Nextera Rapid Capture Enrichment
Protocol Guide

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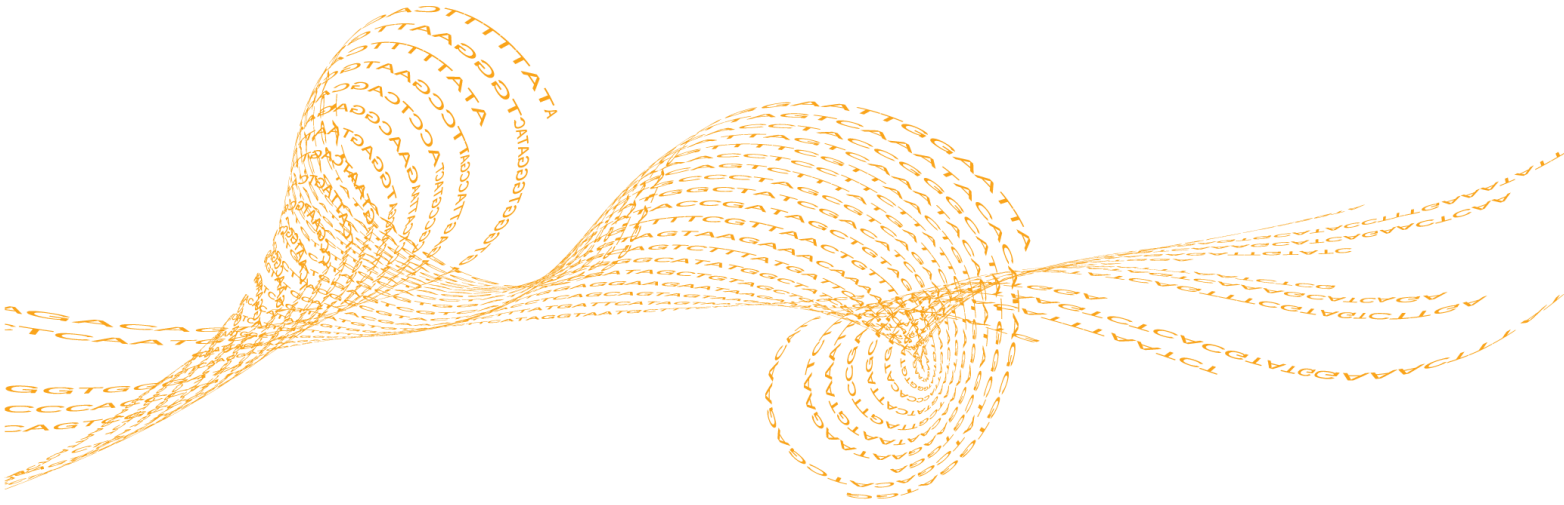

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Tagment Genomic DNA

Preparation

1 Preheat a microheating system with midi plate insert to 58°C.

Procedure

1 Quantify gDNA using a fluorometric method.

2 Dilute gDNA in Tris-HCl 10 mM, pH 8.5 to a final volume of 10 µl at 5 ng/µl.

3 Add the following items in the order listed to the NLT plate.

Item	Volume (µl)
Normalized gDNA	10
TD	25
TDE1	15

4 Shake at 1800 rpm for 1 minute.

5 Centrifuge at 280 × g for 1 minute.

6 Place on the 58°C microheating system with the lid closed for 10 minutes.

7 Add 15 µl ST.

8 Shake at 1800 rpm for 1 minute.

9 Centrifuge at 280 × g for 1 minute.

10 Incubate at room temperature for 4 minutes.

Clean Up Tagmented DNA

Procedure

1. Add 65 µl SPB.
2. Shake at 1800 rpm for 1 minute.
3. Incubate at room temperature for 8 minutes.
4. Centrifuge at 280 × g for 1 minute.
5. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
6. Remove and discard all supernatant.
7. Wash 2 times with 200 µl 80% EtOH.
8. Using a 20 µl pipette, remove residual 80% EtOH.
9. Air-dry on the magnetic stand for 10 minutes.
10. Remove from the magnetic stand.
11. Add 22.5 µl RSB.
12. Shake at 1800 rpm for 1 minute.
13. Incubate at room temperature for 2 minutes.
14. Centrifuge at 280 × g for 1 minute.
15. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
16. Transfer 20 µl supernatant to the NLA plate.

Amplify Tagmented DNA

Preparation

1 Save the following NLM AMP program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 72°C for 3 minutes
- 98°C for 30 seconds
- 10 cycles of:
  - 98°C for 10 seconds
  - 60°C for 30 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 10°C

Procedure

1 Arrange Index 1 (i7) adapters in columns 1–12 of the TruSeq Index Plate Fixture.

2 Arrange Index 2 (i5) adapters in rows A–H of the TruSeq Index Plate Fixture.

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 20 µl NLM.

7 Shake at 1200 rpm for 1 minute.

8 Centrifuge at 280 × g for 1 minute.

9 Place on the thermal cycler and run the NLM AMP program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Amplified DNA

Procedure

1. Centrifuge at 280 × g for 1 minute.
2. Transfer 50 µl supernatant to the NLC plate.
3. Add 90 µl SPB.
4. Shake at 1800 rpm for 1 minute.
5. Incubate at room temperature for 10 minutes.
6. Centrifuge at 280 × g for 1 minute.
7. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
8. Remove and discard all supernatant.
9. Wash 2 times with 200 µl 80% EtOH.
10. Using a 20 µl pipette, remove residual 80% EtOH.
11. Air-dry on the magnetic stand for 10 minutes.
12. Add 27 µl RSB.
13. Shake at 1800 rpm for 1 minute.
14. Incubate at room temperature for 2 minutes.
15. Centrifuge at 280 × g for 1 minute.
16. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
17. Transfer 25 µl supernatant to the NIL plate.
18. Quantify the library using a fluorometric method.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at -25°C to -15°C for up to 14 days.

Hybridize Probes

Preparation

1 Save the NRC HYB program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 95°C for 10 minutes
- 18 cycles of 1 minute each, starting at 94°C, then decreasing 2°C per cycle
- Hold at 58°C

Pool Libraries

1 Combine 500 ng of each DNA library. Make sure that each library has a unique index.
- If the total volume is > 40 µl, concentrate the pooled sample to 40 µl.
- If the total volume is < 40 µl, increase the volume to 40 µl with RSB.

Procedure

1 Add the following items in the order listed to the NEH1 plate.

Item	Volume (µl)
DNA library sample or pool	40
EHB	50
CEX, EEX, or RCO	10

2 Shake at 1200 rpm for 1 minute.

3 Centrifuge at 280 × g for 1 minute.

4 Place on the thermal cycler and run the NRC HYB program.

5 Keep at the 58°C holding temperature for at least 90 minutes and up to 24 hours.

Capture Hybridized Probes

Preparation

1 Preheat a microheating system with midi plate insert to 50°C.

Procedure

1 Centrifuge at 280 × g for 1 minute.
2 Transfer all (~100 µl) to the NEW1 plate.
3 Add 250 µl SMB.
4 Shake at 1200 rpm for 5 minutes.
5 Incubate at room temperature for 25 minutes.
6 Centrifuge at 280 × g for 1 minute.
7 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
8 Remove and discard all supernatant.
9 Remove from the magnetic stand.
10 Wash 2 times with 200 µl EWS.
11 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex to mix.
12 Add 23 µl elution premix.
13 Shake at 1800 rpm for 2 minutes.
14 Incubate at room temperature for 2 minutes.
15 Centrifuge at 280 × g for 1 minute.
16 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
17 Transfer 21 µl supernatant to the NEH2 plate.
18 Add 4 µl ET2.
19 Shake at 1200 rpm for 1 minute.
20 Centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

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

Perform Second Hybridization

Procedure

1 Add the following reagents in the order listed.

Reagent	Volume (µl)
RSB	15
EHB	50
CEX, EEX, or RCO	10

2 Shake at 1200 rpm for 1 minute.

3 Centrifuge at 280 × g for 1 minute.

4 Place on the thermal cycler and run the NRC HYB program.

5 Keep at the 58°C holding temperature for at least 14.5 hours and up to 24 hours.

Perform Second Capture

Preparation

1 Preheat a microheating system with midi plate insert to 50°C.

Procedure

1 Centrifuge at 280 × g for 1 minute.
2 Transfer all (~100 µl) supernatant to the NEW2 plate.
3 Add 250 µl SMB.
4 Shake at 1200 rpm for 5 minutes.
5 Incubate at room temperature for 25 minutes.
6 Centrifuge at 280 × g for 1 minute.
7 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
8 Remove and discard all supernatant.
9 Remove from the magnetic stand.
10 Wash 2 times with 200 µl EWS.
11 Mix 28.5 µl EE1 and 1.5 µl HP3, and then vortex to mix.
12 Add 23 µl elution premix.
13 Shake at 1800 rpm for 2 minutes.
14 Incubate at room temperature for 2 minutes.
15 Centrifuge at 280 × g for 1 minute.
16 Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
17 Transfer 21 µl supernatant to the NEC1 plate.
18 Add 4 µl ET2.
19 Shake at 1800 rpm for 1 minute.
20 Centrifuge at 280 × g for 1 minute.

Clean Up Captured Library

Procedure

1. Add 45 µl SPB.
2. Shake at 1800 rpm for 1 minute.
3. Incubate at room temperature for 10 minutes.
4. Centrifuge at 280 × g for 1 minute.
5. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
6. Remove and discard all supernatant.
7. Wash 2 times with 200 µl 80% EtOH.
8. Use a 20 µl pipette to remove residual EtOH.
9. Air-dry on the magnetic stand for 10 minutes.
10. Remove from the magnetic stand.
11. Add 27.5 µl RSB.
12. Shake at 1800 rpm for 1 minute.
13. Incubate at room temperature for 2 minutes.
14. Centrifuge at 280 × g for 1 minute.
15. Place on a magnetic stand and wait until the liquid is clear (~2 minutes).
16. Transfer 25 µl supernatant to the NEA plate.

SAFE STOPPING POINT

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

Amplify Enriched Library

Preparation

1. Determine the appropriate number of PCR cycles:
   - For capture target sizes > 2 Mb, perform 10 cycle
   - For capture target sizes < 2 Mb, perform 12 cycles
2. Save the following AMP10 or AMP12 program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 98°C for 30 seconds
   - 10 or 12 cycles of:
     - 98°C for 10 seconds
     - 60°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 10°C

Procedure

1. Add 5 µl PPC.
2. Add 20 µl NEM.
3. Shake at 1200 rpm for 1 minute.
4. Centrifuge at 280 × g for 1 minute.
5. Place on the thermal cycler and run the AMP10 or AMP12 program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days.

Clean Up Amplified Enriched Library

Procedure

1. Centrifuge at 280 × g for 1 minute.
2. Transfer 50 µl to the NEC2 plate.
3. Add 90 µl SPB.
4. Shake at 1800 rpm for 1 minute.
5. Incubate at room temperature for 10 minutes.
6. Centrifuge at 280 × g for 1 minute.
7. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
8. Remove and discard all supernatant.
9. Wash 2 times with 200 µl 80% EtOH.
10. Use a 20 µl pipette to remove residual EtOH.
11. Air-dry on the magnetic stand for 10 minutes.
12. Remove from the magnetic stand.
13. Add 32 µl RSB.
14. Shake at 1800 rpm for 1 minute.
15. Incubate at room temperature for 2 minutes.
16. Centrifuge at 280 × g for 1 minute.
17. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
18. Transfer 30 µl supernatant to the NEL plate.

SAFE STOPPING POINT

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

Check Enriched Libraries

Quantify Libraries

1. Quantify the enriched library using a fluorometric method.

Assess Quality [Optional]

1. If the library concentration is higher than the supported quantitative range for the High Sensitivity DNA chip, dilute the library 1:10 with RSB.
2. Run 1 µl of post enriched library using a High Sensitivity DNA chip.

Acronyms

Acronym	Definition
CEX	Coding Exome Oligos
EE1	Enrichment Elution Buffer 1
EEX	Expanded Exome Oligos
EHB	Enrichment Hybridization Buffer
ET2	Elute Target Buffer 2
EWS	Enrichment Wash Solution
HP3	2N NaOH
NEA	Nextera Enrichment Amplification Plate
NEC1	Nextera Enriched Clean Up Plate 1
NEC2	Nextera Enriched Clean Up Plate 2
NEH1	Nextera Enrichment Hyb Plate 1
NEH2	Nextera Enrichment Hyb Plate 2
NEL	Nextera Enrichment Library Plate
NEM	Enrichment Amp Mix
NEW1	Nextera Enrichment Wash Plate 1
NEW2	Nextera Enrichment Wash Plate 2
NIL	Nextera Index Library Plate
NLA	Nextera Library Amplification Plate
NLC	Nextera Library Clean Up Plate
NLM	Library Amp Mix
NLT	Nextera Library Tagment Plate
PPC	PCR Primer Cocktail
RCO	Rapid Capture Oligos
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
SPB	Sample Purification Beads
ST	Stop Tagment Buffer
TD	Tagment DNA Buffer
TDE1	Tagment DNA Enzyme TDE

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