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Matthias Harbers and Günter Kahl

Tag-based Next Generation Sequencing

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Cover

The scheme in the foreground symbolizes the events at a silenced and an activated promoter (adapted with kind permission from Macmillan Publishers Ltd./Shelley L. Berger: The complex language of chromatin regulation during transcription, *Nature* 447, 2007, and with kind permission from Shelley L. Berger, University of Pennsylvania, Philadelphia). The background (modified with kind permission from Steven Henikoff, Fred Hutchinson Cancer Research Center and University of Washington, Seattle) symbolizes the C-methylation patterns of some *Arabidopsis thaliana* chromosomes.

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19
Second-Generation Sequencing Library Preparation: *In Vitro* Tagmentation via Transposome Insertion

Fraz Syed

Abstract

The advent of next-generation sequencing (NGS) has made it possible to analyze the genome at previously unattainable depth. Numerous companies have developed well-established platforms for massively parallel deep sequencing. Regardless of the instrument, one of the bottlenecks for NGS is the amount of time and resources required for template and library preparation. Current methods generally consist of distinct steps that involve significant sample loss and hands-on time: DNA fragmentation, end-polishing, and adapter ligation. *In vitro* transposition with Nextera Transposomes simultaneously fragments and covalently tags the target DNA, combining these three distinct steps into a single reaction. Platform-specific adapters can be added, and the sample can be enriched and barcoded using limited-cycle polymerase chain reaction to prepare ditagged DNA fragment libraries. Nextera technology offers a streamlined, efficient, and high-throughput method for generating barcoded libraries compatible with multiple NGS platforms.

19.1
Introduction

Over the past 5 years, the need to lower the cost of sequencing a human genome has led to development of many massively parallel sequencing (MPS) platforms. The field of MPS, more commonly known as next (or second)-generation sequencing (next-generation sequencingNGS), has grown rapidly and made it possible to analyze the genome at previously unattainable depth. Since being selected method of the year in 2007 by *Nature Methods*, some of the approaches for MPS have been commercialized and are now well-established. These NGS platforms employ slightly different technologies for sequencing, such as sequencing by ligation (Complete Genomics, Life Technologies, Polonator), sequencing-by-synthesis (Helicos BioSciences, Illumina, Intelligent BioSystems), real-time sequencing-by-synthesis (Pacific Biosciences, VisiGen, now part of Life Technologies), and pyrosequencing (Roche 454) [1–3].

Although different in their sequencing chemistries, most platforms adhere to a common library preparation procedure, with minor modifications, prior to a "run" on the instrument. This includes fragmenting the DNA (sonication, nebulization, or shearing), followed by DNA repair and end-polishing (blunt-end or A-overhang), and, finally, platform-specific adapter ligation. The library preparation procedure is one of the major bottlenecks for NGS, and typically results in significant sample loss with limited throughput due to the number of steps

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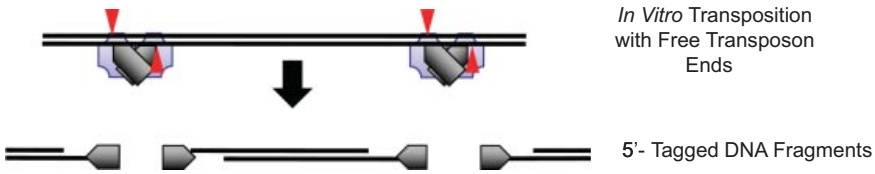

Fig. 19.1 Overview of Nextera fragmentation and tagging technology. Transposon integration and strand transfer occur via a staggered, double-stranded DNA break within the target DNA. When free transposon ends are used in the insertion reaction, the target DNA is cleaved and tagged at the 5′ end with the transposon sequence. The resulting fragments have single-stranded gaps.

involved in making a library [4]. To streamline the work flow, increase throughput, and reduce sample loss, Epicentre has developed the Nextera technology – a transposon-based method for preparing fragmented and tagged DNA libraries. This flexible, scalable, and efficient technique can generate sequencer-ready libraries for multiple platforms in less than 2 h.

In a classic transposition reaction, transposases catalyze the random insertion of excised transposons into DNA targets with high efficiency. During cut-and-paste transposition, a transposase makes random, staggered, double-stranded DNA breaks in the target DNA and covalently attaches the 3′ end of the transferred transposon strand to the 5′ end of the target DNA. The transposase and transposon complex, also referred to as a Transposome complex, inserts an arbitrary DNA sequence at the point of excision. We have discovered that the entire complex is not necessary for excision, and free transposon ends are sufficient for integration.

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 target fragment. The size distribution of the fragments can be controlled by changing the amounts of transposase and transposon ends. Exploiting transposon ends with appended sequences results in DNA libraries which can be used in high-throughput sequencing (Figure 19.1).

The Nextera technology has been used to prepare and sequence libraries from multiple sample types, including genomic DNA [5], cDNA (RNA-seq) [6], amplicons [7], phage DNA (manuscript in preparation), and fosmids [8]. Shendure *et al.* recently published an in-depth comparison of various library preparation methods and found that when compared to conventional methods (mechanical or endonuclease fragmentation), transposase-based "tagmentation" does present slightly greater insertion bias; however, this has minimal impact on coverage [5]. The authors also state that the transposase system is highly versatile and can be adapted to multiple applications, including library preparation from as little as 10 pg of DNA, exome capture, polymerase chain reaction (PCR)-Free and colony PCR library preparation and sample preparation automation. Regarding RNA-seq, with minor modifications, the technology can be adapted for RNA-seq (cDNA) library preparation as well. Briefly, a random-primed method is used to synthesize first-strand cDNA from polyadenylated (poly(A)$^+$) mRNA. Following second-strand synthesis, double-stranded cDNA is used as input for the Nextera "tagmentation" (fragmentation and tagging) reaction. Platform-specific tags are added to the tagmented DNA and ditagged fragments are enriched by limited cycle PCR. Due to the method used to add platform-specific adapters, this library preparation method generates non-directional libraries. Nextera for RNA-seq shows good correlation between the Nextera and standard RNA-seq library preparation methods, and generates libraries of sufficient complexity [6]. When preparing cDNA libraries, coverage of the most distal end is typically reduced because two transposome insertions are required to produce a functional sequencing template. Complete coverage of the linear double-stranded cDNA can be obtained by including one of the adapters in the PCR or cDNA primers. Alternatively, the cDNA can be made slightly larger to compensate for reduced coverage near the ends.

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19.2 Methods and Protocols

19.2.1 Materials

Roche/454-Compatible Library Production

- Target DNA – in water or TE, free of contaminating proteins and salts (see *Note 1*)
- Nextera Enzyme Mix, FLX Titanium-compatible (EZ-Tn5 transposase bound to double-stranded transposon-end DNA; Epicentre):

"METS"	5′-AGATGTGTATAAGAGACAG-3′
"pMENTS"	3′-TCTACACATATTCTCTGTC-PO_4-5′

- 5× Tagmentation reaction buffer (50 mM Tris–OAc, pH 8.0, 25 mM $Mg(OAc)_2$)
- Zymo DNA Clean & Concentrator-5 (Zymo Research; cat. no. D4013 or equivalent)
- Zymo DNA Binding Buffer (Zymo Research; cat. no. D4003-1-L or equivalent)
- Zymo Wash Buffer (Zymo Research; cat. no. D4003-2-6 or equivalent)
- Nuclease-free water
- 2× Nextera PCR Buffer (Epicentre)
- PCR adapters and primers

FLX Titanium-Compatible Adapters and Primers

- Adapter 1 (Ti-compatible library); 0.5 μM in water
  5′-CCATCTCATCCCTGCGTGTCTCCGACTCAGAGATGTGTATAAGAGA-CAG-3′
- Adapter 2 (Ti-compatible library); 0.5 μM in water
  5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAGAGATGTGTATAAGAGA-CAG-3′
- Primer 1 (Ti-compatible library); 10 μM in water
  5′-CCATCTCATCCCTGCGTGTCTCCGAC-3′
- Primer 2 (Ti-compatible library); 10 μM in water
  5′-CCTATCCCCTGTGTGCCTTGGCAGTC-3′
- Nextera PCR Enzyme (Epicentre)

Illumina GAIIx or HiSeq 2000-Compatible Library Production

- Target DNA – in water or TE, free of contaminating proteins and salts (see *Note 1*)
- Nextera Enzyme Mix, Illumina GAII-compatible (EZ-Tn5 transposase bound to appended, double-stranded transposon-end DNA; Epicentre). Equal ratios of:

"A-METS"	5′-GCCTCCCTCGCGCCATCAGAGATGTGTATAAGAGACAG-3′
"pMENTS"	3′-TCTACACATATTCTCTGTC-PO_4-5′

and

"B-METS"	5′-GCCTTGCCAGCCCGCTCAGAGATGTGTATAAGAGACAG-3′
"pMENTS"	3′-TCTACACATATTCTCTGTC-PO_4-5′

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- 5× Tagmentation Reaction Buffer (50 mM Tris–OAc, pH 8.0, 25 mM $Mg(OAc)_2$)
- Zymo DNA Clean & Concentrator-5 (Zymo Research; cat. no. D4013 or equivalent)
- Zymo DNA Binding Buffer (Zymo Research; cat. no. D4003-1-L or equivalent)
- Zymo Wash Buffer (Zymo Research; cat. no. D4003-2-6 or equivalent)
- Nuclease-free water
- 2× Nextera PCR Buffer (Epicentre)
- PCR adapters and primers

Illumina GAIIx or HiSeq-Compatible Adapters and Primers*

The sequences of Primer 1 and Primer 2 and parts of Adapter 1 and Adapter 2 correspond to Illumina bridge PCR (bridge polymerase chain reaction bPCR) sequences and are copyrighted by Illumina, Inc. Oligonucleotide sequences © 2006–2010 Illumina, Inc. All rights reserved.

- Adapter 1 (GAII-compatible library); 0.5 μM in water
  5′-AATGATACGGCGACCACCGAGATCTACACGCCTCCCTCGCGCCATCAG-3′
- Adapter 2 (GAII-compatible library); 0.5 μM in water
  5′-CAAGCAGAAGACGGCATACGAGATCGGTCTGCCTTGCCAGCCCGCT-CAG-3′
- Primer 1 (GAII-compatible library); 10 μM in water
  5′-AATGATACGGCGACCACCGA-3′
- Primer 2 (GAII-compatible library); 10 μM in water
  5′-CAAGCAGAAGACGGCATACGA-3′
- Nextera Read 1 Primer; 100 μM in water
  5′-GCCTCCCTCGCGCCATCAGAGATGTGTATAAGAGACAG-3′
- Nextera Read 2 Primer; 100 μM in water
  5′-GCCTTGCCAGCCCGCTCAGAGATGTGTATAAGAGACAG-3′
- Nextera Index Read Primer; 100 μM in water
  5′-CTGTCTCTTATACACATCTCTGAGCGGGCTGGCAAGGCAGACCG-3′
- Nextera PCR Enzyme (Epicentre)

19.2.2 Methods

Roche/454-Compatible Library Preparation

Target DNA is fragmented and tagged with Transposomes containing free transposon ends. Limited-cycle PCR with a four-primer reaction adds Roche/454-compatible adapter sequences (GS FLX Titanium). Optional barcoding is added between the upstream emulsion PCR (emulsion polymerase chain reaction emPCR) adapter and the transposon end (Figure 19.2).

1. Tagmentation reaction
   Tagmentation with Roche/454-compatible Transposomes should predominantly yield fragments of approximately 500–2000 bp (Figure 19.3). The actual fragment size distribution will depend on a number of factors, including the amount and quality of starting DNA.
   1.1 Assemble the following reaction components on ice, in the order listed (see *Note 2*).

Nuclease-free water	*x* μl
Target DNA (see *Note 1*)	50 ng
5× Tagmentation Reaction Buffer	4 μl
Nextera Transposomes (Roche FLX Ti-Compatible)	1 μl
Total reaction volume	20 μl

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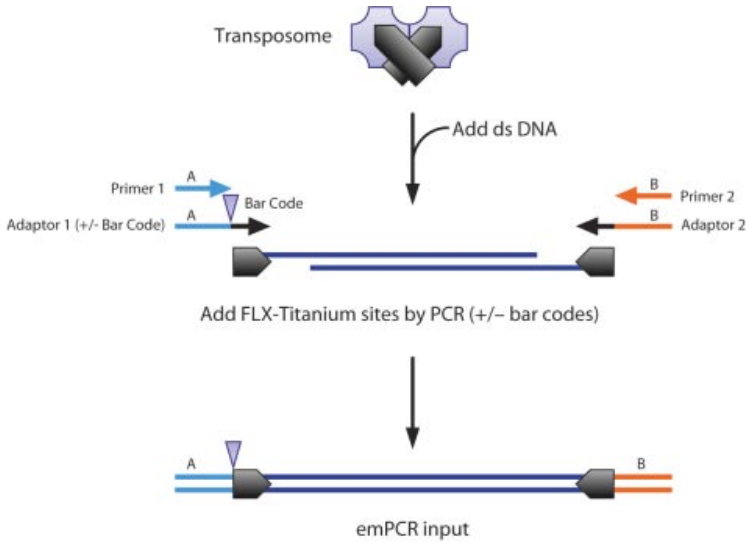

Fig. 19.2 Generation of Roche/454-compatible sequencing libraries. Target DNA is fragmented and tagged with the core Transposomes containing free transposon ends. Limited-cycle PCR with a four-primer reaction adds Roche/454-compatible adapters sequences. Optional barcoding (triangle) is added between the upstream emPCR adapter (blue) and the transposon end (gray).

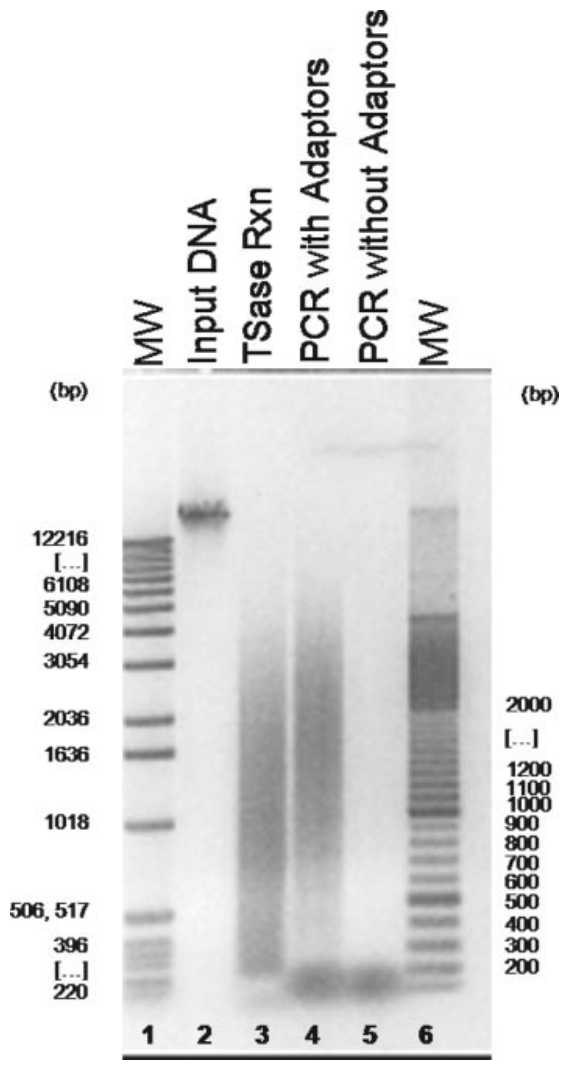

Fig. 19.3 Fragment size distribution of a Roche/454-compatible sequencing library. HeLa DNA (lane 2) was tagmented with Roche/454-compatible Transposomes (lane 3). Limited-cycle PCR added Roche/454 FLX Titanium-compatible adapters (lane 4). The recovered DNA was used directly in emPCR prior to pyrosequencing. Control PCR without Adapter 1 and Adapter 2 (including Primer 1 and Primer 2) confirmed reaction specificity (lane 5).

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1.2 Mix briefly by vortexing and incubate at 55 °C for 5 min. To prevent evaporation, the reaction should be carried out in a thermocycler with a heated lid or the reaction should be overlaid with mineral oil. Adding DNA Binding Buffer in Step 2 stops the reaction.

1.3 Purify the tagmented DNA using a Zymo DNA Clean & Concentrator-5 Kit (or equivalent) according to the manufacturer's instructions (see *Note 3*).

2. Brief Zymo protocol (perform at room temperature)
   - Add 100 μl of DNA Binding Buffer to the 20 μl Tagmentation Reaction from Step 1.2.
   - Mix briefly by vortexing, and transfer the mixture to a Zymo-Spin Column in a collection tube.
   - Centrifuge at 10 000 × *g* for 60 s. Discard the flow-through.
   - Add 250 μl of Wash Buffer to the column. Centrifuge at 10 000 × *g* for 60 s. Discard the flow-through.
   - Repeat the wash step once for a total of two washes.
   - Centrifuge the empty column again at 10 000 × *g* for 60 s to dry and to eliminate any residual Wash Buffer.
   - Transfer the column to a clean and sterile 1.5-ml microcentrifuge tube.
   - Add 11 μl of nuclease-free water directly to the column and incubate at room temperature for 1–2 min. Centrifuge at 10 000 × *g* for 60 s to elute the DNA into the clean microcentrifuge tube.
   - The final eluted volume should be ~10 μl. Use 5 μl of recovered DNA as PCR template (Step 3.1 below).
   - The recovered DNA may be stored at −20 °C.
3. Addition of emPCR-compatible adapters and library enrichment

3.1 Assemble the following reaction components at room temperature:

Nuclease-free water	15 μl
Recovered DNA fragment library	5 μl
2× Nextera PCR Buffer	25 μl
0.5 μM Adapter 1 (FLX or Ti-compatible, See *Note 4*)	1 μl
0.5 μM Adapter 2 (FLX or Ti-compatible)	1 μl
10 μM Primer 1 (FLX or Ti-compatible)	1 μl
10 μM Primer 2 (FLX or Ti-compatible)	1 μl
Nextera PCR Enzyme (2.5 U/μl)	1 μl
Total reaction volume	50 μl

3.2 Incubate the sample in a thermocycler under the following conditions:

72 °C	3 min	(see *Note 5*)
95 °C	30 s	
95 °C	10 s	15 cycles
55 °C	30 s	15 cycles
72 °C	3 min	15 cycles
4 °C	hold	

3.3 Purify the DNA sequencing library using a Zymo DNA Clean & Concentrator-5 kit or equivalent. The anticipated yield is ~300–500 ng of PCR-amplified DNA (Figure 19.3).

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3.4 The recovered DNA is ready to be quantified and used as input for Roche/454 emPCR and sequencing. No changes to the standard Roche/454 protocol are needed (see *Note 6*). Prior to loading onto the sequencer, the library should be quantified and, if needed, size-selected. It is critical to accurately quantify the input DNA amount. Variation in input DNA can affect the molecular weight distribution of libraries. The degree of variance will depend on a number of factors including sample type and purity. For accurate DNA quantitation, any of the following methods can be used: quantitative polymerase chain reaction qPCR, Qubit Fluorometric Quantitation, or NanoDrop. For size-selection, if an stringent size distribution is needed, there are a number of methods that can be used to size select for the required fragment size, including AMPure XP (for removing small fragments), Caliper LabChip XT (for very narrow size selection), or gel extraction.

Illumina GAIIx or HiSeq 2000-Compatible Library Preparation

Target DNA is fragmented and tagged with Transposomes containing transposon ends appended with sequencing tags. Limited-cycle PCR with a four-primer reaction adds Illumina bPCR-compatible adapter sequences. Optional barcoding is added between the upstream bPCR adapter and the sequencing tag on Adapter 2. See Figure 19.4.

1. Tagmentation reaction

   Tagmentation with Illumina GAIIx or HiSeq 2000-compatible Transposomes should predominantly yield fragments approximately 250–1000 bp (Figure 19.5). The actual fragment size distribution will depend on a number of factors, including the amount and quality of starting DNA.

   1.1 Assemble the following reaction components on ice, in the order listed (see *Note 2*):

Nuclease-free water	*x* μl
Target DNA (see *Note 1*)	50 ng
5× Tagmentation Reaction Buffer	4 μl
Nextera Transposomes (Illumina-compatible)	1 μl
Total reaction volume	20 μl

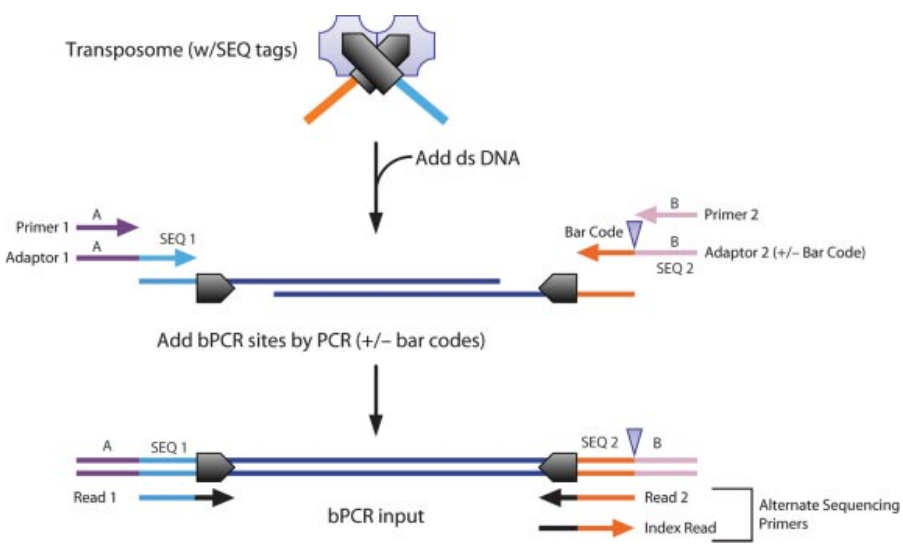

Fig. 19.4 Generation of Illumina GAIIx or HiSeq 2000-compatible sequencing libraries. Target DNA is fragmented and tagged with the core Transposomes containing ends appended with sequencing primer sites. Suppression PCR with a four-primer reaction adds bPCR-compatible adapters to the core sequencing library. Optional barcoding (triangle) is added between the downstream bPCR adapter and the core sequencing library adapter.

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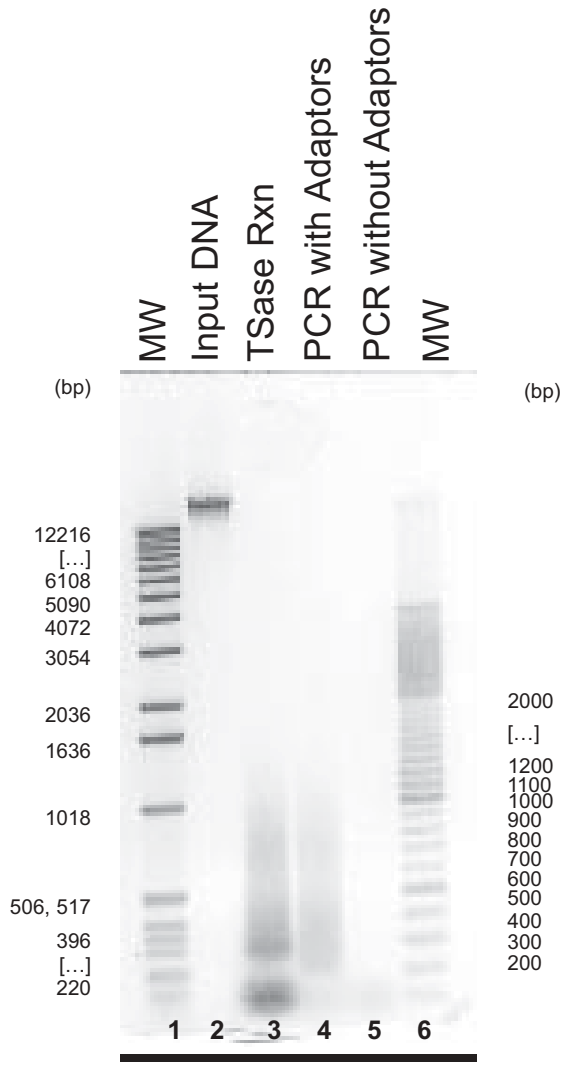

Fig. 19.5 Fragment size distribution of an Illumina GAIIx or HiSeq 2000-compatible sequencing library. HeLa DNA (lane 2) was tagmented with Illumina/Solexa-compatible Transposomes appended with sequencing tags (lane 3). Limited-cycle PCR added Illumina bPCR-compatible adapters (lane 4). The recovered DNA was used directly in bPCR prior to sequencing on the Illumina GAII or HiSeq 2000 instrument. Control PCR without Adapter 1 and Adapter 2 (including Primer 1 and Primer 2) confirmed reaction specificity (lane 5).

1.2 Mix briefly by vortexing, and incubate at 55 °C for 5 min. To prevent evaporation, the reaction should be carried out in a thermocycler with a heated lid or the reaction should be overlaid with mineral oil. Adding DNA Binding Buffer in Step 2 stops the reaction.

1.3 Purify the DNA sequencing library using a Zymo DNA Clean & Concentrator-5 Kit (or equivalent) according to the manufacturer's instructions (see *Note 3*).

2. Brief Zymo protocol (perform at room temperature)
   - Add 100 μl of DNA Binding Buffer to the 20 μl tagmentation reaction from Step 1.2.
   - Mix briefly by vortexing and transfer the mixture to a Zymo-Spin Column in a collection tube.
   - Centrifuge at 10 000 × *g* for 60 s. Discard the flow-through.
   - Add 250 μl of Wash Buffer to the column. Centrifuge at 10 000 × *g* for 60 s. Discard the flow-through.
   - Repeat the wash step once for a total of two washes.
   - Centrifuge the empty column again at 10 000 × *g* for 60 s to dry and to eliminate any residual Wash Buffer.
   - Transfer the column to a clean and sterile 1.5-ml microcentrifuge tube.
   - Add 11 μl of nuclease-free water directly to the column and incubate at room temperature for 1–2 min. Centrifuge at 10 000 × *g* for 60 s to elute the DNA into the clean microcentrifuge tube.

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- The final eluted volume should be ~10 μl. Use 5 μl of recovered DNA as PCR template (see Step 3.1 below).
- The recovered DNA may be stored at −20 °C.

3. Addition of bPCR-compatible adapters and library enrichment

3.1 Assemble the following reaction components at room temperature:

Nuclease-free water	15 μl
Recovered DNA fragment library	5 μl
2× Nextera PCR Buffer	25 μl
0.5 μM Adapter 1 (Illumina GAIIx or HiSeq 2000-compatible)	1 μl
0.5 μM Adapter 2 (Illumina GAIIx or HiSeq 2000-compatible, See *Note 7*)	1 μl
10 μM Primer 1 (Illumina GAIIx or HiSeq 2000-compatible)	1 μl
10 μM Primer 2 (Illumina GAIIx or HiSeq 2000-compatible)	1 μl
Nextera PCR Enzyme (2.5 U/μl)	1 μl
Total reaction volume	50 μl

3.2 Incubate the samples in a thermocycler under the following conditions:

72 °C	3 min	(see *Note 8*)
95 °C	30 s	
95 °C	10 s	9 cycles
62 °C	30 s	9 cycles
72 °C	3 min	9 cycles
4 °C	hold	

3.3 Purify the tagged DNA fragments using a Zymo DNA Clean & Concentrator-5 kit or equivalent. The anticipated yield is ~300–500 ng of PCR-amplified DNA (Figure 19.5).

3.4 Prior to loading onto the sequencer, the library should be quantified and, if needed, size-selected. It is critical to accurately quantify the input DNA amount. Variation in input DNA can affect the molecular weight distribution of libraries. The degree of variance will depend on a number of factors including sample type and purity. For accurate DNA quantitation, any of the following methods can be used: quantitative PCR, Qubit Fluorometric Quantitation, or NanoDrop. For size-selection, if an stringent size distribution is needed, there are a number of methods that can be used to size select for the required fragment size, including AMPure XP (for removing small fragments), Caliper LabChip XT (for very narrow size selection), or gel extraction.

3.5 The recovered DNA is ready to be used as input for Illumina GAII or HiSeq 2000 bPCR and cluster formation. No changes to the standard Illumina protocol are need for cluster formation. However, alternate sequencing primers must be included during the sequencing reactions as described below (see *Note 8*).

a. *For paired-end reads.* Illumina paired-end sequencing mixes (HP6 and HP7) contain the Illumina GAII or HiSeq 2000 sequencing primers.

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The Nextera sequencing primers are compatible with the Illumina GAII or HiSeq 2000 sequencing primers and can be used together in the same flow cell channel.

- Paired-end read 1. Dilute 100 μM Nextera Read 1 Primer 1:200 into Illumina GAIIx or HiSeq 2000 Sequencing Mix HP6.
- Index read. Dilute 100 μM Nextera Index Read Primer 1:200 into Illumina GAIIx or HiSeq 2000 Sequencing Mix HP8.
- Paired-end read 2. Dilute 100 μM Nextera Read 2 Primer 1:200 into Sequencing Mix HP7.
- Alternatively, if it is required to perform sequencing in the presence of only the Nextera primers, the 100 μM Nextera sequencing primers can be diluted 1:200 into Hybridization Buffer (Illumina; cat. no. GA0084204-HT1) or 5× SSC, 0.05% Tween-20.

b. *For Single Reads.* Illumina single-read sequencing mix does not contain the Illumina sequencing primer.

- Single-read sequencing. Dilute Nextera Read 1 Primer 1:200 into Sequencing Mix HP4.
- Index read. Dilute Nextera Index Read Primer 1:200 into Hybridization Buffer (Illumina; cat. no. GA0084204-HT1) or 5× SSC, 0.05% Tween-20.

Notes

1. The quality of the starting DNA is critical. Contaminants such as protein and RNA may interfere with accurate quantification and/or inhibit the transposome fragmentation and tagging reaction if present in the DNA preparation. If DNA purity is in question, the DNA should be cleaned using Zymo Genomic DNA Clean & Concentrator, or equivalent (Zymo Research; cat. no. D4010) prior to the tagmentation reaction.
2. The tagmentation reaction does occur, although very slowly, at room temperature. Therefore, it is recommended that the components be assembled on ice and immediately incubated at 55 °C.
3. The tagmentation reaction results in the simultaneous tagging and fragmentation of the target DNA. However, the transposase remains tightly bound to the ends of the resulting fragment. It is necessary to remove the transposase with chaotropic salts prior to tagging the 3′ ends and adding platform-specific adapters by PCR. Equivalent kits can also be used; however, care must be taken not to denature the double-stranded DNA fragments.
4. For a barcoded Roche-compatible library, replace Adapter 1 with a barcoded Adapter 1 with the sequence composition 5′-CCATCTCATCCCTGCGTGTCTCC-GACTCAG-[BARCODE]-AGATGTGTATAAGAGACAG-3′. The other three primers and the cycling conditions remain unchanged.
5. It is critical to perform the 72 °C extension step to tag the 3′ ends of the DNA fragments *before* denaturing the PCR templates. The 3′ ends are tagged by polymerase extension from the 3′ end of the genomic fragment and displacement of the MENTS oligonucleotide.
6. Nextera-generated and "standard" libraries can be sequenced in the same region of a plate. Perform the sequencing reactions with the standard Roche/454 sequencing primers. Sequencing reads will contain any added barcode sequence and the 19-base transposon end sequence at the 5′ end of all reads. The barcode sequence can be used to bin the sequence reads. The 19-base transposon end sequence (5′-AGATGTGTATAAGAGACAG-3′) should be filtered out prior to assembly and analysis.
7. For a barcoded Illumina-compatible library, replace Adapter 2 with a barcoded Adapter 2 with the sequence composition 5′-CAAGCAGAAGACGGCATAC-GAGA-[BARCODE]-TCGGTCTGCCTTGCCAGCCCGCTCAG-3′. The other three primers and the cycling conditions remain unchanged.

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8. It is *critical* to use the Nextera Read 1, Nextera Read 2, and Index Read primers during cluster sequencing. While the resulting libraries are compatible with Illumina GA II bPCR and cluster formation, the transposon-derived sequencing primer sites are *not compatible with Illumina GAII or HiSeq 2000 sequencing primers*. The 19-bp transposon DNA sequence is present at the 5′ end of all Illumina-compatible libraries. However, the 19-bp transposon DNA sequence is NOT sequenced on the Illumina platform. The Nextera Read 1 and Read 2 primers anneal to this sequence so that the first nucleotide sequenced is target DNA.

19.3 Perspectives

The current library preparation methods for NGS are time-consuming and prone to considerable sample loss. Even before library preparation, the recovered DNA must be purified and end-polished. Epicentre's Nextera technology offers many advantages over current library preparation methods, such as a streamlined workflow that can result in substantial savings in time and cost. The method is scalable and requires less starting DNA than current procedures.

As described here, Nextera technology adapts *in vitro* transposition, a powerful technique that can simultaneously fragment and tag genomic DNA, by using optimized transposases and incorporating engineered free transposon ends. By additional manipulation, libraries containing complementary or independent adapter sequences can also be efficiently constructed and amplified before sequencing on NGS platforms.

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