TruSeq Stranded Total RNA Checklist

Deplete and Fragment RNA

- ☐ 1 Dilute the total RNA in water to a final volume of 10 µl in the BRP plate.
- ☐ 2 Add 5 µl RBB.
- ☐ 3 Add 5 µl GRM, RRM,RRM G, or RRM P.
- ☐ 4 Mix thoroughly.
- ☐ 5 Centrifuge at 280 × g for 1 minute.
- ☐ 6 Place on the thermal cycler and run the RNA Denaturation program.
- ☐ 7 Incubate at room temperature for 1 minute.
- ☐ 8 Add 35 µl RRB to the RRP plate.
- ☐ 9 Transfer all to the RRP plate and mix.
- ☐ 10 [LS] Incubate at room temperature for 1 minute.
- ☐ 11 Place on a magnetic stand for 1 minute.
- ☐ 12 Transfer all to the RCP plate.
- ☐ 13 Place on a magnetic stand for 1 minute.
- ☐ 14 Add 99 µl RNAClean XP Beads and mix.
- ☐ 15 Incubate at room temperature for 15 minutes.
- ☐ 16 Place on a magnetic stand until liquid is clear.
- ☐ 17 Remove and discard all of the supernatant.
- ☐ 18 Wash with 200 µl 70% EtOH.
  - ☐ a Add 200 µl freshly prepared 70% EtOH.
  - ☐ b Incubate on the magnetic stand for 30 seconds.
  - ☐ c Remove and discard all supernatant.
- ☐ 19 Use a 20 µl pipette to remove residual EtOH.
- ☐ 20 Air-dry for 15 minutes.
- ☐ 21 Remove from the magnetic stand.
- ☐ 22 Add 11 µl ELB and mix.
- ☐ 23 Incubate at room temperature for 2 minutes.
- ☐ 24 Centrifuge at 280 × g for 1 minute.
- ☐ 25 Place on a magnetic stand until liquid is clear.
- ☐ 26 Transfer 8.5 µl supernatant to the DFP plate.
- ☐ 27 Add 8.5 µl EPH and mix.
- ☐ 28 Place on the thermal cycler and run the Elution 2-Frag-Prime program.
- ☐ 29 Centrifuge briefly.

Synthesize First Strand cDNA

- ☐ 1 Add 50 µl SuperScript II to one tube of FSA. Pipette to mix, and then centrifuge briefly.
- ☐ 2 Add 8 µl FSA and SuperScript II mixture and mix.
- ☐ 3 Centrifuge at 280 × g for 1 minute.
- ☐ 4 Place on the thermal cycler and run the Synthesize 1st Strand program.

Synthesize Second Strand cDNA

- ☐ 1 Dilute CTE to 1:50 in RSB.
- ☐ 2 Add 5 µl diluted CTE.
- ☐ 3 Add 5 µl RSB.
- ☐ 4 Add 20 µl SMM and mix.
- ☐ 5 Centrifuge at 280 × g for 1 minute.
- ☐ 6 Place on the thermal cycler and incubate at 16°C for 1 hour.
- ☐ 7 Place on the bench and let stand to bring to room temperature.
- ☐ 8 [HS] Add AMPure XP beads as follows.
  - ☐ a Add 90 µl AMPure XP beads to the CCP plate.
  - ☐ b Transfer all to the CCP plate.
- ☐ 9 [LS] Add 90 µl AMPure XP beads.
- ☐ 10 Mix thoroughly.
- ☐ 11 Incubate at room temperature for 15 minutes.
- ☐ 12 Centrifuge at 280 × g for 1 minute.
- ☐ 13 Place on a magnetic stand until liquid is clear.
- ☐ 14 Remove and discard 135 µl supernatant.
- ☐ 15 Wash two times with 200 µl 80% EtOH.
- ☐ 16 Use a 20 µl pipette to remove residual EtOH.
- ☐ 17 Air-dry for 15 minutes. Do not over dry beads.
- ☐ 18 Remove from the magnetic stand.
- ☐ 19 Add 17.5 µl RSB and mix.
- ☐ 20 Incubate at room temperature for 2 minutes.
- ☐ 21 Centrifuge at 280 × g for 1 minute.
- ☐ 22 Place on a magnetic stand until liquid is clear.
- ☐ 23 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 Dilute CTA to 1:100 in RSB.
- ☐ 2 Add 2.5 µl diluted CTA.
- ☐ 3 Add 12.5 µl ATL and mix.
- ☐ 4 Seal the ALP plate with a Mircoseal 'B' adhesive seal.
- ☐ 5 Centrifuge at 280 × g for 1 minute.
- ☐ 6 [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 1 minute.
- ☐ 7 [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 Dilute CTL 1:100 in RSB.
- ☐ 2 Add the following.
  - Diluted CTL (2.5 µl)
  - LIG (2.5 µl)
  - RNA adapters (2.5 µl)
- ☐ 3 Mix thoroughly.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 [HS] Place on the 30°C microheating system for 10 minutes, and then place on ice.
- ☐ 6 [LS] Place on the thermal cycler and run the LIG program.
- ☐ 7 Add 5 µl STL and mix.
- ☐ 8 Centrifuge at 280 × g for 1 minute.
- ☐ 9 Perform steps 10 through 24 using the **Round 1** volumes.
- ☐ 10 Add AMPure XP beads.

	Round 1	Round 2
AMPure XP beads	42 µl	50 µl

- ☐ 11 Incubate at room temperature for 15 minutes.
- ☐ 12 Centrifuge at 280 × g for 1 minute.
- ☐ 13 Place on a magnetic stand until 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 15 minutes.
- ☐ 18 Remove from the magnetic stand.
- ☐ 19 Add RSB.

	Round 1	Round 2
RSB	52.5 µl	22.5 µl

- ☐ 20 Mix.
- ☐ 21 Incubate at room temperature for 2 minutes.
- ☐ 22 Centrifuge at 280 × g for 1 minute.

- ☐ 23 Place on a magnetic stand until liquid is clear.
- ☐ 24 Transfer 50 µl supernatant to the CAP plate.
- ☐ 25 Repeat steps 10 through 24 with the new plate using the **Round 2** volumes.
- ☐ 26 Transfer 20 µl supernatant to the PCR plate.

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Enrich DNA Fragments

- ☐ 1 Place on ice and add 5 µl PPC.
- ☐ 2 Add 25 µl PMM and mix.
- ☐ 3 Centrifuge at 280 × g for 1 minute.
- ☐ 4 Place on the thermal cycler and run the PCR program.
- ☐ 5 Centrifuge at 280 × g for 1 minute.
- ☐ 6 Add AMPure XP beads.

Adapter Type	Volume AMPure XP beads
Adapter tubes	50 µl
Index Adapter Plate	47.5 µl

- ☐ 7 Mix thoroughly.
- ☐ 8 Incubate at room temperature for 15 minutes.
- ☐ 9 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 two times with 200 µl 80% EtOH.
- ☐ 13 Use a 20 µl pipette to remove residual EtOH.
- ☐ 14 Air-dry for 15 minutes.
- ☐ 15 Remove from the magnetic stand.
- ☐ 16 Add 32.5 µl RSB and mix.
- ☐ 17 Incubate at room temperature for 2 minutes.
- ☐ 18 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.

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Check Libraries

- ☐ 1 Quantify the libraries or fluorometric method.
- ☐ 2 If using a Standard Sensitivity NGS Fragment Analysis Kit:
  - ☐ a Dilute the DNA library 1:1 with RSB.
  - ☐ b Run 1 µl diluted DNA library.
- ☐ 3 If using a DNA 1000 chip, run 1 µl undiluted DNA library.
- ☐ 4 Check the size and purity of the sample. Expect the final product to be a band at ~260 bp.

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 Centrifuge at 280 × g for 1 minute.
- ☐ 4 Transfer 10 µl to a single well of the PDP plate.
- ☐ 5 Mix thoroughly.
- ☐ 6 [HS] Centrifuge at 280 × g for 1 minute.
- ☐ 7 Proceed to cluster generation.
- ☐ 8 Transfer 5 µl to column 1 of the PDP plate and mix.
  - ▶ [HS] Shake at 1800 rpm for 2 minutes.
  - ▶ [LS] Pipette up and down 10 times.
- ☐ 9 Centrifuge at 280 × g for 1 minute.
- ☐ 10 Transfer column 1 to well A2.
- ☐ 11 Mix thoroughly.
- ☐ 12 Centrifuge at 280 × g for 1 minute.
- ☐ 13 Proceed to cluster generation.

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Acronyms

Acronym	Definition
ALP	Adapter Ligation Plate
ATL	A-Tailing Mix
BRP	Bind rRNA Plate
CAP	Clean Up ALP Plate
CCP	cDNA Clean Up Plate
CPP	Clean Up PCR Plate
CTA	A-Tailing Control
CTE	End Repair Control
CTL	Ligation Control
DCT	Diluted Cluster Template
DFP	Depleted RNA Fragmentation Plate
ELB	Elution Buffer
EPH	Elute, Prime, Fragment High Mix
EPM	Enhanced PCR Mix
FSA	First Strand Synthesis Act D Mix
GRM	Globin Removal Mix
H/M/R	Human/Mouse/Rat
HS	High Sample
IEM	Illumina Experiment Manager
LIG	Ligation Mix
LRM	Local Run Manager
LS	Low Sample
PCR	Polymerase Chain Reaction
PDP	Pooled Dilution Plate
PMM	PCR Master Mix
PPC	PCR Primer Cocktail
RBB	rRNA Binding Buffer
RCP	RNA CleanUp Plate
RMB	rRNA Removal Mix - Bacteria
RME	rRNA Removal Mix - Epidemiology
RMY	rRNA Removal Mix - Yeast
RRB	rRNA Removal Beads
RRM	rRNA Removal Mix
RRM G	rRNA Removal Mix - Gold
RRM P	rRNA Removal Mix - Plant
RRP	rRNA Removal Plate
RSB	Resuspension Buffer
SMM	Second Strand Marking Master Mix
SPB	Sample Purification Beads
STL	Stop Ligation Buffer
TSP1	Target Sample Plate