TruSeq Stranded mRNA Checklist

For Research Use Only. Not for use in diagnostic procedures.

Purify and Fragment mRNA

- ☐ 1 Dilute the total RNA in nuclease-free ultrapure water to a final volume of 50 µl in the RBP plate.
- ☐ 2 Add 50 µl RPB and mix.
- ☐ 3 Incubate as follows.
  - [HS] Place on the 65°C microheating system for 5 minutes, and then place on ice for 1 minute.
  - [LS] Place on the thermal cycler and run the mRNA Denaturation program.
- ☐ 4 Seal the RBP plate with a Microseal 'B' adhesive seal before running the mRNA denaturation program.
- ☐ 5 Place on the bench and incubate at room temperature for 5 minutes.
- ☐ 6 [HS] Preheat the microheating system to 80°C.
- ☐ 7 [LS] Centrifuge at 280 × g for 1 minute.
- ☐ 8 Place on a magnetic stand until liquid is clear.
- ☐ 9 Remove and discard all supernatant.
- ☐ 10 Remove from the magnetic stand.
- ☐ 11 Add 200 µl BWB and mix.
- ☐ 12 Place on a magnetic stand until liquid is clear.
- ☐ 13 Remove and discard all supernatant.
- ☐ 14 Remove from the magnetic stand.
- ☐ 15 Add 50 µl ELB and mix.
- ☐ 16 [LS] Centrifuge at 280 × g for 1 minute.
- ☐ 17 Incubate as follows.
  - [HS] Place on the 80°C microheating system for 2 minutes, and then place on ice for 1 minute.
  - [LS] Place on the thermal cycler and run the mRNA Elution 1 program.
- ☐ 18 Place on the bench.
- ☐ 19 Add 50 µl BBB and mix.
- ☐ 20 Seal the RBP plate with a Microseal 'B' adhesive seal before running the mRNA denaturation program.
- ☐ 21 Incubate at room temperature for 5 minutes.
- ☐ 22 Place on a magnetic stand until liquid is clear.
- ☐ 23 Remove and discard all supernatant.
- ☐ 24 Remove from the magnetic stand.
- ☐ 25 Add 200 µl BWB and mix.
- ☐ 26 Place on a magnetic stand until liquid is clear.
- ☐ 27 Remove and discard all supernatant.
- ☐ 28 Remove from the magnetic stand.
- ☐ 29 Add 19.5 µl FPF and mix.
- ☐ 30 [LS] Centrifuge at 280 × g for 1 minute.
- ☐ 31 [HS] Transfer all to the RFP plate.
- ☐ 32 Place on the thermal cycler and run the Elution 2 - Frag - Prime program.
- ☐ 33 Centrifuge briefly.

Synthesize First Strand cDNA

- ☐ 1 Place the RBP plate on the magnetic stand until liquid is clear.
- ☐ 2 Transfer 17 µl supernatant to the CDP plate.
- ☐ 3 Add 50 µl SuperScript II to one tube of FSA. Pipette to mix, and then centrifuge briefly.
- ☐ 4 Add 8 µl FSA and SuperScript II mixture and mix.
- ☐ 5 Centrifuge at 280 × g for 1 minute.
- ☐ 6 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 20 µl SMM and mix.
- ☐ 4 Centrifuge at 280 × g for 1 minute.
- ☐ 5 Place on the thermal cycler and incubate at 16°C for 1 hour.
- ☐ 6 Place on the bench and let stand to bring to room temperature.
- ☐ 7 [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.
- ☐ 8 [LS] Add 90 µl AMPure XP beads.
- ☐ 9 Mix thoroughly.
- ☐ 10 Incubate at room temperature for 15 minutes.
- ☐ 11 Centrifuge at 280 × g for 1 minute.
- ☐ 12 Place on a magnetic stand until liquid is clear.
- ☐ 13 Remove and discard 135 µl supernatant.
- ☐ 14 Wash two times with 200 µl 80% EtOH.
- ☐ 15 Use a 20 µl pipette to remove residual EtOH.
- ☐ 16 Air-dry for 15 minutes. Do not over dry beads.
- ☐ 17 Remove from the magnetic stand.
- ☐ 18 Add 17.5 µl RSB and mix.
- ☐ 19 Incubate at room temperature for 2 minutes.
- ☐ 20 Centrifuge at 280 × g for 1 minute.
- ☐ 21 Place on a magnetic stand until liquid is clear.
- ☐ 22 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 mRNA 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
BBB	Bead Binding Buffer
BWB	Bead Washing Buffer
CAP	Clean Up ALP Plate
CCP	cDNA Clean Up Plate
CDP	cDNA Plate
CTA	A-Tailing Control
CTE	End Repair Control
CTL	Ligation Control
DCT	Diluted Cluster Template
ELB	Elution Buffer
FPF	Fragment, Prime, Finish Mix
FSA	First Strand Synthesis Act D Mix
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
RBP	RNA Bead Plate
RFP	RNA Fragmentation Plate
RPB	RNA Purification Beads
RSB	Resuspension Buffer
SSM	Second Strand Master Mix
STL	Stop Ligation Buffer
TSP	Target Sample Plate