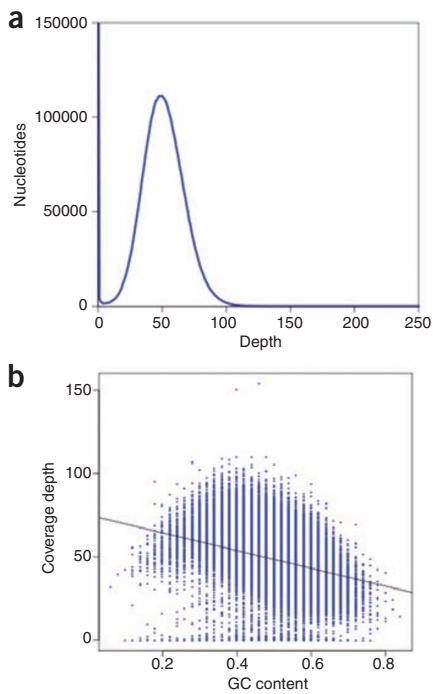

Figure 2 | Deep sequencing of Nextera-generated Illumina-compatible sequencing library. A library prepared from *E. coli* genomic DNA was sequenced using Illumina GAII chemistry. (**a**) Coverage plot (nucleotides vs. depth) shows a near-Poisson distribution. (**b**) Coverage depth vs. GC content; the solid black line is a linear regression curve.

Table 1 | Summary data from deep sequencing of Nextera-generated Roche 454–compatible libraries

Sample	Total reads	% total nucleotides identified	Reference sequence length	× coverage	% mapped reads
E. coli	472,007	99.95	4.64 Mb	33.21	88.74
Plasmid 1	10,657	99.93	19.7 kb	151.38	93.74
Plasmid 2	6,291	99.89	6.3 kb	284.17	86.73
Soy	572,162	99.90	973 Mb	0.16	87.64
Cosmid	63,594	99.51	43 kb	161	96.19

454–compatible adaptors were added, and di-tagged fragments were enriched by limited-cycle PCR. The library was used directly as input for Roche 454 FLX Titanium emPCR. Deep sequencing of the Nextera-fragmented libraries produced coverage (**Table 1**) comparable to that of libraries prepared by physical shearing.

Deep sequencing of Nextera Illumina–compatible libraries

E. coli DNA was used to validate Nextera technology on the Illumina Genome Analyzer. Genomic DNA from *E. coli* B strain (REL606) was fragmented and 5′ end–tagged with Nextera Enzyme Mix (Illumina-Compatible). Illumina-compatible adaptors were added, and di-tagged fragments were enriched by limited-cycle PCR. The library was used as input for cluster formation without size selection. Deep sequencing of the Nextera-fragmented libraries was performed with alternative sequencing primers (included in the kit), and the results were mapped to the reference sequence (**Fig. 2**). The GC bias of the Nextera library is comparable to that observed with physical shearing.

Conclusions

Current library preparation methods for next-generation sequencing are time-consuming and prone to significant sample loss. Epicentre's Nextera technology offers many advantages over these methods, such as a streamlined workflow that can provide significant time and cost savings. The Nextera method, a single-tube reaction, is scalable and requires less starting DNA than other procedures. Validation data—including GC bias, coverage, mapped reads and comparison with existing methods (unpublished data)—show that the Nextera system is not only a significant improvement over current procedures, but also a complete solution for next-generation sequencing library preparation.

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