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Nextera® Mate Pair Library Prep Reference Guide

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Document # 15035209 v02
January 2016

Customize a short end-to-end workflow guide with the Custom Protocol Selector
support.illumina.com/custom-protocol-selector.html

Revision History

Part #	Date	Description of Change
Document # 15035209 v02	January 2016	• Removed reference to obsolete Experienced User Cards and added reference to the Custom Protocol Selector. • Renamed and combined some procedures as needed to improve continuity. • Identified where in the protocol the kit boxes are used. See Supporting Information. • Modified protocol to reflect current reagents.
Document # 15035209 v01	October 2015	• Added storage times for safe stopping points. • Updated the run time in the Pippin Prep size selection step. • Added a purify DNA step to the Pippin Prep size selection step. • Updated volumes for water and circularization ligase in the Circularize DNA step. • Updated volume for water and changed the type of PCR mix and volume in the Amplify DNA step. • Removed box and tube part numbers from Kit Contents. • Updated PCR reagent in the TruSeq DNA LT Library Prep Kit - PCR Box. • Revised step-by-step instructions to be more succinct.
Part # 15035209 Rev. D	May 2013	• Reorganized information to improve usability. • Explained the degree of difficulty using the gel-plus protocol with larger fragment lengths. • Removed the Illumina Nebulizers and Nebulization buffer kit. This kit is no longer available. Alternate sources are listed. • Corrected the dilution factor calculation in the Clean Up DNA step. • Updated nebulization buffer volume from 400 µl to 550 µl in the Sheer Circularized DNA step.
Part # 15035209 Rev. C	February 2013	• Corrected the location of the Select Fragment Size step in the workflow. It is now before the Circularize DNA step. • Correctly identified index adaptor AD016 in the Index Adaptor Sequences section and the Pooling Guidelines section. • Updated Zymo DNA Binding Buffer volume from 2000 ml to 2000 µl in the alternative procedure to Shear Circularized DNA using nebulization.
Part # 15035209 Rev. B	January 2013	• Updated the End Repair Reaction mix volume to 100 µl in the End Repair step. • Updated the PCR Reaction Mix volume to 50 µl in the Amplify DNA step.
Part # 15035209 Rev. A	December 2012	Initial release

Material # 20002336
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Overview

Chapter 1

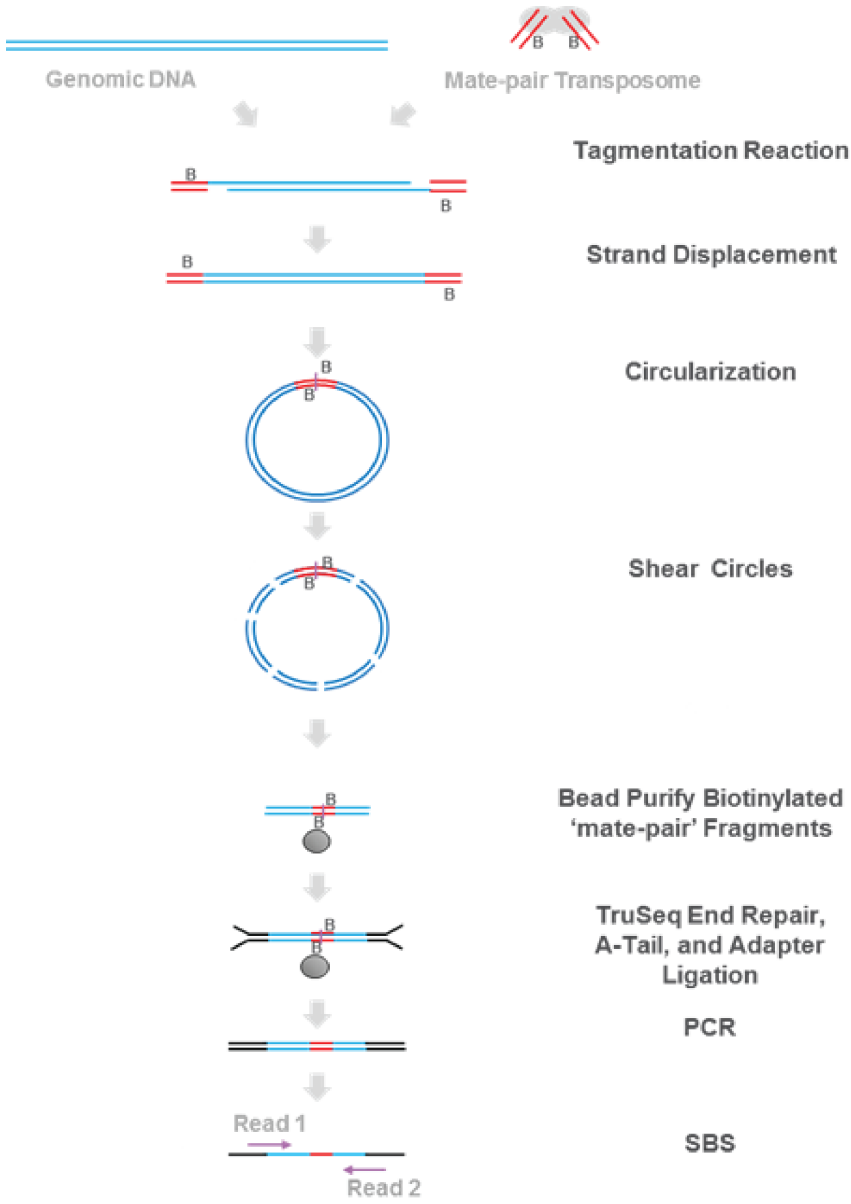
Introduction

This protocol explains how to generate mate pair libraries from genomic DNA for paired-end sequencing using the Illumina Nextera Mate Pair Library Prep Kit.

The Nextera Mate Pair protocol includes the following features:

- Transposome DNA fragmentation and adapter tagging
- An identifiable mate pair junction sequence
- TruSeq DNA Library Prep master-mixed reagents
- TruSeq DNA Library Prep adapter indexing compatibility (includes 12 indexes)
- On-bead reactions for ease of automation, reduced sample loss, and simple purification steps
- Gel-free protocol
- [Optional] Gel-plus protocol

Figure 1 Nextera Mate Pair Library Prep Kit Procedure



Gel-Free and Gel-Plus Versions

- The gel-free protocol is shorter, more robust, yields a higher diversity of fragments, and requires less input DNA. This protocol produces a broader range of fragment sizes (2 kb to 15 kb). Median fragment size is 2.5 kb to 4 kb. This protocol can generate 48 libraries from 48 independent samples.
- The gel-plus protocol allows fragment size selection. This protocol is used for mate pair applications requiring a narrower range of fragment sizes or larger fragment sizes. Libraries with larger fragment sizes have lower library yield and diversity. The range of fragment size is determined by the gel-based size selection process. This protocol can prepare 12 libraries with single size selections per sample or 48 libraries with multiple size selections from up to 12 samples.

Workflow and final application of data determine which protocol to use.

Table 1 Summary Table

Protocol	DNA Input	Number of Samples	Number of Size-Selections per Sample	Number of Libraries
Gel-Free Protocol	1 µg	48	n/a	48
Gel-Plus Protocol Using Pippin Prep Size Selection	4 µg	12	1	12
Gel-Plus protocol Using Agarose Gel Size Selection	4 µg	12	up to 4	up to 48

DNA Input Recommendations

The Nextera Mate Pair Library Prep Kit Kit protocol is optimized as follows:

- Gel-free protocol—1 µg input DNA
- Gel-plus protocol—4 µg input DNA

Quantify the input DNA.

Input DNA Quantification

The enzymatic DNA fragmentation used in the Nextera Mate Pair library prep protocol is more sensitive to DNA input compared to mechanical fragmentation. Success depends on accurate quantification of input DNA.

Use a fluorometric-based method to quantify input DNA. Avoid methods that measure total nucleic acid, such as NanoDrop or other UV absorbance methods. For example, if you use the Qubit dsDNA BR Assay system, use 2 µl of each DNA sample with 198 µl of the Qubit working solution.

Assess DNA Quality

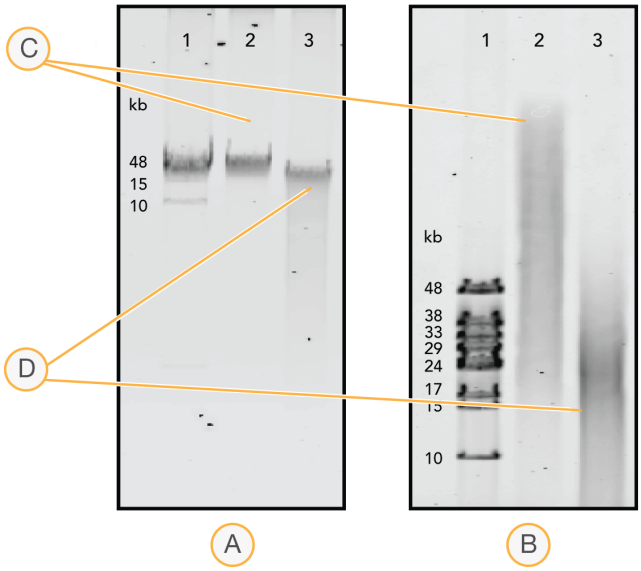
For successful library generation, use high-quality, high molecular weight genomic input DNA. Degraded DNA can result in fragment sizes below the desired size range and diminished library yields and diversity.

Run a small amount of input DNA on a low-percentage agarose gel. Run high-quality DNA as a high molecular weight band with the majority of DNA greater than 50 kb in size and minimal lower molecular weight smearing. If the majority of the DNA is below 50 kb or smearing is visible, the DNA might be degraded. You can still use the Nextera Mate Pair Library Prep Kit protocol with partially degraded DNA, but you might need to reduce the amount of transposome during the tagmentation step.

The following figure shows agarose gel analysis of 2 genomic DNA samples, where approximately 200 ng of sample was loaded per lane.

- Figure A is a 0.6% standard agarose gel stained with ethidium bromide.
- Figure B is a higher resolution Pulse Field Gel, which more clearly shows the differences in quality and integrity.

Figure 2 Analysis of Genomic DNA Sample Integrity



A 0.6% Standard Agarose gel stained with ethidium bromide
B Higher resolution pulse field gel
C Intact, high-quality DNA has large fragments (> 50 kb)
D Partially degraded DNA has small fragments (< 29 kb)

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Additional Resources

Visit the Nextera Mate Pair Library Prep kit support page on the Illumina website for documentation, software downloads, training resources, and information about compatible Illumina products.

The following resources are available for download from the Illumina website.

Resource	Description
Custom Protocol Selector	http://support.illumina.com/custom-protocol-selector.html A wizard for generating customized end-to-end documentation that is tailored to the library prep method, run parameters, and analysis method used for the sequencing run.
Nextera Mate Pair Library Prep Protocol Guide (document # 1000000003672)	Provides only protocol instructions. The protocol guides are intended for experienced users.
Nextera Mate Pair Library Prep Checklist for Gel-Free (document # 1000000003674)	Provides a checklist of the protocol steps for the gel-free option. The checklist is intended for experienced users.
Nextera Mate Pair Library Prep Checklist for Gel-Plus (document # 1000000003675)	Provides a checklist of the protocol steps for the gel-plus option. The checklist is intended for experienced users.

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Protocol

Introduction

This chapter describes the Nextera Mate Pair protocol.

- Review Best Practices before proceeding. See *Additional Resources* on page 7 for information about accessing Nextera Mate Pair Best Practices on the Illumina website.
- Follow the protocols in the order shown using the specified volumes and incubation parameters.

Prepare for Pooling

If you plan to pool libraries, record information about your samples before beginning library prep. Different methods are available depending on the sequencing instrument you are using. See the Nextera Mate Pair Library Prep support page for more information.

Review the planning steps in the *Pooling Guide* when preparing libraries for Illumina sequencing systems that require balanced index combinations.

Nextera Mate Pair Library Prep Workflow

Tagment Genomic DNA

This step uses the Nextera Mate Pair transposome to tagment gDNA, which is a process that fragments DNA and then tags the DNA with an adapter sequence in a single step.

Consumables

- Mate Pair Tagment Enzyme
- Tagmentation Buffer
- RSB (Resuspension Buffer)
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tubes (2)
- gDNA:
  - [Gel-free] 1 µg
  - [Gel-plus] 4 µg
- Zymo Genomic DNA Clean & Concentrator kit

NOTE

See *Purify the Tagmentation Reaction [Alternative Procedure]* on page 37 for an alternative tagmentation purification procedure using AMPure XP beads as an alternate to Zymo columns. When using the gel-free protocol, the alternative procedure offers a more automation friendly alternative.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
Tagment Buffer Mate Pair	-25°C to -15°C	Place on ice.
Mate Pair Tagment Enzyme	-25°C to -15°C	Place on ice.
gDNA	-25°C to -15°C	Place on ice.

2 Preheat a heat block to 55°C.

3 Quantify DNA using a fluorometric-based method.

Procedure

1 Add the following items in the order listed to a new 1.7 ml microcentrifuge tube.

Item	Gel-Free Volume (µl)	Gel-Plus Volume (µl)
gDNA	x µl (1 µg)	x µl (4 µg)
Water	76–x	308–x
Tagment Buffer Mate Pair	20	80
Mate Pair Tagment Enzyme	4	12
Total	100	400

2 Flick to mix, and then centrifuge briefly. Repeat.

3 Incubate at 55°C for 30 minutes.

Purify the Tagmentation Reaction

This step uses a Zymo Genomic DNA Clean & Concentrator to purify the tagmented DNA.

1 Add 2 volumes of Zymo ChIP DNA Binding Buffer to the tagmentation reaction. Pipette to mix.

2 Transfer up to 800 µl of mixture to a Zymo-Spin IC-XL column in a collection tube.

3 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.

4 Transfer remaining tagmentation mixture to the same Zymo-Spin IC-XL column.

5 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.

6 Wash 2 times as follows.

   a Add 200 µl Zymo DNA Wash Buffer.
   b Centrifuge at 10,000–16,000 × g for 1 minute.
   c Discard the flow-through.

7 Centrifuge the empty column at 10,000–16,000 × g for 1 minute with lid open. Discard the flow-through and the collection tube.

8 Transfer the column to a new 1.7 ml microcentrifuge tube.

9 Add 30 µl RSB.

10 Incubate at room temperature for 1 minute.

11 Centrifuge at 10,000–16,000 × g for 1 minute.

12 To assess tagmentation, dilute 1 µl DNA with water and run on an Agilent Technology 2100 Bioanalyzer using a DNA 12000 LabChip.
   - [Gel-free] 1 µl water
   - [Gel-plus] 7 µl water

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 24 hours.

Strand Displacement

The previous step left a short single-stranded gap in the tagmented DNA. This step repairs that gap and ensures that all fragments are ready for circularization.

Consumables

- 10X Strand Displacement Buffer
- dNTPs
- Strand Displacement Enzyme Mix
- RSB (Resuspension Buffer)

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
10X Strand Displacement Buffer	-25°C to -15°C	Thaw at room temperature. Place on ice.
dNTPs	-25°C to -15°C	Thaw at room temperature. Place on ice.
Strand Displacement Polymerase	-25°C to -15°C	Place on ice.

2 Preheat a heat block to 20°C.

Procedure

1 Add the following items in the order listed to the microcentrifuge tube.

Item	Gel-Free Volume (µl)	Gel-Plus Volume (µl)
Tagmented DNA Sample	30	30
Water	10.5	132
10x Strand Displacement Buffer	5	20
dNTPs	2	8
Strand Displacement Polymerase	2.5	10
Total	50	200

2 Flick to mix, and then centrifuge briefly.

3 Incubate at 20°C for 30 minutes.

Purify the DNA

This step uses AMPure XP beads to purify the DNA from the Strand Displacement Reaction mix and remove short fragments (< 1500 bp).

Consumables

- RSB (Resuspension Buffer)
- AMPure XP beads
- Freshly prepared 70% ethanol (EtOH)
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube

About Reagents

- Vortex AMPure XP beads before each use.
- Vortex AMPure XP beads frequently to make sure that beads are evenly distributed.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
AMPure XP Beads	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 Prepare fresh 70% ethanol (800 µl per sample).

Procedure

1 Add the following items in the order listed to the 1.7 ml microcentrifuge tube.

Item	Gel-Free Volume (µl)	Gel-Plus Volume (µl)
Strand Displaced DNA	50	200
Water	50	0
AMPure XP Beads	40	100
Total	140	300

Success of this step depends on accurate ratio of beads to DNA (eg, 0.4x).

2 Flick to mix, and then centrifuge briefly.

3 Incubate at room temperature for 15 minutes. Flick every 2 minutes.

4 Centrifuge briefly.

5 Place on a magnetic rack for 5 minutes.

6 Remove and discard all supernatant.

7 Wash 2 times as follows.

   a Add 400 µl freshly prepared 70% EtOH.
   b Incubate on the magnetic rack for 30 seconds.
   c Remove and discard all supernatant.

8 Air-dry on the magnetic rack for 10–15 minutes.

9 Remove from the magnetic rack.

10 Add 30 µl RSB. Flick the tube to mix.

11 Centrifuge briefly.

12 Incubate at room temperature for 5 minutes.

13 Place on the magnetic rack for 5 minutes.

14 Transfer all supernatant to a new 1.7 ml microcentrifuge tube.

15 Select from the following options:
- [Gel-free] Proceed to *Circularize DNA* on page 22.
- [Gel-plus] Proceed to *Select Fragment Size (Gel-Plus Only)* on page 17.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 7 days.

Select Fragment Size (Gel-Plus Only)

This step offers a precise size-selection process and allows you to generate libraries with large fragment sizes and tight distributions.

The size of the fragments selected determines the distance between the paired reads during sequencing. Libraries with larger fragment sizes have lower yields and diversity. The fragment size you decide to use depends on the design of your experiment, the application of the data set, and the fragment size distribution generated by the tagmentation process.

In mate pair library prep, fragment size selection and inefficiencies in the purification step can result in sample loss and sample-to-sample variability in the final libraries. Success depends on using the correct amount of input DNA, accurately quantifying the input DNA, and selecting the appropriate fragment size.

To avoid having too little DNA in the protocol and low library yield, use a broad range of fragment sizes or increase the number of PCR cycles at the end of the procedure. The goal is to recover 150–500 ng of DNA per size selection. Select a broader range of fragment sizes to increase the chance of recovering DNA within this range.

The following size selection procedures are appropriate for the gel-plus protocol.

Size Selection Procedure	Mate Pair Fragment Size
Sage Science Pippin Prep with 0.75% Cassette	up to 8 kb
Agarose gel electrophoresis and DNA extraction with Zymo Purification kit	up to 10 kb

NOTE
Other electrophoresis conditions and DNA extraction methods might yield comparable or superior results. If you have an optimized gel sizing protocol that produces consistent results, you can use your protocol for size selection.

Pippin Prep Size Selection

This size selection method allows only a single size selection per sample.

Elute fragments with a broad range of sizes (3–6 kb in width), increasing in width with increasing fragment length (eg 2–5 kb, 4–8 kb, or 6–12 kb). Select a broad fragment size range to increase the amount of recovered DNA and to generate higher diversity libraries. Narrower fragment size ranges result in a smaller amount of recovered DNA, diminished library yields, and lower diversity libraries.

Verify the size of the purified DNA sample before selecting the elution range. To achieve maximum recoveries of DNA, use the peak fragment size from the Bioanalyzer electropherogram to select the elution range.

Consumables

- Pippin Prep 0.75% agarose cassette and solutions (catalog # CSD7510)
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tubes
- Zymo Genomic DNA Clean & Concentrator kit

Preparation

1 Review the Sage Science Pippin Prep system documentation.

Procedure

1 Load 30 µl AMPure purified DNA on single lane of a Pippin Prep 0.75% agarose cassette.

2 Use the Pippin Prep Protocol range mode to define the start and end of the desired sample elution size.

NOTE
To avoid sample loss, make sure that you seal the elution wells with the adhesive tape provided with the 0.75% cassette.

3 Run for the maximum run time allowed for the cassette.

4 When the run finishes, transfer the elution to a new 1.7 ml microcentrifuge tube.

Purify the DNA

This step purifies the DNA using a Zymoclean Genomic DNA Clean & Concentrator Kit.

1 Add 5 volumes of ChIP Binding Buffer to each volume of DNA (eg, 5:1, 500 µl to 100 µl DNA). Pipette to mix.

2 Transfer to a Zymo-Spin IC-XL column in a collection tube.

3 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.

4 Wash 2 times as follows.

a Add 200 µl Zymo DNA Wash Buffer.
b Centrifuge at 10,000–16,000 × g for 1 minute.
c Discard the flow-through.

5 Discard the collection tube.

6 Transfer the column to a new 1.7 ml microcentrifuge tube.

7 Add 10 µl RSB to each column.

8 Incubate at room temperature for 1 minute.

9 Centrifuge at 10,000–16,000 × g for 30 seconds.

10 [Optional] To quantify DNA, run 1 µl undiluted elution on an Agilent Technology 2100 Bioanalyzer using a DNA 12000 LabChip.

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 24 hours.

Agarose Size Selection

This size selection method allows multiple size selections per sample.

Select a fragment range of several kb in width (eg 4–6 kb, 7–10 kb or 9–12 kb). A broader range of fragment size increases the yield of DNA recovered and increases the chances of generating a high diversity mate pair library.

This agarose size selection procedure has been optimized for the following equipment.

Equipment	Dimensions	Supplier and Part Number
Gel tray and electrophoresis unit	12 cm width × 14 cm length	Fisher Scientfic, part # 09-528-110B
Gel comb with wide wells	9 mm width × 1 mm length	Fisher, Scientific part # OWB212

If you use alternative electrophoresis equipment, make sure that the agarose gel and well dimensions are similar. Optimize the voltage and run times before processing a sample.

Consumables

- RSB (Resuspension Buffer)
- 0.6% Megabase Agarose
- 50 X TAE Buffer
- 1 kb plus DNA ladder
- 6X Gel Loading Dye
- Agarose Gel Electrophoresis Equipment
- SYBR safe DNA Gel Stain
- Clean scalpels
- Zymoclean Large Fragment DNA Recovery Kit
- 3.5 ml screw cap tubes
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tubes

Preparation

1. Prepare a 100 ml, 0.6% megabase agarose gel as follows.
   a. Add 0.6 g of agarose powder to 100 ml of 1X TAE buffer.
   b. Microwave the gel buffer until agarose powder is dissolved.
   c. Incubate at room temperature for 5 minutes.
   d. Add 10 µl SYBR Safe DNA gel stain. Swirl to Mix.
   e. Pour the solution into the gel tray.
   f. Allow to cool.
2. When the agarose gel is set, place it in the gel electrophoresis unit and fill the tank with 1X TAE Buffer to the maximum fill mark.
3. Clean the tray, comb, and the gel tank with ethanol and rinse thoroughly with deionized water to avoid cross-contamination.
4. Dilute 1 kb plus DNA ladder 1:10 in a 1X solution of Gel Loading Dye.
5. Set an incubator oven (or a heat block suitable for 3.5 ml tubes) to 50°C.

Procedure

1. Add 6 µl 6X Loading Dye to 30 µl DNA.
2. Load over 2 consecutive lanes of the gel. Pipette 18 µl per well.
3. Load 20 µl diluted prepared 1 kb plus ladder into the lanes on either side of the sample lanes.
4. Run the gel at 100 V (constant voltage) for 120 minutes.
5. View the gel on a Dark Reader transilluminator.

6 Use a new scalpel blade and the 1 kb plus DNA ladder as a size guide to excise DNA fractions from the gel containing the desired fragment sizes.

7 Transfer agarose gel fraction to a new 3.5 ml screw cap tube.

Purify the DNA

This step purifies the agarose fractions using a Zymoclean Large Fragment DNA Recovery Kit.

1 Add 3 volumes of Zymo ADB to each volume of agarose excised from the gel (eg, for a 600 mg agarose gel, add 1800 µl ADB).

2 Incubate at 50°C until the gel is dissolved (~10–15 minutes). Invert the tubes every 2 minutes to mix.

3 Transfer up to 800 µl melted agarose solution to each Zymo-Spin IC-XL Columns per gel fraction. Distribute evenly across both columns.

4 Centrifuge at 10,000–16,000 × g for 1 minute. Discard the flow-through.

5 Transfer remaining melted agarose to the columns.

6 Centrifuge at 10,000–16,000 × g for 1 minute. Discard the flow-through.

7 Wash 2 times as follows.

   a Add 200 µl Zymo DNA Wash Buffer.
   b Centrifuge at 10,000–16,000 × g for 1 minute.
   c Discard the flow-through.

8 Centrifuge the empty columns at 10,000–16,000 × g for 1 minute with the lid open.

9 Remove residual EtOH.

10 Discard the flow-through and the collection tube.

11 Transfer the columns to new 1.7 ml microcentrifuge tubes.

12 Add 30 µl RSB to each column.

13 Incubate at room temperature for 1 minute.

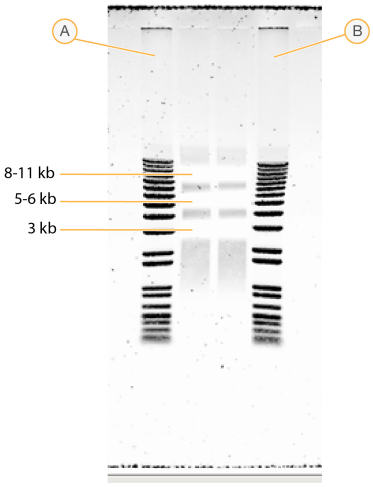
14 Centrifuge at 10,000–16,000 × g for 1 minute.

15 Combine elutions from the 2 matching columns for a total of 60 µl per size selection.

16 To quantify DNA, run 1 µl undiluted elution on an Agilent Technology 2100 Bioanalyzer using a DNA 12000 LabChip.
   The following figure shows an example of agarose gel size-selection.
   - Image A shows an agarose gel with 3 gel fractions removed, 3.5–4.5 kb, 5–7 kb, and 8–11 kb.
   - Image B shows a Bioanalyzer 12000 LabChip trace showing sizing and quantification the same 3 fractions.

   For each fraction 1 µl of the 60 µl elution volume was run on a 12000 LabChip. The total amount of recovered size-selected DNA for the 4 kb, 6 kb, and 9 kb fragments are 167 ng, 234 ng, and 167 ng respectively.
   The degree of difficulty in generating gel-plus libraries increases as the length of fragments increases. Compared to libraries with smaller fragment sizes, libraries with larger fragment lengths are expected to have a lower final library yield and lower library diversity.

Figure 3 Example Agarose Size Selection Gel



SAFE STOPPING POINT

If you are stopping, cap the tube and store at 2°C to 8°C for up to 24 hours.

Circularize DNA

This step uses a blunt ended intramolecular ligation to circularize the DNA fragments. An overnight incubation maximizes the number of fragments that form circular molecules.

Before starting this step, use the Agilent Bioanalyzer or Qubit HS quantification to calculate how much DNA is remaining for each sample.

- Gel-free—Expected yield is 250–700 ng
- Gel-plus—Expected yield is 150–400 ng

Larger volumes of DNA increase library yields and diversity but also increase chimeric read pairs. Smaller volumes of DNA decrease the number of chimeric read pairs but also decrease library yield and diversity. To balance these conditions, use up to 600 ng of DNA in a total circularization volume of 300 µl.

Consumables

- Circularization Buffer 10X
- Circularization Ligase
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tubes

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
Circularization Buffer 10x	-25°C to -15°C	Thaw at room temperature. Place on ice.
Circularization Ligase	-25°C to -15°C	Place on ice.

2 Preheat a heat block to 30°C.

3 Quantify DNA.

Procedure

1 Add the following items in the order listed to a new 1.7 ml microcentrifuge tube.

Item	Volume (µl)
AMPure Purified or Size Selected DNA	x µl (up to 600 ng)
Water	268–x
Circularization Buffer 10x	30
Circularization Ligase	2
Total	300

2 Flick to mix, and then centrifuge briefly.

3 Incubate at 30°C overnight (12–16 hours).

Remove Linear DNA

This step uses a DNA exonuclease treatment to remove linear DNA. Circularized DNA remains intact.

Consumables

- Exonuclease
- Stop Ligation Buffer

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
Stop Ligation Buffer	-25°C to -15°C	Thaw at room temperature. Place on ice.
Exonuclease	-25°C to -15°C	Place on ice.

2 Preheat heat blocks to 37°C and 70°C.

Procedure

1 Add 9 µl Exonuclease directly to the overnight circularization reaction.

2 Flick to mix, and then centrifuge briefly.

3 Incubate at 37°C for 30 minutes.

4 Incubate at 70°C for 30 minutes. Flick to mix.

5 Add 12 µl Stop Ligation Buffer.

6 Flick to mix, and then centrifuge briefly.

Shear Circularized DNA

This step shears large circularized DNA fragments to create smaller fragments (~300–1000 bp). This shearing step also generates dsDNA fragments with 3' or 5' overhangs.

Use a Covaris S2 or S220 device to shear the DNA. If using an alternative device, see the Covaris User Manual for equivalent settings and parameters.

Alternatively, use a nebulizer device to shear DNA. For more information, see *Shear Circularized DNA - Nebulizer Procedure [Alternative Procedure]* on page 39.

Consumables

- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube
- Covaris T6 tube and Snap Cap

NOTE
To ensure a good fit to the Covaris T6 tubes, use Covaris Snap-Caps # 520042 and not the Covaris Snap-Caps # 520030.

Preparation

1. Turn on the Covaris instrument at least 30 minutes before starting.
2. Using the Covaris manufacturer instructions, degas and prechill the water to 6°C.

Procedure

1. Transfer the entire sample to a Covaris T6 tube (~320 µl).
2. Add water to fill the tube to the top, and then cap the tube.
3. Make sure that no air bubbles are present in the tube.
4. Shear the DNA using Covaris S2 or S220 device with the following settings.

Settings	S2	S220
Peak Power Intensity	--	240
Intensity	8	--
Duty Cycle/Duty Factor	20%	20%
Cycles Per Burst	200	200
Time	40 seconds	40 seconds
Temperature	6°C	6°C

5. Transfer the ~320 µl sample to a new 1.7 ml microcentrifuge tube.

Purify the Sheared DNA

This step uses Streptavidin Magnetic Beads to purify the sheared DNA fragments that contain adapters (the mate pair fragments). Fragments without adapters are removed through a series of washes.

NOTE
After binding the mate pair fragments to the beads, all sample processing can be performed on-bead using a 1.7 ml microcentrifuge tube per sample. It is not necessary to transfer the sample to a new tube until the Amplify DNA step.

Consumables

- Bead Bind Buffer
- Bead Wash Buffer
- RSB (Resuspension Buffer)
- Dynabeads M-280 Streptavidin Magnetic Beads
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tubes

About Reagents

- Flick the tubs to mix the reactions and resuspend the beads. Do not pipette to mix.
- Briefly centrifuge tubes to collect the contents to the bottom.

Preparation

1 Preheat a heat block to 20°C.

Procedure

Bead Preparation

This step prepares Streptavidin Magnetic Beads for 1 sample. You can prepare beads for more than 1 sample by multiplying the volumes used by the number of samples you are processing. A 1.7 ml microcentrifuge tube holds enough beads for 5 samples. If you are processing more than 5 samples, use a larger volume tube.

1 Shake the bottle well to resuspend the beads.

2 Transfer 20 µl beads to a new 1.7 ml microcentrifuge tube.

3 Place on a magnetic rack for 1 minute.

4 Remove and discard all supernatant

5 Wash 2 times as follows.

   a Add 40 µl Bead Bind Buffer.
   b Incubate on the magnetic rack for 1 minute.
   c Remove and discard all supernatant.

6 Remove from the magnetic rack.

7 Add 300 µl Bead Bind Buffer.

Bead Binding

1 Add 300 µl beads to the 300 µl sheared DNA.

2 Incubate at 20°C for 15 minutes. Flick to mix every 2 minutes.

3 Centrifuge briefly (5–10 seconds).

4 Place on a magnetic rack for 1 minute.

5 Remove and discard all supernatant.

6 Wash 4 times with Bead Wash Buffer as follows.

   a Add 200 µl Bead Wash Buffer.
   b Remove from the magnet rack.
   c Flick to mix, and then centrifuge briefly (1–2 seconds).
   d Place on a magnetic rack for 30 seconds.
   e Remove and discard all supernatant.

7 Wash with RSB as follows.

   a Add 200 µl RSB.
   b Remove from the magnet rack.
   c Flick to mix, and then centrifuge briefly (5–10 seconds).
   d Place on a magnetic rack for 30 seconds.
   e Remove and discard all supernatant.

8 Repeat the RSB wash, but do not remove and discard the supernatant until you are ready to add the enzyme reaction mix in the next step.

End Repair

This step removes the 3' overhangs and fills in the 5' overhangs that were created in the shearing step. The DNA remains bound to the beads throughout this step and subsequent bead wash steps.

Consumables

- End Repair Mix
- Bead Wash Buffer
- RSB (Resuspension Buffer)
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
End Repair Mix	-25°C to -15°C	Thaw at room temperature. Place on ice.

2 Preheat a heat block to 30°C.

Procedure

End Repair

1 Create the end repair reaction mix in a new 1.7 ml microcentrifuge tube. For multiple samples, prepare a master mix.

Item	Volume (µl)
End Repair Mix	40
Water	60
Total	100

2 Remove and discard all supernatant from the DNA sample.

3 Centrifuge briefly.

4 Place on the magnetic rack.

5 Using a 10 µl pipette, remove residual supernatant.

6 Add 100 µl end repair reaction mix.

7 Remove from the magnetic rack.

8 Flick to mix, and then centrifuge briefly. Do not allow the beads to pellet.

9 Incubate at 30°C for 30 minutes.

Bead Wash

1 Centrifuge briefly (5–10 seconds).

2 Place on a magnetic rack for 1 minute.

3 Remove and discard all supernatant.

4 Wash 4 times with Bead Wash Buffer as follows.

a Add 200 µl Bead Wash Buffer.
b Remove from the magnetic rack.
c Flick to mix, and then centrifuge briefly (5–10 seconds).
d Place on a magnetic rack for 30 seconds.
e Remove and discard all supernatant.

5 Wash with RSB as follows.

a Add 200 µl RSB.
b Remove from the magnetic rack.
c Flick to mix, and then centrifuge briefly (5–10 seconds).
d Place on a magnetic rack for 30 seconds.
e Remove and discard all supernatant.

6 Repeat the RSB wash, but do not remove and discard the supernatant until you are ready to add the enzyme reaction mix in the next step.

A-Tailing

This step adds an A nucleotide to the 3' ends of the blunt fragments, which prevents them from ligating to each another during adapter ligation. The 3' ends of the adapters have a complementary T nucleotide. This process ensures a low rate of chimera (concatenated template) formation. A-tailing is performed on-bead, and the DNA remains bound to the beads throughout this step.

Consumables

- A-Tailing Mix
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
A-Tailing Mix	-25°C to -15°C	Thaw at room temperature. Place on ice.

2 Preheat a heat block to 37°C.

A-Tailing

1 Create the A-tailing reaction mix in a new 1.7 ml microcentrifuge tube. For multiple samples, prepare a master mix.

Item	Volume (µl)
A-Tailing Mix	12.5
Water	17.5
Total	30

2 Remove and discard all supernatant from the sample.

3 Centrifuge briefly.

4 Place on the magnetic rack.

5 Using a 10 µl pipette, remove residual supernatant.

6 Add 30 µl A-tailing reaction mix.

7 Remove from the magnet rack.

8 Flick to mix, and then centrifuge briefly. Do not allow the beads to pellet.

9 Incubate at 37°C for 30 minutes.

Ligate Adapters

This step ligates indexing adapters to the ends of the DNA fragments, which prepares them for amplification and subsequent hybridization onto a flow cell. The adapter ligation reaction is performed on-bead, and the DNA remains bound to the beads throughout this step and subsequent bead wash steps.

Consumables

- Ligation Mix
- DNA Adapter Index
- Bead Wash Buffer
- RSB (Resuspension Buffer)

NOTE

When used in low-plex combinations, not all index combinations are compatible. See *Index Adapter Sequences* on page 46 for more information.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
DNA Adapter Indexes	-25°C to -15°C	Thaw at room temperature. Place on ice.
Stop Ligation Buffer	-25°C to -15°C	Thaw at room temperature. Place on ice.
Ligation Mix	-25°C to -15°C	Place on ice.

2 Preheat a heat block to 30°C.

Procedure

Adapter Ligation

1 Add the following items in the order listed to the tube that contains the A-tailing reaction mix.

Item	Volume (µl)
A-Tailing Reaction/Bead Mix	30
Ligation Mix	2.5
Water	4
DNA Adapter Index	1
Total	37.5

2 Flick to mix, and then centrifuge briefly. Do not allow the beads to pellet.

3 Incubate at 30°C for 10 minutes.

4 Add 5 µl Ligation Stop Buffer.

Bead Wash

1 Centrifuge briefly (5–10 seconds).

2 Place on a magnetic rack for 1 minute.

3 Remove and discard all supernatant.

4 Wash 4 times with Bead Wash Buffer as follows.

a Add 200 µl Bead Wash Buffer.
b Remove from the magnetic rack.
c Flick to mix, and then centrifuge briefly (5–10 seconds).
d Place on a magnetic rack for 30 seconds.
e Remove and discard all supernatant.

5 Wash with RSB as follows.

a Add 200 µl RSB.
b Remove from the magnetic rack.
c Flick to mix, and then centrifuge briefly (5–10 seconds).
d Place on a magnetic rack for 30 seconds.
e Remove and discard all supernatant.

6 Repeat the RSB wash, but do not remove and discard the supernatant until you are ready to add the enzyme reaction mix in the next step.

Amplify Libraries

This step amplifies the DNA fragments with TruSeq DNA adapters on both ends.

Consumables

- Enhanced PCR Mix
- PCR Primer Cocktail
- 0.2 ml thin wall PCR tubes
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
PCR Master Mix	-25°C to -15°C	Thaw at room temperature. Place on ice.
PCR Primer Cocktail	-25°C to -15°C	Thaw at room temperature. Place on ice.

2 Save the following program on a thermal cycler:
- 98°C for 30 seconds
- 10 or 15 cycles of PCR:
  - 98°C for 10 seconds
  - 60°C for 30 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 4°C

PCR Cycle Number Guidelines

Protocol	Circularized DNA	PCR cycles
Gel-Free	200–600 ng	10
Gel-Plus	> 200 ng and < 8 kb	10
	< 200 ng or > 5 kb	15

Procedure

1 Create the PCR reaction mix in a new 1.7 ml microcentrifuge tube. For multiple samples, prepare a master mix.

Item	Volume (µl)
Enhanced PCR Mix	20
PCR Primer Cocktail	5
Water	25
Total	50

2 Remove and discard all supernatant from the DNA sample.

3 Centrifuge briefly.

4 Place on a magnetic rack.

5 Using a 10 µl pipette, remove residual supernatant.

6 Add 50 µl PCR reaction mix. Pipette to mix.

7 Transfer the mix to PCR tubes.

8 Place on the preprogrammed thermal cycler and run the PCR program.

SAFE STOPPING POINT

If you are stopping, cap the tubes and store at -25°C to -15°C for up to 7 days.

Clean Up Libraries

This step uses AMPure XP beads to purify the library DNA and remove short library fragments (< 300 bp).

Consumables

- RSB (Resuspension Buffer)
- AMPure XP beads
- Freshly prepared 70% ethanol (EtOH)
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube

About Reagents

- Vortex AMPure XP beads before each use.
- Vortex AMPure XP beads frequently to make sure that beads are evenly distributed.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
AMPure XP beads	2°C to 8°C	Let stand for at least 30 minutes to bring to room temperature.

2 Prepare fresh 70% ethanol (400 µl per sample).

Procedure

1 Place PCR tubes on a magnetic rack for 1 minute.

2 Transfer 45 µl supernatant to a new 1.7 ml microcentrifuge tube.

3 Add 30 µl AMPure XP beads to the PCR mix.

4 Flick to mix, and then centrifuge briefly.

5 Incubate at room temperature for 5 minutes.

6 Place a magnetic rack for 5 minutes.

7 Remove and discard all supernatant.

8 Wash 2 times as follows.

   a Add 200 µl freshly prepared 80% EtOH.
   b Incubate on the magnetic rack for 30 seconds.
   c Remove and discard all supernatant.

9 Air dry on the magnetic rack for 10–15 minutes.

10 Remove from the magnetic rack.

11 Add 20 µl RSB. Flick the tube to mix.

12 Incubate at room temperature for 5 minutes.

13 Place on the magnetic rack for 5 minutes.

14 Transfer supernatant to a new 1.7 ml microcentrifuge tube.

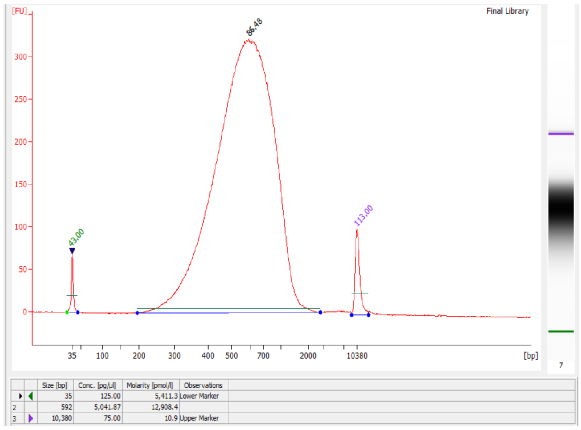
Check Libraries

1 Run 1 µl undiluted library on a gel or an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA LabChip.

- [Gel-free] Load 1 µl undiluted library on a 7500 or 12000 High Sensitivity DNA chip. The expected library size range is 300–1500 bp, with a concentration of 5–50 nM.
- [Gel-plus] Load 1 µl undiluted library on a High Sensitivity DNA chip. The expected library size range is 300–1500 bp, with a concentration of 1.5–20 nM.
- If validating by gel, load 10% of the library volume on a gel and make sure that the size range is 300–1000 bp.

The following figure shows an Agilent Technology 2100 Bioanalyzer High Sensitivity DNA LapChip profile of a typical mate pair library. Typical libraries show a broad size distribution of ~300–1200 bp. The concentration of the final library is 12.9 nM.

Figure 4 Example of a gel-plus mate pair library bioanalyzer profile



Quantify Libraries

To achieve high quality sequencing data, create optimum cluster densities across every lane of the flow cell. For best results, quantify your library using qPCR according to the *Illumina Sequencing Library qPCR Quantification Guide*.

1 Calculate concentration of library using qPCR or Bioanalyzer analysis.

2 Normalize the libraries to 2 nM by diluting with Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.

3 Select from the following options:

- Proceed to *Pool Libraries* on page 35.
- Proceed to cluster generation. For more information, see the appropriate user guide for the sequencing platform being used.

Pool Libraries

This step pools indexed libraries for cluster generation and sequencing.

NOTE
Make sure that libraries are pooled using compatible index adapters. For more information see *Index Adapter Sequences* on page 46.

1 Make sure that all libraries have been accurately quantified and normalized to 2 nM.

2 Combine 10 µl of each library in a new 1.7 ml microcentrifuge tube.

3 Vortex to mix, and then centrifuge briefly.

4 Proceed to cluster generation and sequencing. To prepare, see the Denature and Dilute Libraries guide for the Illumina sequencing system you are using.

Purify the Tagmentation Reaction [Alternative Procedure]

This step uses AMPure beads to purify the tagmentation reaction and applies only to the gel-free protocol. If you perform this step manually, it takes more time than using the Zymo column.

Consumables

- Neutralize Tagment Buffer
- RSB (Resuspension Buffer)
- AMPure XP beads
- Freshly prepared 70% ethanol (EtOH)
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube

About Reagents

- Vortex AMPure XP beads before each use.
- Vortex AMPure XP beads frequently to make sure that beads are evenly distributed.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
AMPure XP beads	2°C to 8°C	Let stand for at least 30 minutes to bring to room temperature.

2 Prepare fresh 70% ethanol (400 µl per sample).

Procedure

1 Incubate tagmentation reaction at 55°C for 30 minutes.

2 Add 25 µl neutralize tagment buffer. Pipette to mix.

3 Incubate at room temperature for 5 minutes.

4 Add 125 µl AMPure XP beads. Flick the tube for 5 seconds.

5 Incubate at room temperature for 15 minutes. Flick the tubes to mix every 2 minutes.

6 Place a magnetic rack for 5 minutes.

7 Remove and discard all supernatant.

8 Wash 2 times as follows.

   a Add 200 µl freshly prepared 80% EtOH.
   b Incubate on the magnetic rack for 30 seconds.
   c Remove and discard all supernatant.

9 Air-dry on the magnetic rack for 10–15 minutes.

10 Remove from the magnetic rack.

11 Add 30 µl RSB. Flick the tube to mix.

12 Incubate at room temperature for 5 minutes.

13 Place on the magnetic rack for 5 minutes.

14 Transfer supernatant to a new 1.7 ml microcentrifuge tube.

15 [Optional] To assess tagmentation, dilute 1 µl DNA with 1 µl water and run on an Agilent Technology 2100 Bioanalyzer using a DNA 12000 LabChip.

Shear Circularized DNA - Nebulizer Procedure [Alternative Procedure]

This step uses nebulization to shear large circularized DNA fragments to create smaller fragments (~300–1000 bp). This step is an alternative to the Covaris sonication method for shearing the circularized DNA.

Consumables

- RSB (Resuspension Buffer)
- Nebulizers and nebulization buffer (65% Glycerol, 25 mM Tris HCL pH 7.5, 5 mM EDTA)
- PVC tubing or equivalent
- Compressed nitrogen or air source (32 psi or above)
- Zymo Genomic DNA Clean & Concentrator-5
- Axygen Maxymum Recovery 1.7 ml microcentrifuge tube

Procedure

1. Remove a nebulizer from the packaging. Remove the blue lid.
2. Using gloves, remove a piece of vinyl tubing from the packaging and slip it over the central atomizer tube. Push it all the way to the inner surface of the blue lid.
3. Transfer the exonuclease-treated DNA to the nebulizer.
4. Add 550 µl nebulization buffer. Pipette to mix.
5. Attach the blue lid to the nebulizer (finger tight).
6. Set aside on ice while performing the next step.
7. Connect the compressed air source to the inlet port on the top of the nebulizer with the PVC tubing. Ensure a tight fit.
8. Bury the nebulizer in an ice bucket and place in a fume hood.
9. Make sure that the compressed air is delivered at 32 psi.
10. Nebulize for 6 minutes.
11. Centrifuge at 450 × g for 2 minutes.
12. Collect the droplets from the side of the nebulizer.
13. Measure the recovered volume (~400 µl).

Purify Sheared DNA

This step uses a Zymo Kit and RSB purify the tagmentation reaction.

1. Add 5 volumes (~2000 µl) of Zymo DNA Binding Buffer to the tagmentation reaction. Pipette to mix.
2. Transfer up to 750 µl of mixture to a Zymo-Spin column in a collection tube.
3. Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.
4. Transfer remaining tagmentation mixture to the same Zymo-Spin column.

5 Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.

6 Wash 2 times as follows.

   a Add 200 µl Zymo DNA Wash Buffer.
   b Centrifuge at 10,000–16,000 × g for 1 minute.
   c Discard the flow-through.

7 Add 50 µl RSB.

8 Incubate at room temperature for 1 minute.

9 Transfer the column to a new 1.7 ml microcentrifuge tube.

10 Centrifuge at 10,000–16,000 × g for 30 seconds.

11 Add 250 µl RSB.

Supporting Information

Appendix A

Introduction

The protocols described in this guide assume that you have reviewed the contents of this appendix, confirmed your kit contents, and obtained all the required consumables and equipment.

Acronyms

Acronym	Definition
ATL2	A-Tailing Mix
BBB	Bead Bind Buffer
BWB	Bead Wash Buffer
CB	Circularization Buffer 10X
CCL	Circularization Ligase
CTA	A-Tailing Control
CTE	End Repair Control
CTL	Ligation Control
EPM	Enhanced PCR Mix
ERP3	End Repair Mix
LIG2	Ligation Mix
MTP	Mate Pair Tagment Enzyme
NPT	dNTPs
NT	Neutralize Tagment Buffer
PPC	PCR Primer Cocktail
PS1	Exonuclease
RSB	Resuspension Buffer
SDB	10X Strand Displacepment Buffer
SDP	Strand Displacement Polymerase
STL	Stop Ligation Buffer
TB1	Tagment Buffer Mate Pair

Nextera Mate Pair Library Prep Kit (FC-132-1001)

Make sure that you have all the reagents identified in this section before proceeding. The Nextera Mate Pair Library Prep Kit contains 4 boxes.

- Nextera Mate Pair Library Prep Kit - Box 1
- Nextera Mate Pair Library Prep Kit - Box 2 Wash Solutions
- TruSeq DNA LT Library Prep Kit - Set A
- TruSeq DNA Library Prep Kit - PCR Box

Use the reagents in Nextera Mate Pair Library Prep Kit Box 1 and Box 2 for *Tagment Genomic DNA* through *Purify the Sheared DNA*.

Use the reagents in Nextera Mate Pair Library Prep Kit Box 2 and the TruSeq Library Prep Kit boxes for *End Repair* through *Clean Up DNA*.

Nextera Mate Pair Library Prep Kit - Box 1

Reagent	Storage Temperature	Description
MTP	-25°C to -15°C	Mate Pair Tagment Enzyme
NPT	-25°C to -15°C	dNTPs
SDP	-25°C to -15°C	Strand Displacement Polymerase
PS1	-25°C to -15°C	Exonuclease
TB1	-25°C to -15°C	Tagment Buffer Mate Pair
NT	-25°C to -15°C	Neutralize Tagment Buffer
CB	-25°C to -15°C	Circularization Buffer 10X
SDB	-25°C to -15°C	10X Strand Disp Buffer
CCL	-25°C to -15°C	Circularization Ligase
STL	-25°C to -15°C	Stop Ligation Buffer

Nextera Mate Pair Library Prep Kit – Box 2 Wash Solutions

Reagent	Storage Temperature	Description
RSB	Room temperature	Resuspension Buffer
BBB	Room temperature	Bead Bind Buffer
BWB	Room temperature	Bead Wash Buffer

TruSeq DNA LT Library Prep Kit - Set A Box

Reagent	Storage Temperature	Description
RSB	-25°C to -15°C	Resuspension Buffer
ERP3	-25°C to -15°C	End Repair Mix
ATL2	-25°C to -15°C	A-Tailing Mix
LIG2	-25°C to -15°C	Ligation Mix
CTE	-25°C to -15°C	End Repair Control
CTA	-25°C to -15°C	A-Tailing Control
CTL	-25°C to -15°C	Ligation Control
STL	-25°C to -15°C	Stop Ligation Buffer
AD002	-25°C to -15°C	DNA Adapter Index 2
AD004	-25°C to -15°C	DNA Adapter Index 4
AD005	-25°C to -15°C	DNA Adapter Index 5
AD006	-25°C to -15°C	DNA Adapter Index 6
AD007	-25°C to -15°C	DNA Adapter Index 7
AD012	-25°C to -15°C	DNA Adapter Index 12
AD013	-25°C to -15°C	DNA Adapter Index 13
AD014	-25°C to -15°C	DNA Adapter Index 14
AD015	-25°C to -15°C	DNA Adapter Index 15
AD016	-25°C to -15°C	DNA Adapter Index 16
AD018	-25°C to -15°C	DNA Adapter Index 18
AD019	-25°C to -15°C	DNA Adapter Index 19

NOTE
The control reagents provided in the TruSeq LT Library Prep Kit are not used with the Nextera Mate Pair Library Prep protocol.

TruSeq DNA LT Library Prep Kit - PCR Box

Reagent	Storage Temperature	Description
EPM	-25°C to -15°C	Enhanced PCR Mix
PPC	-25°C to -15°C	PCR Primer Cocktail

Index Adapter Sequences

The Nextera Mate Pair Library Prep Kit comes with a TruSeq DNA LT Library Prep Kit containing DNA Adapter Index tubes (Set A) that enable pooled libraries.

Each tube contains a unique 6-base index adapter and enough reagent for 20 reactions.

Libraries prepared with these adapters can be sequenced on Illumina sequencing platforms using a single-indexed sequencing workflow.

TruSeq DNA LT Index Adapter Sequences

The TruSeq DNA LT Library Prep Kit Set A contains the following the index adapter sequences.

NOTE

The index numbering is not contiguous.

The base in parentheses () indicates the base for the seventh cycle and is not considered as part of the index sequence. Record the index in the sample sheet as only 6 bases. For indexes 13 and above, the seventh base (in parentheses) might not be A and is seen in the seventh cycle of the Index Read.

Table 2 TruSeq DNA LT Library Prep Kit Index Adapter Sequences Set A

Index Adapter	Sequence
AD002	CGATGT(A)
AD004	TGACCA(A)
AD005	ACAGTG(A)
AD006	GCCAAT(A)
AD007	CAGATC(A)
AD012	CTTGTA(A)
AD013	AGTCAA(C)
AD014	AGTTCC(G)
AD015	ATGTCA(G)
AD016	CCGTCC(C)
AD018	GTCCGC(A)
AD019	GTGAAA(C)

Pooling Guidelines

Follow the pooling guidelines in this section for single-indexed sequencing to ensure base diversity during single-indexed sequencing.

The TruSeq DNA LT Library Prep Kit Set A contains 12 unique index adapter tubes. When designing low-plexity index pools for single-indexed sequencing, always use at least 2 unique and compatible indexes.

The following table describes possible pooling strategies for 2–4 samples generated with the index adapter tubes provided with the TruSeq DNA LT Kit.

For 5–11 plex pools, use 4-plex options with any other available adapters.

Table 3 Single-Indexed Pooling Strategies for 2–4 Samples

Plexity	Option	Set A Only
2	1	AD006 and AD012
	2	AD005 and AD019
3	1	AD002 and AD007 and AD019
	2	AD005 and AD006 and AD015
	3	2-plex options with any other adapter
4	1	AD005 and AD006 and AD012 and AD019
	2	AD002 and AD004 and AD007 and AD016
	3	3-plex options with any other adapter

Consumables and Equipment

Make sure that you have the required user-supplied consumables and equipment before starting the protocol.

The protocol has been optimized and validated using the items listed. Comparable performance is not guaranteed when using alternate consumables and equipment.

Consumables

Consumables	Supplier
1.7 ml Axygen Maxymum Recovery Microcentrifuge tubes	Axygen Scientific, part # MCT-175-L-C
0.2 ml thin wall PCR tubes	Axygen Scientific, part # PCR-02-C or equivalent
Qubit dsDNA BR Assay Kit (recommended)	Invitrogen, catalog # Q32850
Agilent High Sensitivity DNA Kit (recommended)	Agilent Technologies, catalog # 5067-4626
Agilent DNA 12000 Kit (recommended)	Agilent Technologies, catalog # 5067-1508
Zymo Genomic DNA Clean & Concentrator	Zymo Research, catalog # D4010 (25 preps) or D4011 (100 preps)
PCR grade water (for gel-free option)	General lab supplier
AMPure XP Beads	Beckman Coulter, catalog # A63880
Dynabeads M-280 streptavidin magnetic beads	Invitrogen, part # 112-05D

Consumables for Sage Pippin Prep (Gel-Plus)

Consumable	Supplier
Pippin Prep 0.75% Agarose Cassettes and Marker	Sage Science, catalog # CSD7510

Consumables for Agarose Gel Method (Gel-Plus)

Consumables	Supplier
Megabase Agarose	Bio-Rad, catalog # 161-3108
50 X TAE Buffer	Bio-Rad, catalog # 161-0743
1 kb plus DNA ladder	Invitrogen, catalog # 10787-018
6X Gel Loading Dye	BioLabs, catalog # B7021S
SYBR Safe	Invitrogen, catalog # S33102

Consumables	Supplier
3.5 ml screw cap tubes (or equivalent)	Sarstedt, catalog # 62.613
DNA Gel Extraction kit - Zymoclean Large Fragment DNA Recovery Kit	Zymo Research, catalog # D4045

Consumables for Nebulization Protocol

Consumable	Supplier
Glycerol	Sigma, part # G5516
PVC tubing or equivalent	Intersurgical, part # 1174-003
Nebulizers	Life Technologies, catalog # K7025-05
Nebulization buffer	General lab supplier
Zymo DNA Clean & Concentrator-5	Zymo Research, catalog # D4013

Consumables for Covaris Protocol

If you are performing the Covaris shearing protocol, make sure that you have the necessary user-supplied consumables before proceeding to library prep.

Table 4 User-Supplied Consumables for the Covaris Shearing Protocol

Consumables	Supplier
Covaris T6 (6 x 32 mm) glass tubes	Covaris, part # 520031
Covaris Snap-Cap - Teflon Silicone Septa 8 mm	Covaris, part # 520042

Equipment

Equipment	Supplier
Heat blocks (20–70°C)	General lab supplier
Magnetic rack for 1.7 ml microcentrifuge tubes	Invitrogen, part # CS15000
Microcentrifuge for 1 minute spins > 16,000 g	General lab supplier (eg, Eppendorf catalog # 5424 000.410)
Thermal cycler or PCR machine	General lab supplier
Minicentrifuge for quick ~2000 g spins (recommended)	General lab supplier (eg, Fisher, catalog # 05-090-100)
2100 Bioanalyzer (recommended)	Agilent
Qubit Fluorometer or equivalent (recommended)	Invitrogen, catalog # Q32866

Equipment for Sage Pippin Prep (Gel-Plus)

Equipment	Supplier
Sage Pippin Prep DNA size selection system	Sage Science
Zymo Genomic DNA Clean & Concentrator	Zymo Research, catalog # D4010 (25 preps) or D4011 (100 preps)

Equipment for Agarose Gel Method (Gel-Plus)

Equipment	Supplier
Gel tray and electrophoresis unit (12 cm width x 14 cm length)	Fisher Scientific, catalog # 09-528-110B (or similar)
Gel comb with wide wells (9 mm width x 1 mm length)	Fisher, catalog # OWB212 (or similar)
Dark reader transilluminator	Clare Chemical Research, model # D195M

Equipment for Covaris Shearing Protocol

Equipment	Supplier
Covaris AFA Ultrasonicator	Covaris, model # S2 or S220

Equipment for Nebulization Protocol

Equipment	Supplier
Compressed Nitrogen or Air source of at least 32 psi	General lab supplier
Benchtop centrifuge with swing-out rotor - Capable of holding nebulizer units.	General lab supplier

Sequencing and Data Analysis

Mate pair libraries are generated using unique molecular biology protocols that share characteristics with other Illumina library generation workflows. Although the initial fragmentation of the DNA uses Nextera transposomes, the final library contains TruSeq adapter sequences. Therefore, sequence the final libraries using TruSeq DNA workflows and sequencing chemistry.

You can sequence the libraries on any Illumina platform. There is no specified read length limit when sequencing; however, long read lengths increase the risk of sequencing into the mate pair junction adapter. Sequence data on the far side of the adapter are trimmed during analysis. Up to a 2 x 250 bp run is possible, but data might be lost on a 250 bp read length.

When analyzing and interpreting sequence data from a Nextera Mate Pair library, consider the following characteristics that differentiate these libraries from other Illumina libraries:

- The presence of a junction adapter sequence can occur at a random position within the template. Recognition of the adapter during sequencing depends on its location within a template, the length of a cluster template, and the length of the reads.
- Sequenced read pairs align in an outward-facing (or 'reverseforward', RF) orientation to one another rather than inward facing (or 'forward-reverse', FR). This outward-facing alignment is a consequence of circularization, whereby the fragment ends are inverted and linked together.

For more information, see *Data processing of Nextera Mate Pair reads on Illumina sequencing platforms* available on the Nextera Mate Pair Library Prep support page.

Material # 20002336
Document # 15035209 v02

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 5 Illumina General Contact Information

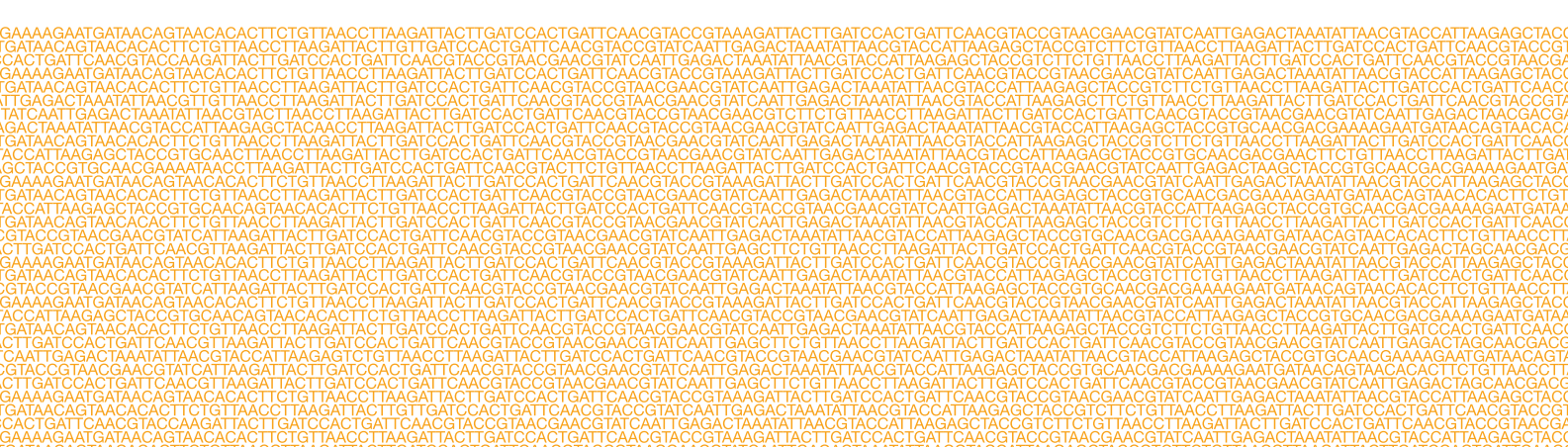
Website	www.illumina.com
Email	techsupport@illumina.com

Table 6 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Japan	0800.111.5011
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
China	400.635.9898	Singapore	1.800.579.2745
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	Taiwan	00806651752
Hong Kong	800960230	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000
Italy	800.874909		

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.



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