Nextera DNA Library Prep

Protocol Guide

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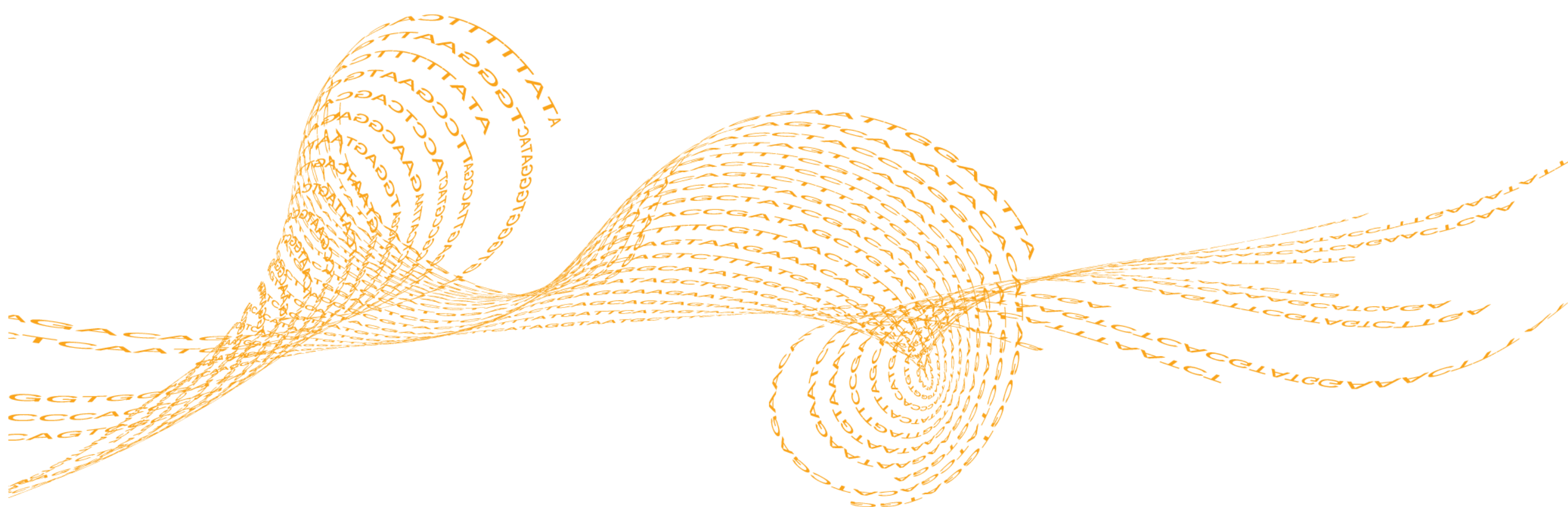

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Tagment Genomic DNA

Preparation

1. Label a new 96-well TCY plate NSP1.
2. Save the following program as TAG NSP1 on a thermal cycler with a heated lid.
   - Choose the preheat lid option and set to 55°C
   - 55°C for 5 minutes
   - Hold at 10°C

Procedure

1. Add 20 µl of genomic DNA at 2.5 ng/µl (50 ng total) to each sample well of the NSP1 plate.
2. Add 25 µl of TD Buffer to the wells containing genomic DNA.
3. Add 5 µl of TDE1 to the wells containing genomic DNA and TD Buffer.
4. Pipette up and down 10 times to mix.
5. Centrifuge at 280 × g at 20°C for 1 minute.
6. Place on the preprogrammed thermal cycler and run the TAG NSP1 program.

Clean Up Tagmented DNA

Preparation

1. Label a new midi plate NSP2.
2. Label a new TCY plate NSP3.
3. Add 180 µl Zymo DNA binding buffer to each well of the NSP2 plate with a sample in the corresponding well of the NSP1 plate.

Procedure

1. Transfer 50 µl from each well of NSP1 to the corresponding well of NSP2. Pipette up and down 10 times to mix.
2. Place the Zymo-Spin I-96 Plate on the Collection Plate.
3. Transfer sample mixture from NSP2 to the Zymo-Spin I-96 Plate.
4. Centrifuge at 1300 × g at 20°C for 2 minutes.
5. Discard the flow-through.
6. Wash 2 times with 300 µl Zymo wash buffer.
7. Centrifuge at 1300 × g for 2 minutes.
8. Place the Zymo-Spin I-96 Plate on NSP3.
9. Add 25 µl of RSB directly to the column matrix in each well.
10. Incubate for 2 minutes at room temperature.
11. Centrifuge at 1300 × g at 20°C for 2 minutes.

Amplify Tagmented DNA

Preparation

1. Label a new 96-well microplate NAP1.
2. Save the following program as PCR AMP on a thermal cycler with a heated lid.
   - Choose the preheat lid option and set to 100°C
   - 72°C for 3 minutes
   - 98°C for 30 seconds
   - 5 cycles of:
     - 98°C for 10 seconds
     - 63°C for 30 seconds
     - 72°C for 3 minutes
   - Hold at 10°C

Procedure

1. Arrange Index 1 (i7) adapters in the TruSeq Index Plate Fixture as follows:
   - 24 libraries—Columns 1–6
   - 96 libraries—Columns 1–12
2. Arrange Index 2 (i5) adapters in the TruSeq Index Plate Fixture as follows:
   - 24 libraries—Rows A–D
   - 96 libraries—Rows A–H
3. Place the plate on the TruSeq Index Plate Fixture.
4. Using a multichannel pipette, add 5 µl of each Index 1 (i7) adapter down each column. Replace the cap on each i7 adapter tube with a new orange cap.
5. Using a multichannel pipette, add 5 µl of each Index 2 (i5) adapter across each row. Replace the cap on each i5 adapter tube with a new white cap.
6. Add 15 µl NPM.
7. Add 5 µl PPC.
8. Transfer 20 µl from each well of NSP3 to NAP1. Pipette up and down 3–5 times to mix.
9. Centrifuge at 280 × g at 20°C for 1 minute.
10. Transfer the NAP1 plate to the post-amplification area.
11. Place on the preprogrammed thermal cycler and run the PCR AMP program.

Clean Up Libraries

Preparation

1. Prepare fresh 80% ethanol from absolute ethanol.
2. Label a new midi plate NAP2.
3. Label a new TCY plate NLP.

Procedure

1. Centrifuge NAP1 at 280 × g at 20°C for 1 minute.
2. Transfer the contents of NAP1 to NAP2.
3. Vortex AMPure XP beads for 30 seconds. Add beads to a trough.
4. Add 30 µl AMPure XP beads to NAP2.
5. Add 30 µl AMPure XP beads to NAP2.
6. Pipette up and down 10 times to mix.
7. Incubate at room temperature for 5 minutes.
8. Place on a magnetic stand and wait until the liquid is clear (~2 minutes). Keep on magnetic stand until step 13.
9. Remove and discard supernatant.
10. Wash 2 times with 200 µl 80% EtOH.
11. Remove residual EtOH.
12. Air-dry the beads for 15 minutes.
13. Remove from the magnetic stand.
14. Add 32.5 µl RSB.
15. Pipette up and down 10 times to mix.
16. Incubate at room temperature for 2 minutes.
17. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
18. Transfer 30 µl supernatant from NAP2 to NLP.

Check Libraries

Quantify Libraries

1 Quantify your libraries. Convert library concentration using the formula 1 ng/ul = 3 nM.

Quality Control

1 Run 1 µl of 1:3 diluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip.

Library Size from Bioanalyzer in bp	Conversion Factor for ng/µl > nM	DNA Concentration for Cluster Generation
250	1 ng/µl = 6 nM	6–12 pM
500	1 ng/µl = 3 nM	6–12 pM
1000–1500	1 ng/µl = 1.5 nM	12–20 pM

Normalize and Pool Libraries

Preparation

1. Apply the NDP barcode label to a new 96-well midi plate.
2. Apply the NPP barcode label to a new 96-well midi plate (for indexed libraries).
3. If NLP was stored frozen, thaw at room temperature and then centrifuge at 280 × g for 1 minute.

Make NDP

1. Transfer 10 µl library from each well of NLP to the corresponding well of NDP.
2. Normalize the concentration in each well to 2 nM using Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.
3. Shake at 1000 rpm for 2 minutes.
4. Centrifuge at 280 × g for 1 minute.

Make NPP [For Indexed Libraries]

1. Transfer 5 µl from each well in column 1 of NDP to column 1 of NPP.
2. Repeat step 1 for the remaining columns of NDP until samples are pooled in column 1 of NPP.
3. Combine the contents of column 1 into A2 of NPP.
4. Shake at 1800 rpm for 2 minutes.
5. Denature and dilute pooled libraries to the loading concentration for the instrument you are using. See the denature and dilute libraries guide for your instrument.

Acronyms

Acronym	Definition
NAP1	Nextera Amplification Plate 1
NAP2	Nextera Amplification Plate 2
NDP	Nextera Dilution Plate
NLP	Nextera Library Plate
NPM	Nextera PCR Master Mix
NSP1	Nextera Sample Plate 1
NSP2	Nextera Sample Plate 2
NSP3	Nextera Sample Plate 3
NPP	Nextera Pooled Plate
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
TD	Tagment DNA Buffer
TDE1	Tagment DNA Enzyme

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**.

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