Nextera® Mate Pair Library Preparation Kit

An optimized library preparation method for long-insert libraries, empowering *de novo* sequencing and structural variant detection.

Highlights

- **Fast and Simple Mate Pair Preparation**
  A simple tagmentation reaction and low DNA input enable library preparation in less than 2 days
- **Dual Protocol Flexibility**
  Gel-free and gel-plus protocols enable a range of applications, including *de novo* assembly and structural variation detection
- **High Data Quality**
  Highly diverse libraries maximize data yield
- **End-to-End Mate Pair Solution**
  Conveniently bundled kit includes reagents and indexes for efficient mate pair preparation

Introduction

Mate pair library preparation generates long-insert paired-end libraries for sequencing. The Nextera Mate Pair Library Preparation Kit offers two methods, gel-free and gel-plus, to support various applications and input requirements. The robust, low-input, gel-free protocol yields high-diversity libraries that enable deeper sequencing. The size-selection step in the gel-plus protocol generates fragments with a narrow size distribution for structural variation detection. Libraries prepared with the gel-plus protocol also provide sequence information for larger repeat regions, empowering *de novo* genome assembly.

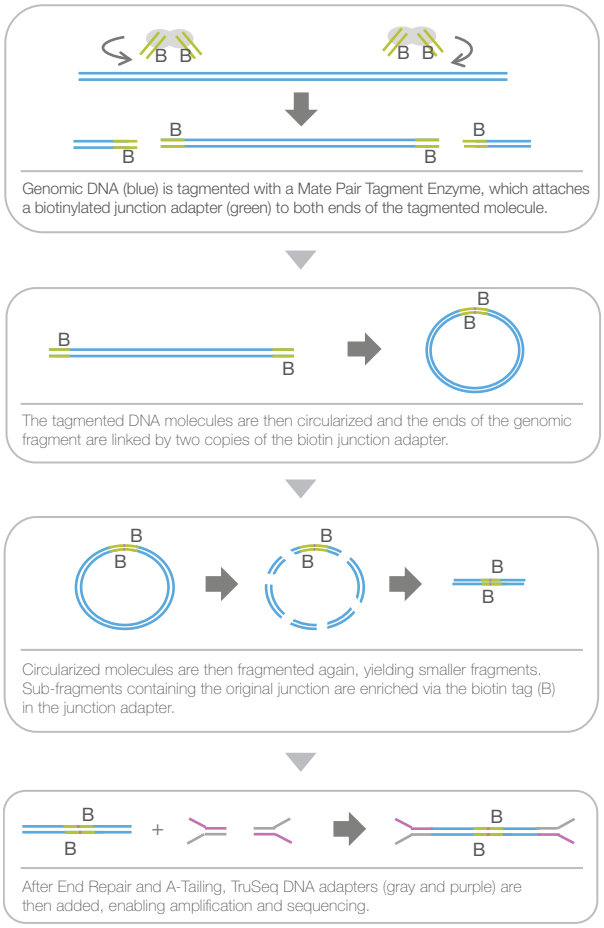
Simplified Mate Pair Workflow

The Nextera Mate Pair protocol provides a simple mate pair workflow for preparing sequencing-ready libraries in less than 2 days (Figure 1). Master-mixed TruSeq® DNA Library Preparation reagents minimize the number of assay steps, reducing hands-on time to as little as 3 hours. The Nextera "tagmentation" reaction utilizes a specially engineered transposome, the Mate Pair Tagment Enzyme, to simultaneously fragment and tag the DNA sample. This simplified method only biotinylates DNA molecules at the sites of fragmentation, avoiding troublesome internal biotinylation.

Dual Protocol Flexibility

The flexibility of the Nextera Mate Pair Library Preparation Kit stems from the availability of two different size-selection options (Table 1). The gel-free protocol, which requires only 1 µg DNA, provides highly diverse mate pair libraries with a broad range of fragment sizes (Figure 2A). This protocol is ideal for routine *de novo* assembly of small bacterial genomes, or for the robust generation of mate pair data for samples with limited DNA. The gel-free protocol offers a faster, simplified option with a lower DNA input requirement to streamline mate pair studies.

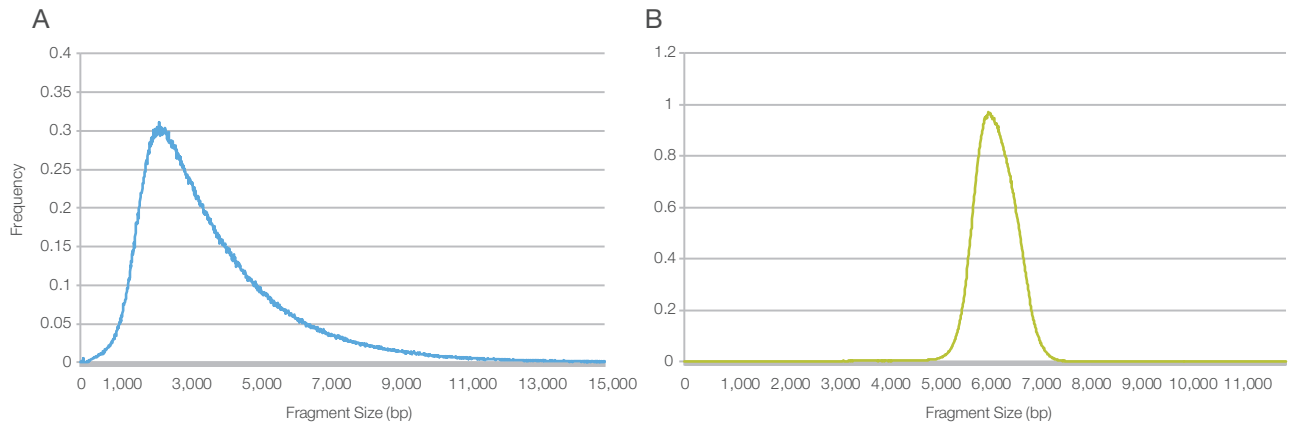
Figure 1: Nextera Mate Pair Workflow



The Nextera Mate Pair Library Preparation Kit features a simple workflow that enables library preparation in less than 2 days. It supports a range of fragment sizes ~2–8 kb, though 2–5 kb fragments are observed at higher frequencies. Observed fragments may be up to 12 kb in length.

The gel-plus protocol, which requires 4 µg DNA and standard agarose gels or Sage Pippin Prep gels[1], offers a more stringent size selection process. The gel-plus protocol produces libraries with narrower size distributions to facilitate structural variation detection (Figure 2B and Figure 3). However, creating gel-plus libraries becomes more difficult as the fragment lengths increase. Greater control over fragment sizes is ideal for more challenging mate pair applications, such as *de novo* assembly of complex genomes and structural variation detection.

Figure 2: Fragment Size Distribution with Dual Protocols



Panel A shows the fragment size distribution of an *E. coli* mate pair library prepared using the Nextera Mate Pair gel-free protocol, resulting in a broad fragment size distribution. Panel B shows the narrow fragment size distribution of an *E. coli* mate pair library generated with the Nextera Mate Pair gel-plus protocol with automated size selection using the Pippin Prep platform.

Highly Diverse Libraries

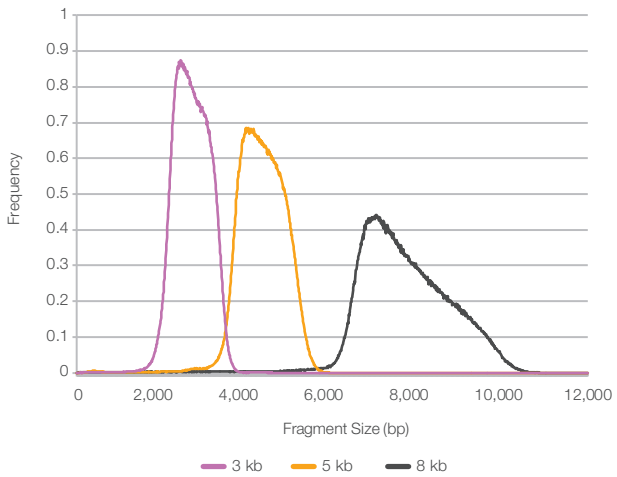
The Nextera tagmentation reaction drives the creation of highly diverse libraries (Table 2) that are compatible with all Illumina sequencing systems. Library diversity is defined as the number of unique fragments in a given library. The Nextera Mate Pair protocol allows for the creation of millions of unique fragments. Such high library diversity generates fewer duplicate reads and yields larger volumes of data.

The Nextera Mate Pair Library Preparation Kit also provides identifiable junction sequences that mark fragment ends, drastically simplifying data analysis. The presence of searchable junction sequences allows for accurate fragment identification and enables sequencing of longer read lengths, as mate pair junctions can be precisely identified and trimmed accordingly.

Mate Pair Preparation Solution

In addition to Nextera Mate Pair reagents, the comprehensive Nextera kit contains TruSeq DNA library preparation reagents and indexes. TruSeq on-bead reactions follow the tagmentation and circularization steps (Figure 1), simplifying the purification workflow and reducing sample loss. This integrated solution streamlines the library preparation workflow, maximizing sequencing efficiency with more samples per lane and enabling rapid multiplexed sequencing of small genomes. The Nextera Mate Pair Library Preparation Kit is compatible with TruSeq DNA Library Preparation adapter indexing, supporting 12 indexes per kit for a scalable experimental approach. With all necessary reagents included in one convenient, cost-effective bundle, the Nextera Mate Pair Library Preparation Kit is an all-in-one solution for fast and simple mate pair library preparation.

Figure 3: Fragment Size Distribution



This figure shows fragment size distributions of three *E. coli* mate pair libraries (3 kb, 5 kb, and 8 kb) created from the same tagmentation reaction. These distributions were generated following the Nextera Mate Pair gel-plus protocol with agarose gel size selection. Though 8 kb fragments are possible with this protocol, 2–5 kb fragments generate libraries with the highest yield and diversity.

Table 1: Nextera Mate Pair Protocols

Protocol	DNA Input	Number of Samples	Size Selections Per Sample	Number of Libraries
Gel-Free	1 µg	48	N/A	48
Gel-Plus with Pippin Prep size selection	4 µg	12	1	12
Gel-Plus with agarose size selection	4 µg	12	Up to 4	Up to 48

Table 2: Nextera Mate Pair Library Diversity*

Preparation	Input DNA	Fragment Size	Diversity†
Nextera Mate Pair Gel-Free	1 µg	~2–8 kb	860 million
Nextera Mate Pair Gel-Plus	4 µg	~2–4 kb	568 million
Nextera Mate Pair Gel-Plus	4 µg	~5–7 kb	396 million
Nextera Mate Pair Gel-Plus	4 µg	~6–10 kb	102 million

* This table demonstrates example diversity values, with diversity reported in number of unique fragments. Actual diversities achieved with this kit may vary and depend on several factors, including DNA input quantity, DNA quality, and precise execution of the protocol.

† Library diversity was calculated from the number of unique read pairs observed in a data set, using a method based on the Lander-Waterman equation[2].

Ordering Information

Product	Catalog No.
Nextera Mate Pair Library Preparation Kit	FC-132-1001

This kit contains Nextera Mate Pair reagents and TruSeq reagents and indexes.

Summary

With a fast and easy workflow, the Nextera Mate Pair Library Preparation Kit allows the construction of high-quality sequencing libraries in less than 2 days. The gel-free and gel-plus options provide flexibility for various applications. Transposome-mediated tagmentation, identifiable junction sequences, and indexing capability make the Nextera Mate Pair Library Preparation Kit a simple and easy solution for mate pair applications.

References

1. www.sagescience.com/products/pippin-prep
2. Lander ES, Waterman MS (1988) Genomic mapping by fingerprinting random clones: a mathematical analysis. Genomics 2: 231–9.

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