Poster #P104

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DNA Library Preparation: Simultaneous DNA Fragmentation and Adaptor Tagging by *In Vitro* Transposition

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Introduction

DNA library preparation is a common entry point for next-generation sequencing. Current methods generally consist of distinct DNA fragmentation, end-polishing, and adaptor-ligation steps; often with significant sample loss and hands-on time.

EPICENTRE Biotechnologies has developed Nextera™ technology, a streamlined, scalable, and efficient method that generates adaptor-tagged libraries for multiple next-generation sequencing platforms, whole-genome amplification, and other applications.

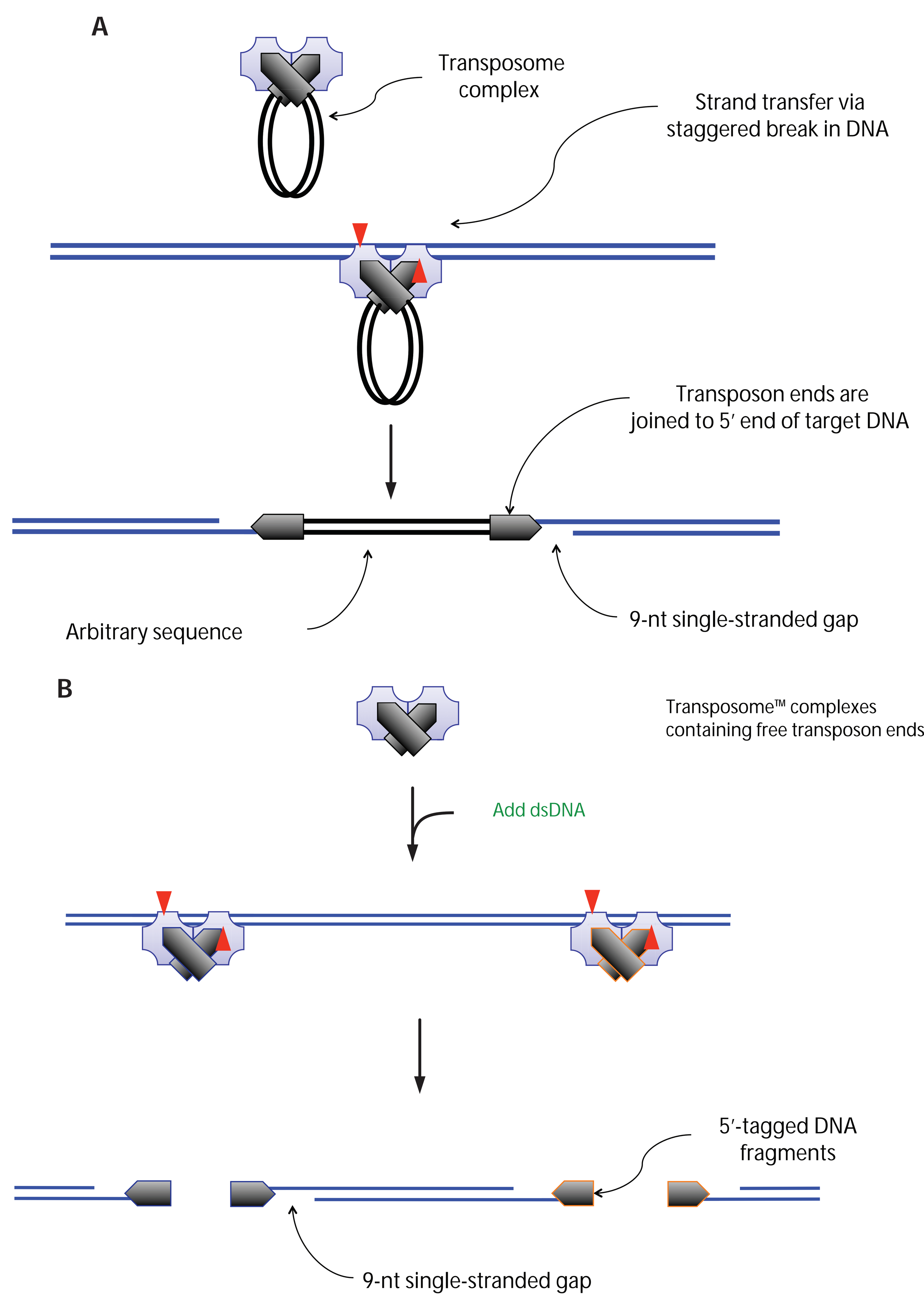
In vitro transposition with Nextera Transposomes™ fragments and covalently tags the target DNA in a single step. Di-tagged sequencing libraries compatible with the Roche/454 and Illumina/Solexa platforms can be enriched and bar-coded using limited-cycle PCR.

Deep sequencing of the transposon-fragmented libraries produces a single contig of the expected size with read-length, accuracy, and coverage comparable to a control library produced using nebulization and the recommended protocol.

Methods and Results

DNA Fragmentation and Tagging with Free Transposon Ends

During *in vitro* transposition with hyperactive Transposomes, strand-transfer occurs via random, staggered, dsDNA breaks in the target DNA and covalent attachment of the 3′end of the transferred (top) transposon strand to the 5′end of the target DNA (Figure 1A). 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 (Figures 1B and 1C). The size distribution of the fragments can be controlled by changing the amount of Transposomes (Figure 1C) and reaction time (data not shown). Genomic DNA can be fragmented to <1 kb in 5 minutes with enhanced reaction conditions (Figure 1D).



C — Input DNA, MW — 5 Minute Reaction

D — MW, LMW Rxn Buffer, HMW Rxn Buffer — 5 Minute Reaction

Figure 1. Simultaneous DNA fragmentation and tagging by *in vitro* cut-and-paste transposition. A) Transposon integration and strand transfer occur via a staggered, dsDNA break within the target DNA (red triangles). The 3′end of the transferred strand is joined to the free 5′end of the target DNA resulting in a 9-nucleotide gap. B) When free transposon ends are used in the insertion reaction, the target DNA is cleaved and tagged at the 5′ end with the transposon sequence. The resulting fragments have single-stranded gaps. C) Dose-dependent fragmentation and 5′-end-tagging of HeLa genomic DNA by Transposomes containing free transposon ends (MEs). Genomic DNA was incubated with varying amounts of Transposomes for 5 minutes at 55˚C. Reaction products were resolved by electrophoresis in a 2% agarose gel and staining with SYBR® Gold. D) Molecular weight distribution of sequencing libraries prepared from transposome-fragmented HeLa genomic DNA. Fifty nanograms of HeLa genomic DNA was incubated with ME-containing Transposomes for 5 minutes at 55˚C in either "LMW" or "HMW" reaction buffer prior to adding Roche/454 FLX adaptors by PCR (see Figure 2A). Reaction products were resolved by electrophoresis in a 2% agarose gel and staining with SYBR® Gold.

Production of Bar-Coded Libraries Compatible with Roche and Illumina Platforms

Bar-coded adaptors compatible with Roche/454 or Illumina/Solexa sequencing platforms were added by PCR. The primer design uses limited-cycle PCR to enrich di-tagged libraries. PCR products are compatible with emulsion PCR (Roche/454) or bridge PCR (Illumina/Solexa). Validation is in progress.

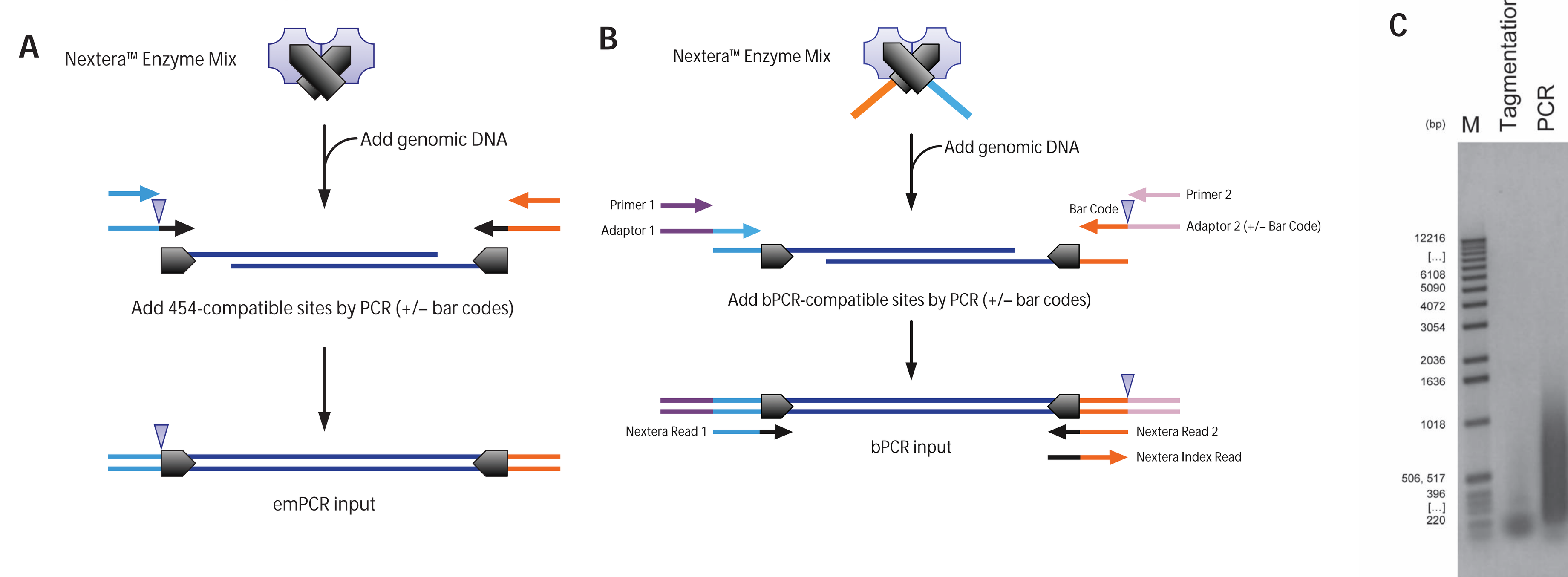
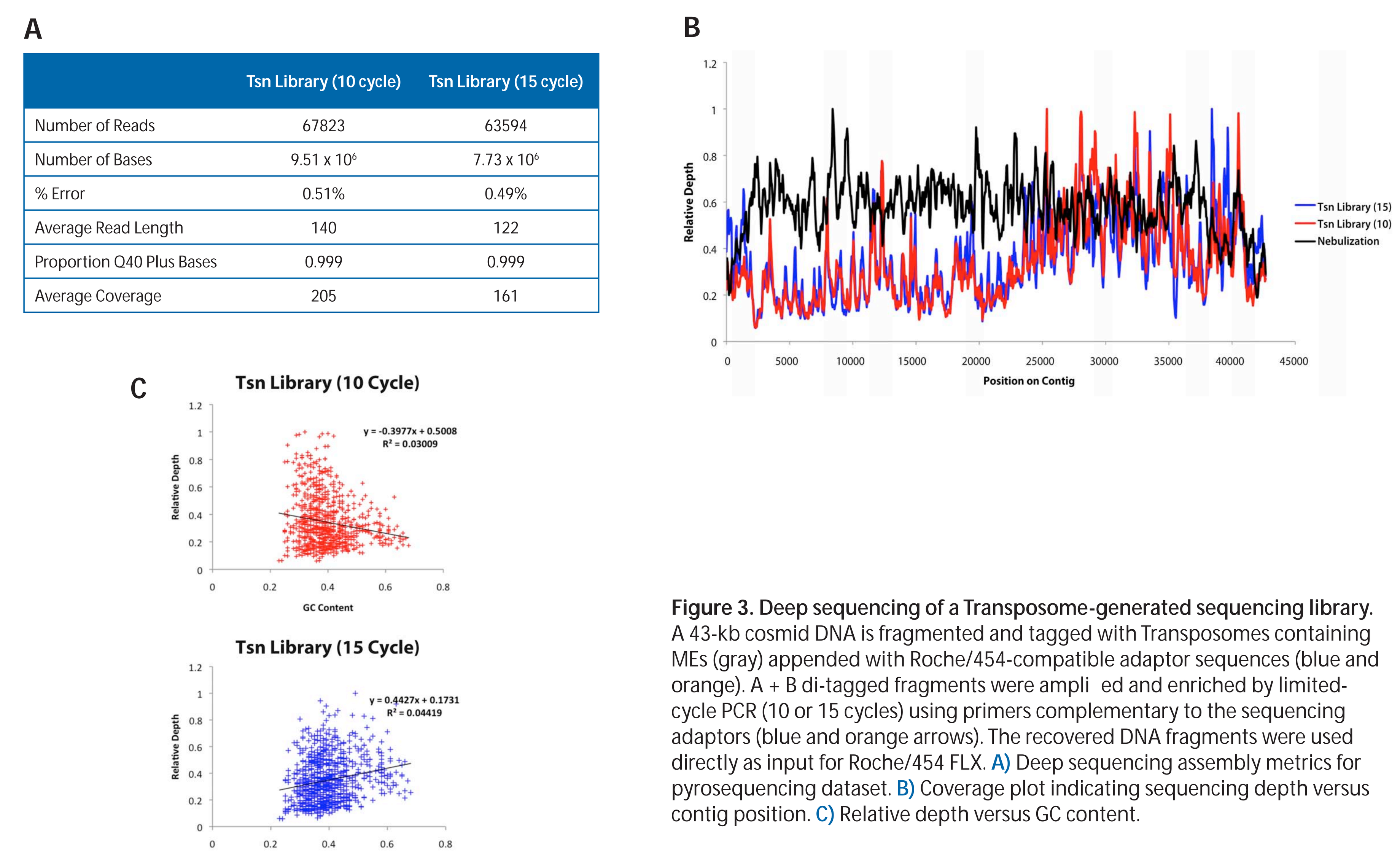


Figure 2. Addition of bar-coded Roche/454-compatible and Illumina/Solexa-compatible adaptors for multiplexed sequencing. A) Roche/454-compatible libraries. Target DNA is fragmented and tagged with the core Transposomes containing MEs. Limited-cycle PCR with a four-primer reaction adds Roche/454-compatible adaptors sequences (blue and orange). Optional bar coding (triangle) is added between the upstream emPCR adaptor (blue) and the transposon ME (gray). B) Illumina/Solexa-compatible libraries. Target DNA is fragmented and tagged with the core Transposomes containing MEs appended with sequencing primer sites (blue and orange). Limited-cycle PCR with a four-primer reaction adds bPCR-compatible adaptors (purple and pink) to the core sequencing library. Optional bar coding (triangle) is added between the downstream bPCR adaptor (pink) and the core sequencing library (orange). Alternative sequencing primers are required for the Illumina/Solexa-compatible libraries: Read 1 (blue/gray arrow), Read 2 (orange/gray arrow), Index Read (gray/orange arrow). C) Production of a sequencing library. Fifty nanograms of genomic DNA was 5′-end-tagged and fragmented with Transposomes under enhanced reaction conditions for 5 minutes. After Qiagen purification, one-fourth of the recovered DNA was amplified using the Illumina/Solexa-compatible primer set. Reaction products were resolved by electrophoresis in a 1% agarose gel and staining with SYBR® Gold.

454-Compatible Libraries Produced by *In Vitro* Transposition

A 43-kb cosmid was fragmented and 5′-end-tagged with Transposomes appended with Roche/454-compatible adaptor sequences. Di-tagged fragments were enriched by limited-cycle PCR and used directly as input for Roche/454 FLX emulsion PCR. Deep sequencing of the transposon-fragmented libraries produced a single contig of the expected size with read length, accuracy, and coverage that was comparable to the control library.



A

	Tsn Library (10 cycle)	Tsn Library (15 cycle)
Number of Reads	67823	63594
Number of Bases	9.51 x 10⁶	7.73 x 10⁶
% Error	0.51%	0.49%
Average Read Length	140	122
Proportion Q40 Plus Bases	0.999	0.999
Average Coverage	205	161

Figure 3. Deep sequencing of a Transposome-generated sequencing library. A 43-kb cosmid DNA is fragmented and tagged with Transposomes containing MEs (gray) appended with Roche/454-compatible adaptor sequences (blue and orange). A + B di-tagged fragments were amplified and enriched by limited-cycle PCR (10 or 15 cycles) using primers complementary to the sequencing adaptors (blue and orange arrows). The recovered DNA fragments were used directly as input for Roche/454 FLX. A) Deep sequencing assembly metrics for pyrosequencing dataset. B) Coverage plot indicating sequencing depth versus contig position. C) Relative depth versus GC content.

Illumina-Compatible Libraries Produced by *In Vitro* Transposition

E. coli CC118 genomic DNA was fragmented and 5′-end-tagged with Transposomes appended with sequencing adaptors. Illumina-compatible adaptors were added and di-tagged fragments were enriched by limited-cycle PCR. The produced library was used as input for cluster formation with and without size selection. Deep sequencing of the transposon-fragmented libraries was performed with alternate sequencing primers (Figure 2) and mapped genomic deletions in *E. coli* CC118 relative to the reference sequence.

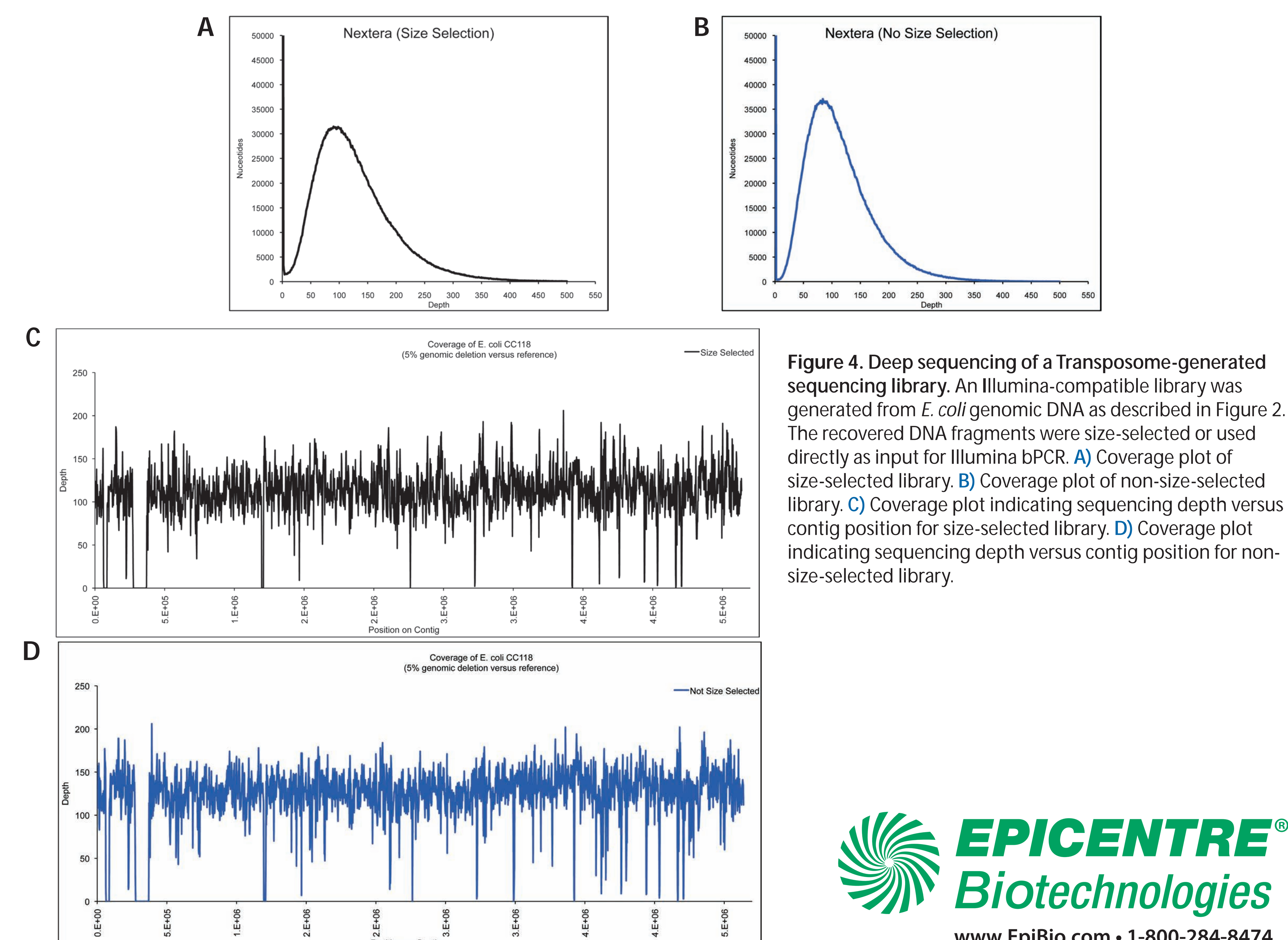


Figure 4. Deep sequencing of a Transposome-generated sequencing library. An Illumina-compatible library was generated from *E. coli* genomic DNA as described in Figure 2. The recovered DNA fragments were size-selected or used directly as input for Illumina bPCR. A) Coverage plot of size-selected library. B) Coverage plot of non-size-selected library. C) Coverage plot indicating sequencing depth versus contig position for size-selected library. D) Coverage plot indicating sequencing depth versus contig position for non-size-selected library.

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