Fragment DNA

- ☐1 Normalize gDNA with RSB to 55 µl in the DNA plate.
  - 20 ng/µl for a 350 bp insert size
  - 40 ng/µl 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 52.5 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 50 µl supernatant to the IMP plate.

Repair Ends and Select Library Size

- ☐1 Add 10 µl CTE.
- ☐2 Add 40 µl ERP2/ERP3 and mix.
- ☐3 [HS] Centrifuge at 280 × g for 1 minute.
- ☐4 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.
- ☐5 Dilute SPB with PCR grade water to 160 µl per 100 µl of sample.
- ☐6 Vortex diluted SPB until well-dispersed.
- ☐7 Add 160 µl diluted SPB and mix.
- ☐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 Transfer 250 µl supernatant to the CEP plate.
- ☐12 Add 30 µl SPB and mix.
- ☐13 Incubate at room temperature for 5 minutes.
- ☐14 [HS] Centrifuge at 280 × g for 1 minute.
- ☐15 Place on a magnetic stand until liquid is clear.
- ☐16 Remove and discard all supernatant.
- ☐17 Wash 2 times with 200 µl 80% EtOH.
- ☐18 Use a 20 µl pipette to remove residual EtOH.
- ☐19 Air-dry for 5 minutes.
- ☐20 Add 17.5 µl RSB.
- ☐21 Remove from the magnetic stand and mix.
- ☐22 Incubate at room temperature for 2 minutes.
- ☐23 [HS] Centrifuge at 280 × g for 1 minute.
- ☐24 Place on a magnetic stand until liquid is clear.
- ☐25 Transfer 15 µl supernatant to the ALP plate.

Adenylate 3′ Ends

☐1 Add 2.5 µl CTA.
☐2 Add 12.5 µl ATL/ATL2 and mix.
☐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.

Ligate Adapters

☐1 Add the following and mix.

Reagent	Volume (µl)
CTL	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	22.5 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 20 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 using qPCR, with the following modifications.
- Use at least 2 µl of the original library stock.
- Perform 2 additional dilutions.
- Determine the concentration of the diluted library.
- Perform a size adjustment calculation.
- Calculate the concentration of the undiluted library.

☐2 Verify fragment size by checking the library size distribution.
- ☐a Dilute the DNA library 1:5 with water.
- ☐b Run 1 µl diluted DNA library on a High Sensitivity DNA chip.

Normalize and Pool Libraries

☐1 Transfer 5 µl library to the DCT plate.
☐2 Normalize with Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20 to 2 nM and mix.
☐3 [HS] Centrifuge at 280 × g for 1 minute.
☐4 If pooling 2–24 samples, transfer 5 µl to a single well of the PDP plate.
☐5 If pooling 25–96 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 well A2.

☐6 Mix thoroughly.
☐7 [HS] Centrifuge at 280 × g for 1 minute.
☐8 Proceed to cluster generation.

SAFE STOPPING POINT

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

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
CTA	A-Tailing Control
CTE	End Repair Control
CTL	Ligation Control
DAP	DNA Adapter Plate
DCT	Diluted Cluster Template Plate
DNA	Customer Sample DNA Plate
ERP	End Repair Mix
IMP	Insert Modification Plate
LIG	Ligation Mix
PDP	Pooled Dilution Plate
RSB	Resuspension Buffer
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
TSP1	Target Sample Plate 1