Nextera Mate Pair Library Prep
Protocol Guide

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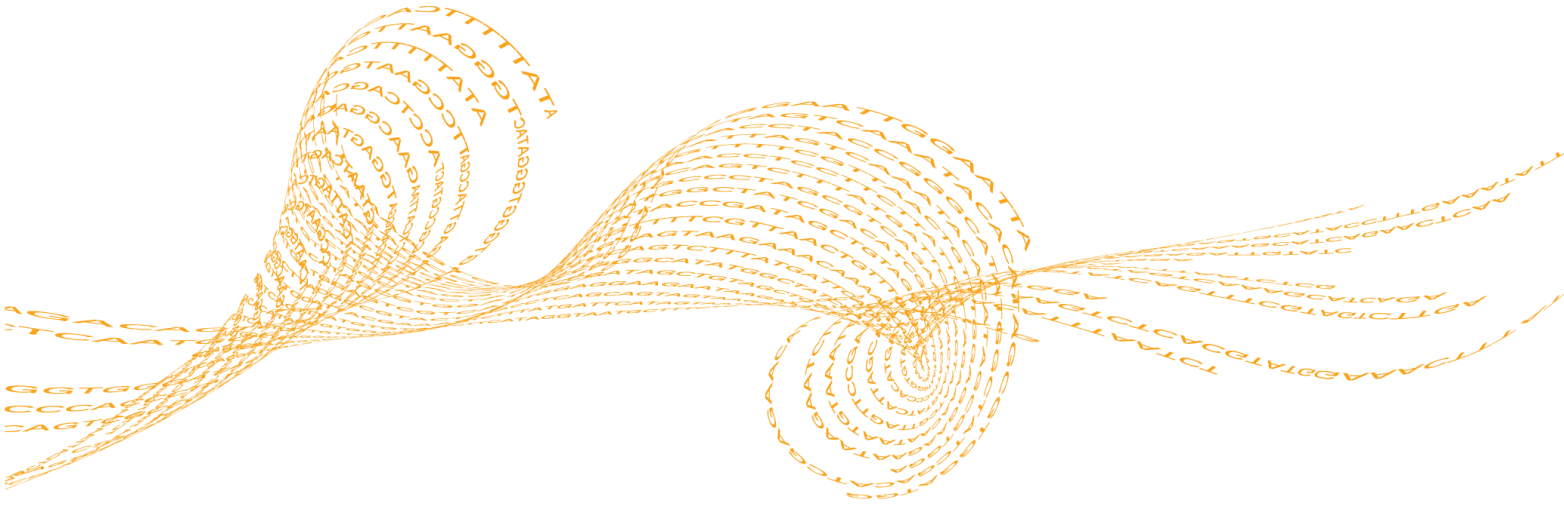

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Tagment Genomic DNA

Preparation

1 Preheat a heat block to 55°C.

Procedure

1 Add the following items in the order listed to a 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.

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

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

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

7 Transfer remaining tagmentation mixture.

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

9 Wash 2 times with 200 µl Zymo DNA Wash Buffer.

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

11 Transfer column to a 1.7 ml microcentrifuge tube.

12 Add 30 µl RSB.

13 Incubate at room temperature for 1 minute.

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

15 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

Preparation

1 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

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 with 400 µl 70% EtOH.

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

9 Remove from the magnetic rack.

10 Add 30 µl RSB. Flick 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 1.7 ml microcentrifuge tube.

15 Select from the following options:

- [Gel-free] Proceed to *Circularize DNA* on page 8.
- [Gel-plus] Proceed to *Select Fragment Size* on page 6.

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

Pippin Prep Size Selection

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.
3. Run for the maximum run time allowed for the cassette.
4. Transfer the elution to a new 1.7 ml microcentrifuge tube.
5. Add 5 volumes of ChIP Binding Buffer to DNA. Pipette to mix.
6. Transfer to a Zymo-Spin IC-XL column in a collection tube.
7. Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.
8. Wash 2 times with 200 µl Zymo DNA Wash Buffer.
9. Discard the collection tube.
10. Transfer the column to a 1.7 ml microcentrifuge tube.
11. Add 10 µl RSB.
12. Incubate at room temperature for 1 minute.
13. Centrifuge at 10,000–16,000 × g for 30 seconds.
14. [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

1. Set an incubator oven (or a heat block suitable for 3.5 ml tubes) to 50°C.
2. Add 6 µl 6X Loading Dye to 30 µl DNA.
3. Load over 2 consecutive lanes of the gel. Pipette 18 µl per well.
4. Load 20 µl diluted prepared 1 kb plus ladder into the lanes on either side of the sample lanes.
5. Run the gel at 100 V (constant voltage) for 120 minutes.
6. View the gel on a Dark Reader transilluminator.
7. Use a new scalpel blade and the 1 kb plus DNA ladder as a size guide to excise DNA fractions containing the desired fragment sizes.
8. Transfer agarose gel fraction to a new 3.5 ml screw cap tube.
9. Add 3 volumes of Zymo ADB to each volume of agarose excised from the gel.
10. Incubate at 50°C until the gel is dissolved (~10–15 minutes). Invert every 2 minutes to mix.
11. Transfer up to 800 µl melted agarose solution. Distribute evenly.

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

13 Transfer remaining melted agarose.

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

15 Wash 2 times with 200 µl Zymo DNA Wash Buffer.

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

17 Remove residual EtOH.

18 Discard the flow-through and the collection tube.

19 Transfer the columns to 1.7 ml microcentrifuge tubes.

20 Add 30 µl RSB to each column.

21 Incubate at room temperature for 1 minute.

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

23 Combine elutions from the 2 matching columns.

24 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.

Circularize DNA

Preparation

1 Preheat a heat block to 30°C.

Procedure

1 Add the following items in the order listed to a 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

Preparation

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

Procedure

1 Add 9 µl Exonuclease 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

Procedure

1. Transfer the entire sample to a Covaris T6 tube (~320 µl).
2. Add water to fill to the top, and then cap the tube.
3. Make sure that no air bubbles are present.
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 1.7 ml microcentrifuge tube.

Purify the Sheared DNA

Preparation

1. Preheat a heat block to 20°C.

Procedure

1. Shake to resuspend the beads.
2. Transfer 20 µl beads to a 1.7 ml microcentrifuge tube.
3. Place on a magnetic rack for 1 minute.
4. Remove and discard all supernatant
5. Wash 2 times with 40 µl Bead Bind Buffer.
6. Remove from the magnetic rack.
7. Add 300 µl Bead Bind Buffer.
8. Add 300 µl beads to the 300 µl sheared DNA.
9. Incubate at 20°C for 15 minutes. Flick to mix every 2 minutes.
10. Centrifuge briefly (5–10 seconds).
11. Place on a magnetic rack for 1 minute.
12. Remove and discard all supernatant.
13. Wash 4 times with 200 µl Bead Wash Buffer.
14. Wash with 200 µl RSB.
15. Repeat the RSB wash, but do not remove and discard the supernatant until the next step.

End Repair

Preparation

1 Preheat a heat block to 30°C.

Procedure

1 Create the end repair reaction mix in a 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.

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 beads to pellet.

9 Incubate at 30°C for 30 minutes.

10 Centrifuge briefly (5–10 seconds).

11 Place on a magnetic rack for 1 minute.

12 Remove and discard all supernatant.

13 Wash 4 times with 200 µl Bead Wash Buffer.

14 Wash with 200 µl RSB.

15 Repeat the RSB wash, but do not remove and discard the supernatant until the next step.

A-Tailing

Preparation

1 Preheat a heat block to 37°C.

A-Tailing

1 Create the A-tailing reaction mix in a 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.

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 beads to pellet.

9 Incubate at 37°C for 30 minutes.

Ligate Adapters

Preparation

1 Preheat a heat block to 30°C.

Procedure

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 beads to pellet.

3 Incubate at 30°C for 10 minutes.

4 Add 5 µl Ligation Stop Buffer.

5 Centrifuge briefly (5–10 seconds).

6 Place on a magnetic rack for 1 minute.

7 Remove and discard all supernatant.

8 Wash 4 times with 200 µl Bead Wash Buffer.

9 Wash with 200 µl RSB.

10 Repeat the RSB wash, but do not remove and discard the supernatant until the next step.

Amplify Libraries

Preparation

1 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

Procedure

1 Create the PCR reaction mix in a 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.

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 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

Procedure

1. Place PCR tubes on a magnetic rack for 1 minute.
2. Transfer 45 µl supernatant to a 1.7 ml microcentrifuge tube.
3. Add 30 µl AMPure XP beads to 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 with 200 µl 70% EtOH.
9. Air dry on the magnetic rack for 10–15 minutes.
10. Remove from the magnetic rack.
11. Add 20 µl RSB. Flick to mix.
12. Incubate at room temperature for 5 minutes.
13. Place on the magnetic rack for 5 minutes.
14. Transfer supernatant to a 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.

Quantify Libraries

1. Calculate concentration of library using qPCR or Bioanalyzer analysis.
2. Normalize 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 17.
   - Proceed to cluster generation. For more information, see the appropriate user guide for the sequencing platform being used.

Pool Libraries

1. Make sure that all libraries have been accurately quantified and normalized to 2 nM.
2. Combine 10 µl of each library in a 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]

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 to mix every 2 minutes.
6. Place a magnetic rack for 5 minutes.
7. Remove and discard all supernatant.
8. Wash 2 times with 200 µl 70% EtOH.
9. Air-dry on the magnetic rack for 10–15 minutes.
10. Remove from the magnetic rack.
11. Add 30 µl RSB. Flick to mix.
12. Incubate at room temperature for 5 minutes.
13. Place on the magnetic rack for 5 minutes.
14. Transfer supernatant to a 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]

Procedure

1. Remove nebulizer from packaging. Remove blue lid.
2. Using gloves, remove a piece of tubing from packaging and slip it over the central atomizer tube. Push it to the inner surface of the lid.
3. Transfer the 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.
7. Connect the compressed air source to the nebulizer with the 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 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

1. Add 5 volumes (~2000 µl) of Zymo DNA Binding Buffer to 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.
5. Centrifuge at 10,000–16,000 × g for 30 seconds. Discard the flow-through.
6. Wash 2 times with 200 µl Zymo Wash Buffer.
7. Add 50 µl RSB.
8. Incubate at room temperature for 1 minute.
9. Transfer to a 1.7 ml microcentrifuge tube.
10. Centrifuge at 10,000–16,000 × g for 30 seconds.
11. Add 250 µl RSB.

Notes

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 1 Illumina General Contact Information

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

Table 2 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**.

Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com