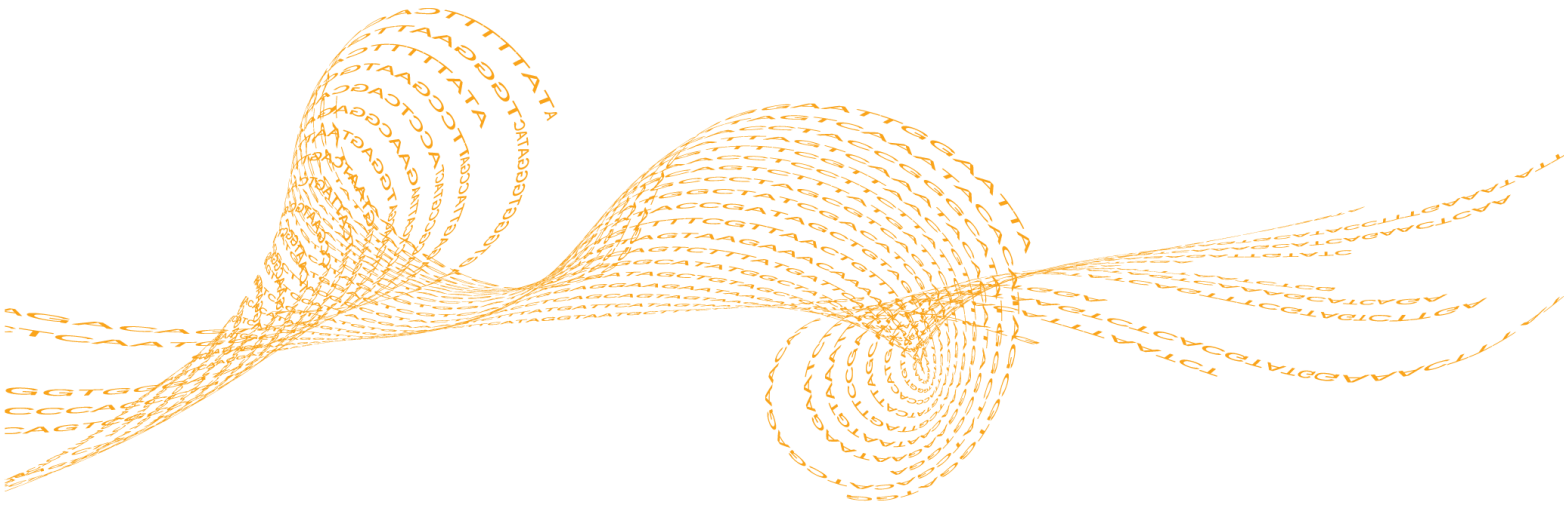
TruSeq Nano DNA Library Prep
Protocol Guide

For Research Use Only. Not for use in diagnostic procedures.



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Fragment DNA

Procedure

1. Quantify gDNA using a fluorometric-based method.
2. Normalize gDNA with RSB to 52.5 µl in the DNA plate.
   - 100 ng for a 350 bp insert size
   - 200 ng for a 550 bp insert size
3. Mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.
4. Centrifuge as follows.
   - [HS] Centrifuge at 280 × g for 1 minute.
   - [LS] Centrifuge briefly.
5. Transfer 52.5 µl DNA to Covaris tubes.
6. Centrifuge at 280 × g for 5 seconds.
7. Fragment the DNA using the following Covaris settings.

Table 1 350 bp Insert Settings

Covaris Setting	M220	S220	S2	E210
Duty Factor (%)	20	5	10	
Intensity	—	—	5.0	
Peak/Displayed Power (W)	50	175	23	14
Cycles/Burst	200			
Duration (seconds)	65	50	45	
Mode	—	Frequency sweeping		
Temperature (°C)	20	5.5–6		

Table 2 550 bp Insert Settings

Covaris Setting	M220	S220	S2	E210
Duty Factor (%)	20	5	10	
Intensity	—	—	2.0	
Peak/Displayed Power (W)	50	175	9	7
Cycles/Burst	200			
Duration (seconds)	45	25	45	
Mode	—	Frequency sweeping		
Temperature (°C)	20	5.5–6		

8. Centrifuge at 280 × g for 5 seconds.
9. Transfer 50 µl supernatant to the CSP plate.
10. Add 80 µl SPB, and then mix thoroughly as follows.
    - [HS] Shake at 1800 rpm for 2 minutes.
    - [LS] Pipette up and down.
11. Incubate at room temperature for 5 minutes.
12. [HS] Centrifuge at 280 × g for 1 minute.
13. Place on a magnetic stand and wait until the liquid is clear (~8 minutes).

14 Remove and discard all supernatant.

15 Wash 2 times with 200 µl 80% EtOH.

16 Use a 20 µl pipette to remove residual EtOH.

17 Air-dry on the magnetic stand for 5 minutes.

18 Add 62.5 µl RSB.

19 Remove from the magnetic stand, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

20 Incubate at room temperature for 2 minutes.

21 [HS] Centrifuge at 280 × g for 1 minute.

22 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

23 Transfer 60 µl supernatant to the IMP plate.

Repair Ends and Select Library Size

Preparation

1 [HS] Preheat the microheating system to 30°C.

2 [LS] Save the following ERP program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 30°C for 30 minutes
- Hold at 4°C

Procedure

1 Add 40 µl ERP2 or ERP3, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

2 [HS] Centrifuge at 280 × g for 1 minute.

3 Incubate as follows.
- [HS] Place on the 30°C microheating system with the lid closed for 30 minutes, and then place on ice.
- [LS] Place on the thermal cycler and run the ERP program.

4 Dilute SPB with PCR grade water to 160 µl per 100 µl of end-repaired sample.
- When processing ≤ 6 samples, use a new 1.7 ml microcentrifuge tube.
- When processing > 6 samples, use a new 15 ml conical tube.

Determine the volumes using the following formulas, which include 15% excess for multiple samples.

Table 3 Diluted SPB for a 350 bp Insert Size

	Formula	Example Amount per 12 samples	Your Calculation
SPB	# of samples X 109.25 µl	1311 µl	
PCR grade water	# of samples X 74.75 µl	897 µl	

Table 4 Diluted SPB for a 550 bp Insert Size

	Formula	Example Amount per 12 samples	Your Calculation
SPB	# of samples X 92 µl	1104 µl	
PCR grade water	# of samples X 92 µl	1104 µl	

5 Vortex diluted SPB until well-dispersed.

6 Add 160 µl diluted SPB, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

7 Incubate at room temperature for 5 minutes.

8 [HS] Centrifuge at 280 × g for 1 minute.

9 Place on a magnetic stand and wait until the liquid is clear (~5 minutes).

10 Transfer 250 µl supernatant to the CEP plate.

11 Add 30 µl undiluted SPB, and then mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.

12 Incubate at room temperature for 5 minutes.

13 [HS] Centrifuge at 280 × g for 1 minute.

14 Place on a magnetic stand and wait until the liquid is clear (~5 minutes).

15 Remove and discard all supernatant.

16 Wash 2 times with 200 µl 80% EtOH.

17 Use a 20 µl pipette to remove residual EtOH.

18 Air-dry on the magnetic stand for 5 minutes.

19 Add 20 µl RSB.

20 Remove from the magnetic stand, and then mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.

21 Incubate at room temperature for 2 minutes.

22 [HS] Centrifuge at 280 × g for 1 minute.

23 Place on a magnetic stand and wait until the liquid is clear (~5 minutes).

24 Transfer 17.5 µl supernatant to the ALP plate.

SAFE STOPPING POINT

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

Adenylate 3′ Ends

Preparation

1 [HS] Preheat 2 microheating systems, the first to 37°C and the second to 70°C.

2 [LS] Save the following ATAIL70 program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
- 37°C for 30 minutes
- 70°C for 5 minutes
- 4°C for 5 minutes
- Hold at 4°C

Procedure

1 Add 12.5 µl ATL or ATL2, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

2 Centrifuge at 280 × g for 1 minute.

3 Incubate as follows.

[HS]

a Place on the 37°C microheating system with the lid closed for 30 minutes.

b Move to the 70°C microheating system with the lid closed for 5 minutes.

c Place on ice for 5 minutes.

[LS]

a Place on the thermal cycler and run the ATAIL70 program.

b Centrifuge at 280 × g for 1 minute.

Ligate Adapters

Preparation

1. [HS] Preheat a microheating system to 30°C.
2. [LS] Save the following LIG program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - 30°C for 10 minutes
   - Hold at 4°C

Procedure

1. Add the following reagents in the order listed, and then mix thoroughly as follows.

Reagent	Volume (µl)
RSB	2.5
LIG2	2.5
DNA adapters	2.5

   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.
2. Centrifuge at 280 × g for 1 minute.
3. Incubate as follows.
   - [HS] Place on the 30°C microheating system with the lid closed for 10 minutes, and then place on ice.
   - [LS] Place on the thermal cycler and run the LIG program.
4. Add 5 µl STL, and then mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.
5. [HS] Centrifuge at 280 × g for 1 minute.
6. Perform steps 6a through 6m using the **Round 1** volumes.
   a. Add SPB, and then mix thoroughly as follows.

	Round 1	Round 2
SPB	42.5 µl	50 µl

      — [HS] Shake at 1800 rpm for 2 minutes.
      — [LS] Pipette up and down.
   b. Incubate at room temperature for 5 minutes.
   c. [HS] Centrifuge at 280 × g for 1 minute.
   d. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).
   e. Remove and discard all supernatant.
   f. Wash 2 times with 200 µl 80% EtOH.
   g. Use a 20 µl pipette to remove residual EtOH.
   h. Air-dry on the magnetic stand for 5 minutes.
   i. Add RSB.

	Round 1	Round 2
RSB	52.5 µl	27.5 µl

j Remove from the magnetic stand, and then mix thoroughly as follows.
   - [HS] Shake at 1800 rpm for 2 minutes.
   - [LS] Pipette up and down.

k Incubate at room temperature for 2 minutes.

l [HS] Centrifuge at 280 × g for 1 minute.

m Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

7 Transfer 50 µl supernatant to the CAP plate.

8 Repeat steps 6a through 6m with the new plate using the **Round 2** volumes.

9 Transfer 25 µl supernatant to the PCR plate.

SAFE STOPPING POINT

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

Enrich DNA Fragments

Preparation

1 Save the following PCRNano program on the thermal cycler:
- Choose the preheat lid option and set to 100°C
  - 95°C for 3 minutes
- 8 cycles of:
  - 98°C or 20 seconds
  - 60°C for 15 seconds
  - 72°C for 30 seconds
- 72°C for 5 minutes
- Hold at 4°C

Procedure

1 Place on ice and add 5 µl PPC.

2 Add 20 µl EPM, and then mix thoroughly as follows.
- [HS] Shake at 1600 rpm for 20 seconds.
- [LS] Pipette up and down.

3 Centrifuge at 280 × g for 1 minute.

4 Place on the thermal cycler and run the PCRNano program.

5 Centrifuge at 280 × g for 1 minute.

6 Add SPB. The volume depends on the type of adapter used.

Adapter Type	Volume SPB
Adapter tubes	50 µl
DAP	47.5 µl

7 Mix thoroughly, as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

8 Incubate at room temperature for 5 minutes.

9 [HS] Centrifuge at 280 × g for 1 minute.

10 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

11 Remove and discard all supernatant.

12 Wash 2 times with 200 µl 80% EtOH.

13 Use a 20 µl pipette to remove residual EtOH.

14 Air-dry on the magnetic stand for 5 minutes.

15 Add 32.5 µl RSB.

16 Remove from the magnetic stand, and then mix thoroughly as follows.
- [HS] Shake at 1800 rpm for 2 minutes.
- [LS] Pipette up and down.

17 Incubate at room temperature for 2 minutes.

18 [HS] Centrifuge at 280 × g for 1 minute.

19 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

20 Transfer 30 µl supernatant to the TSP1 plate.

SAFE STOPPING POINT

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

Validate Libraries

Procedure

1. Quantify the libraries using a fluorometric quantification method that uses dsDNA binding dyes or qPCR.
2. Do the following on an Agilent Technologies 2100 Bioanalyzer:
   - If using a High Sensitivity DNA chip:
     - Dilute the DNA library 1:100 with water.
     - Run 1 µl diluted DNA library.
   - If using a DNA 7500 chip, run 1 µl undiluted DNA library.

Normalize and Pool Libraries

Procedure

1. Transfer 10 µl library to the DCT plate.
2. Normalize with Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20 to 10 nM, and then mix thoroughly as follows.
   - [HS] Shake at 1000 rpm for 2 minutes.
   - [LS] Pipette up and down.
3. [HS] Centrifuge at 280 × g for 1 minute.
4. If pooling 2–24 samples, transfer 10 µl of each normalized library to a single well of the PDP plate.
5. If pooling 25–48 samples, do the following.
   a. Transfer 5 µl of each column of normalized library to column 1 of the PDP plate, and then mix thoroughly as follows.
      — [HS] Shake at 1800 rpm for 2 minutes.
      — [LS] Pipette up and down.
   b. [HS] Centrifuge at 280 × g for 1 minute.
   c. Transfer the contents of each well of column 1 to well A2.
6. If pooling 49–96 samples, do the following.
   a. Transfer 5 µl of each column of normalized library to column 1 of the PDP plate, and then mix thoroughly as follows.
      — [HS] Shake at 1800 rpm for 2 minutes.
      — [LS] Pipette up and down.
   b. [HS] Centrifuge at 280 × g for 1 minute.
   c. Transfer the contents of each well of column 1 to a 1.7 ml microcentrifuge tube.
7. Mix thoroughly as follows.
   - [HS] Shake plate at 1800 rpm for 2 minutes or vortex the tube.
   - [LS] Pipette up and down.
8. [HS] Centrifuge at 280 × g for 1 minute.
9. Proceed to cluster generation.

SAFE STOPPING POINT

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

Acronyms

Acronym	Definition
ALP	Adapter Ligation Plate
ATL	A-Tailing Mix
CAP	Clean Up ALP Plate
CEP	Clean Up End Repair Plate
CSP	Clean Up Sheared DNA Plate
DAP	DNA Adapter Plate
DCT	Diluted Cluster Template Plate
DNA	Customer Sample DNA Plate
EPM	Enhanced PCR Mix
ERP	End Repair Mix
IMP	Insert Modification Plate
LIG	Ligation Mix
PDP	Pooled Dilution Plate
PPC	PCR Primer Cocktail
RSB	Resuspension Buffer
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
TSP1	Target Sample Plate 1

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	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

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

Product Documentation

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

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