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 Mix thoroughly.
☐3 Centrifuge.
☐4 Transfer 52.5 µl DNA to Covaris tubes.
☐5 Centrifuge at 280 × g for 5 seconds.
☐6 Fragment the DNA using the following settings.

Table 1 350 bp Insert Settings

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

Table 2 550 bp Insert Settings

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

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

Repair Ends and Select Library Size

☐1 Add 40 µl ERP2/ERP3 and mix.
☐2 [HS] Centrifuge at 280 × g for 1 minute.
☐3 Incubate as follows.
- [HS] Place on the 30°C microheating system 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 sample.
☐5 Vortex diluted SPB until well-dispersed.
☐6 Add 160 µl diluted SPB and mix.
☐7 Incubate at room temperature for 5 minutes.
☐8 [HS] Centrifuge at 280 × g for 1 minute.
☐9 Place on a magnetic stand until liquid is clear.
☐10 Transfer 250 µl supernatant to the CEP plate.
☐11 Add 30 µl SPB and mix.
☐12 Incubate at room temperature for 5 minutes.
☐13 [HS] Centrifuge at 280 × g for 1 minute.
☐14 Place on a magnetic stand until liquid is clear.
☐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 for 5 minutes.
☐19 Add 20 µl RSB.
☐20 Remove from the magnetic stand and mix.
☐21 Incubate at room temperature for 2 minutes.
☐22 [HS] Centrifuge at 280 × g for 1 minute.
☐23 Place on a magnetic stand until liquid is clear.
☐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

☐1 Add 12.5 µl ATL/ATL2 and mix.
☐2 Centrifuge at 280 × g for 1 minute.
☐3 Incubate as follows.
[HS]
☐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.
[LS]
☐a Place on the thermal cycler and run the ATAIL70 program.
☐b Centrifuge at 280 × g for 1 minute.

Ligate Adapters

☐1 Add the following and mix.

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

☐2 Centrifuge at 280 × g for 1 minute.
☐3 Incubate as follows.
- [HS] Place on the 30°C microheating system 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 mix.
☐5 [HS] Centrifuge at 280 × g for 1 minute.
☐6 Perform steps 6a through 6m using the **Round 1** volumes.
☐a Add SPB and mix.

	Round 1	Round 2
SPB	42.5 µl	50 µl

☐b Incubate at room temperature for 5 minutes.
☐c [HS] Centrifuge at 280 × g for 1 minute.
☐d Place on a magnetic stand until liquid is clear.
☐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 for 5 minutes.
☐i Add RSB.

	Round 1	Round 2
RSB	52.5 µl	27.5 µl

☐j Remove from the magnetic stand and mix.
☐k Incubate at room temperature for 2 minutes.
☐l [HS] Centrifuge at 280 × g for 1 minute.
☐m Place on a magnetic stand until liquid is clear.
☐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

☐1 Place on ice and add 5 µl PPC.
☐2 Add 20 µl EPM and mix.
☐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.

Adapter Type	Volume SPB
Adapter tubes	50 µl
DAP	47.5 µl

☐7 Mix thoroughly.
☐8 Incubate at room temperature for 5 minutes.
☐9 [HS] Centrifuge at 280 × g for 1 minute.
☐10 Place on a magnetic stand until liquid is clear.
☐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 for 5 minutes.
☐15 Add 32.5 µl RSB.
☐16 Remove from the magnetic stand and mix.
☐17 Incubate at room temperature for 2 minutes.
☐18 [HS] Centrifuge at 280 × g for 1 minute.
☐19 Place on a magnetic stand until liquid is clear.
☐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

☐1 Quantify the libraries.
☐2 Do the following:
- 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

☐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 mix.
☐3 [HS] Centrifuge at 280 × g for 1 minute.
☐4 If pooling 2–24 samples, transfer 10 µl to a single well of the PDP plate.
☐5 If pooling 25–48 samples.
  ☐a Transfer 5 µl to column 1 of the PDP plate and mix.
  ☐b [HS] Centrifuge at 280 × g for 1 minute.
  ☐c Transfer column 1 to well A2.
☐6 If pooling 49–96 samples.
  ☐a Transfer 5 µl to column 1 of the PDP plate and mix.
  ☐b [HS] Centrifuge at 280 × g for 1 minute.
  ☐c Transfer column 1 to a 1.7 ml microcentrifuge tube.
☐7 Mix thoroughly.
☐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