TruSeq DNA PCR-Free Checklist

Fragment DNA

- ☐ 1 Normalize gDNA with RSB to 55 µl in the DNA plate.
  - 1 ug for a 350 bp insert size
  - 2 ug 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 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 50 µl supernatant to the IMP plate.

Repair Ends and Select Library Size

- ☐ 1 Centrifuge CTE at 600 × g for 5 seconds.
- ☐ 2 Add 10 µl CTE or RSB.
- ☐ 3 Add 40 µl ERP 2 or ERP 3 to each well.
- ☐ 4 [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.
- ☐ 5 [LS] Pipette to mix, centrifuge, and then place on the thermal cycler and run the ERP program.
- ☐ 6 Vortex SPB.
- ☐ 7 Using your calculations from the previous step, dilute SPB with PCR-grade water.
- ☐ 8 Vortex diluted SPB.
- ☐ 9 Add 160 µl diluted SPB to each well.
- ☐ 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 Transfer 250 µl supernatant to the CEP plate.
- ☐ 15 Vortex undiluted SPB.
- ☐ 16 Add 30 µl undiluted SPB.
- ☐ 17 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 18 Incubate at room temperature for 5 minutes.
- ☐ 19 Centrifuge at 280 × g for 1 minute.
- ☐ 20 Place on a magnetic stand until the liquid is clear.
- ☐ 21 Remove and discard all supernatant.

- ☐ 22 Wash two times with 200 µl 80% EtOH.
- ☐ 23 Use a 20 µl pipette to remove residual EtOH.
- ☐ 24 Air dry for 5 minutes.
- ☐ 25 Add 17.5 µl RSB, and then remove from the magnetic stand.
- ☐ 26 Mix as follows.
  - [HS] Shake at 1800 rpm for 2 minutes.
  - [LS] Pipette up and down.
- ☐ 27 Incubate at room temperature for 2 minutes.
- ☐ 28 Centrifuge at 280 × g for 1 minute.
- ☐ 29 Place on a magnetic stand until the liquid is clear.
- ☐ 30 Transfer 15 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 CTA at 600 × g for 5 seconds.
- ☐ 2 Add 2.5 µl CTA.
- ☐ 3 Centrifuge ATL or ATL 2 at 600 × g for 5 seconds.
- ☐ 4 Add 12.5 µl ATL or ATL 2 .
- ☐ 5 [HS] Mix and incubate as follows.
  - ☐ a Shake at 1800 rpm for 2 minutes.
  - ☐ b Place on the 37°C microheating system for 30 minutes.
  - ☐ c Move to the 70°C microheating system for 5 minutes.
  - ☐ d Place on ice for 5 minutes.
- ☐ 6 [LS] Pipette to mix, and then place on the thermal cycler and run the ATAIL70 program.

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:
  - CTL (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**—22.5 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 20 µl supernatant to the TSP1 plate.

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Check Libraries

- ☐ 1 Quantify libraries using qPCR, with the following modifications:
  - Use at least 2 µl of the original library stock.
  - Perform two additional dilutions.
  - Determine the concentration of the diluted library.
  - Make 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 or NGS kit.

Normalize and Pool Libraries

- ☐ 1 Transfer 5 µl library to the DCT plate.
- ☐ 2 Normalize to 4 nM using Tris-HCl 10 mM, pH 8.5 with 0.1% Tween 20.
- ☐ 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 Proceed to pooling or clustering:
  - To pool libraries, proceed to .
  - To leave libraries unpooled, skip the remaining library prep steps and proceed to cluster generation.
- ☐ 6 Transfer 5 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.

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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
CTA	A-Tailing Control
CTE	End Repair Control
CTL	Ligation Control
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