Fragment DNA

- ☐ 1 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.
- ☐ 2 [HS] Mix and centrifuge as follows.
  - ☐ a Shake at 1800 rpm for 2 minutes.
  - ☐ b Centrifuge at 280 × g for 1 minute.
- ☐ 3 [LS] Pipette to mix, and then centrifuge briefly.
- ☐ 4 Transfer 52.5 µl DNA to Covaris tubes.
- ☐ 5 Centrifuge at 280 × g for 5 seconds.
- ☐ 6 Fragment using the appropriate settings:

Table 1 350 bp Insert

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

Table 2 550 bp Insert

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

- ☐ 7 Centrifuge at 280 × g for 5 seconds.
- ☐ 8 Transfer 50 µl sample to the CSP plate.
- ☐ 9 Add 80 µl SPB.
- ☐ 10 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 11 Incubate at room temperature for 5 minutes.
- ☐ 12 Centrifuge at 280 × g for 1 minute.
- ☐ 13 Place on a magnetic stand until the liquid is clear.
- ☐ 14 Remove and discard all supernatant.
- ☐ 15 Wash two times with 200 µl 80% EtOH.
- ☐ 16 Use a 20 µl pipette to remove residual EtOH.
- ☐ 17 Air dry for 5 minutes.
- ☐ 18 Add 62.5 µl RSB, and then remove from the magnetic stand.
- ☐ 19 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 20 Incubate at room temperature for 2 minutes.
- ☐ 21 Centrifuge at 280 × g for 1 minute.
- ☐ 22 Place on a magnetic stand until the liquid is clear.
- ☐ 23 Transfer 60 µl supernatant to the IMP plate.

Repair Ends and Select Library Size

- ☐ 1 Add 40 µl ERP 2 or ERP 3 .
- ☐ 2 [HS] Mix, centrifuge, and incubate as follows.
  - ☐ a Shake at 1800 rpm for 2 minutes.
  - ☐ b Centrifuge at 280 × g for 1 minute.
  - ☐ c Place on the 30°C microheating system for 30 minutes.
  - ☐ d Place on ice.
- ☐ 3 [LS] Pipette to mix, centrifuge briefly, and then place on the thermal cycler and run the ERP program.
- ☐ 4 Vortex SPB.
- ☐ 5 Using your calculations from the previous step, dilute SPB with PCR-grade water.
- ☐ 6 Vortex diluted SPB.
- ☐ 7 Add 160 µl diluted SPB to each well.
- ☐ 8 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 9 Incubate at room temperature for 5 minutes.
- ☐ 10 Centrifuge at 280 × g for 1 minute.
- ☐ 11 Place on a magnetic stand until the liquid is clear.
- ☐ 12 Transfer 250 µl supernatant to the CEP plate.
- ☐ 13 Vortex undiluted SPB.
- ☐ 14 Add 30 µl undiluted SPB.
- ☐ 15 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 16 Incubate at room temperature for 5 minutes.
- ☐ 17 Centrifuge at 280 × g for 1 minute.
- ☐ 18 Place on a magnetic stand until the liquid is clear.
- ☐ 19 Remove and discard all supernatant.
- ☐ 20 Wash two times with 200 µl 80% EtOH.
- ☐ 21 Use a 20 µl pipette to remove residual EtOH.

- ☐ 22 Air dry for 5 minutes.
- ☐ 23 Add 20 µl RSB, and then remove from the magnetic stand.
- ☐ 24 Mix as follows.
  - ▶ [HS] Shake at 1800 rpm for 2 minutes.
  - ▶ [LS] Pipette up and down.
- ☐ 25 Incubate at room temperature for 2 minutes.
- ☐ 26 Centrifuge at 280 × g for 1 minute.
- ☐ 27 Place on a magnetic stand until the liquid is clear.
- ☐ 28 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

- ☐ 1 Centrifuge ATL or ATL 2 at 600 × g for 5 seconds.
- ☐ 2 Add 12.5 µl ATL or ATL 2 .
- ☐ 3 Mix as follows.
  - ▶ [HS] Shake at 1800 rpm for 2 minutes.
  - ▶ [LS] Pipette up and down.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 [HS] Incubate as follows.
  - ☐ a Place on the 37°C microheating system for 30 minutes.
  - ☐ b Move to the 70°C microheating system for 5 minutes.
  - ☐ c Place on ice for 5 minutes.
- ☐ 6 [LS] Incubate as follows.
  - ☐ a Place on the thermal cycler and run the ATAIL70 program.
  - ☐ b Centrifuge at 280 × g for 1 minute.

Ligate Adapters

- ☐ 1 [HS] Centrifuge the DAP at 280 × g for 1 minute.
- ☐ 2 [LS] Centrifuge the adapter tubes at 600 × g for 5 seconds.
- ☐ 3 Remove LIG 2 from -25°C to -15°C storage.
- ☐ 4 In the order listed, add the following reagents:
  - RSB (2.5 µl)
  - LIG 2 (2.5 µl)
  - DNA adapters (2.5 µl)
- ☐ 5 Mix as follows.
  - ▶ [HS] Shake at 1800 rpm for 2 minutes.
  - ▶ [LS] Pipette up and down.
- ☐ 6 Centrifuge at 280 × g for 1 minute.
- ☐ 7 Incubate as follows.
  - ▶ [HS] Place on the 30°C microheating system for 10 minutes. Set aside on ice.
  - ▶ [LS] Place on the thermal cycler and run the LIG program.
- ☐ 8 Centrifuge the STL at 600 × g for 5 seconds.
- ☐ 9 Add 5 µl STL to each well.
- ☐ 10 Mix as follows.
  - ▶ [HS] Shake at 1800 rpm for 2 minutes.
  - ▶ [LS] Pipette up and down.
- ☐ 11 Centrifuge at 280 × g for 1 minute.
- ☐ 12 Add SPB.
  - **Round 1**—42.5 µl
  - **Round 2**—50 µl
- ☐ 13 Mix as follows.
  - ▶ [HS] Shake at 1800 rpm for 2 minutes.
  - ▶ [LS] Pipette up and down.
- ☐ 14 Incubate at room temperature for 5 minutes.
- ☐ 15 Centrifuge at 280 × g for 1 minute.
- ☐ 16 Place on a magnetic stand until the liquid is clear.
- ☐ 17 Remove and discard supernatant.
- ☐ 18 Wash two times with 200 µl 80% EtOH.

- ☐ 19 Use a 20 µl pipette to remove residual EtOH.
- ☐ 20 Air dry for 5 minutes.
- ☐ 21 Add RSB.
  - **Round 1**—52.5 µl
  - **Round 2**—27.5 µl
- ☐ 22 Remove from the magnetic stand, and then mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 23 Incubate at room temperature for 2 minutes.
- ☐ 24 Centrifuge at 280 × g for 1 minute.
- ☐ 25 Place on a magnetic stand until the liquid is clear.
- ☐ 26 Transfer 50 µl supernatant to the CAP plate.
- ☐ 27 Repeat steps 12 through 25 using the new plate and the **Round 2** volumes.
- ☐ 28 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

- ☐ 1 Place on ice and add 5 µl PPC.
- ☐ 2 Add 20 µl EPM.
- ☐ 3 Mix as follows.
  - [HS] Shake at 1600 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the thermal cycler and run the PCRNano program.
- ☐ 6 Centrifuge at 280 × g for 1 minute.
- ☐ 7 Vortex SPB.
- ☐ 8 Add the appropriate volume of SPB to each well:

Adapter Type	SPB Volume
Adapter tubes	50 µl
Index Adapter Plate	47.5 µl

- ☐ 9 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 10 Incubate at room temperature for 5 minutes.
- ☐ 11 Centrifuge at 280 × g for 1 minute.
- ☐ 12 Place on a magnetic stand until the liquid is clear.
- ☐ 13 Remove and discard all supernatant.
- ☐ 14 Wash two times with 200 µl 80% EtOH.
- ☐ 15 Use a 20 µl pipette to remove residual EtOH.
- ☐ 16 Air-dry for 5 minutes.
- ☐ 17 Add 32.5 µl RSB, and then remove from the magnetic stand.
- ☐ 18 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 19 Incubate at room temperature for 2 minutes.
- ☐ 20 Centrifuge at 280 × g for 1 minute.
- ☐ 21 Place on a magnetic stand until the liquid is clear.
- ☐ 22 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.

Check Libraries

- ☐ 1 Quantify libraries.
- ☐ 2 If you are using a High Sensitivity DNA chip, dilute the DNA library 1:10 with water.
- ☐ 3 Run on the Advanced Analytical Fragment Analyzer or Agilent Technology 2100 Bioanalyzer:
  - For a High Sensitivity DNA or NGS Kit, run 1 µl diluted DNA library.
  - For a Bioanalyzer DNA 7500 chip, run 1 µl undiluted DNA library.

Normalize and Pool Libraries

- ☐ 1 Transfer 10 µl library to of the DCT plate.
- ☐ 2 Normalize to 10 nM using Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20.
- ☐ 3 Mix as follows.
  - [HS] Shake at 1000 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Do the following:
  - To pool libraries, proceed to .
  - Libraries that are not pooled must be diluted and denatured before proceeding to cluster generation.
- ☐ 6 Transfer 10 µl to one well of the PDP plate.
- ☐ 7 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 8 Centrifuge at 280 × g for 1 minute.
- ☐ 9 Proceed to cluster generation.
- ☐ 10 Transfer 5 µl to column 1 of the PDP plate.
- ☐ 11 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 12 Centrifuge at 280 × g for 1 minute.
- ☐ 13 Transfer column 1 contents to well A2.
- ☐ 14 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 15 Centrifuge at 280 × g for 1 minute.
- ☐ 16 Proceed to cluster generation.
- ☐ 17 Transfer 5 µl to column 1 of the PDP plate.
- ☐ 18 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 19 Centrifuge at 280 × g for 1 minute.
- ☐ 20 Transfer column 1 contents to a 1.7 ml microcentrifuge tube.
- ☐ 21 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes or vortex the tube.
  - [LS] Pipette up and down.
- ☐ 22 Centrifuge at 280 × g for 1 minute.
- ☐ 23 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
CFP	Covaris Fragmentation Plate
CSP	Clean Up Sheared DNA Plate
DCT	Diluted Cluster Template Plate
DNA	Customer Sample DNA Plate
ERP	End Repair Mix
HS	High Sample
IEM	Illumina Experiment Manager
IMP	Insert Modification Plate
LIG	Ligation Mix
LRM	Local Run Manager
LS	Low Sample
PDP	Pooled Dilution Plate
RSB	Resuspension Buffer
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
TSP1	Target Sample Plate 1