Quick Start Protocol

SureSelect XT HS2 mRNA Library Preparation

G9995-90500 Rev A0

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

mRNA Enrichment and cDNA Conversion Workflow

3.5 Hours

Enrich mRNA on oligo(dT) beads

40 min

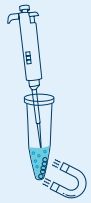
Fragment mRNA

10 min

Convert mRNA to cDNA

2 hrs

Clean-up cDNA



30 min

Stopping point

cDNA Library Preparation Workflow

3 Hours

End repair and dA-tail cDNA

40 min

Ligate MBC-tagged adaptors

40 min

Clean-up ligation

30 min

Amplify and dual-index

35 min

Clean-up library

30 min

Quality control

Varies

Stopping point

Duration estimates are provided as guidelines for 16 reaction runs using 1 µg high-quality input RNA. Your results may vary.

This Quick Start Protocol provides key protocol details for experienced users. Visit SureSelect XT HS2 mRNA System Protocol for the full User Guide with further information including:

- Safety information
- Notices to purchaser
- List of required materials
- Sample qualification guidelines
- QC output examples
- Critical factors for system performance including component handling, mixing, and spinning specifications; pay special attention to the key critical factors such as mixing steps highlighted in this Quick Start Protocol
- Full protocol details including Notes and Caution statements
- Index sequences and other NGS support

mRNA Enrichment, Fragmentation and Conversion to cDNA

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions.

Step 1: Enrich poly-A mRNA with oligo(dT) beads

40 min

- [ ] Set up thermal cycler program (Table 1). Start and pause program.

Table 1: Thermal cycler program for RNA denaturation (vol 50 µl)

Step	Temperature	Time
Step 1	65 °C	5 min
Step 2	4 °C	1 min
Step 3	4 °C	Hold

- [ ] Prepare RNA samples in wells: 25 µl each containing 10–1000 ng total RNA in nuclease-free water. Keep on ice.
  For lower-quality RNA samples (RIN 6–8) use > 50 ng RNA.
- [ ] ● Get Oligo(dT) Microparticles from 4 °C, vortex until homogeneous.
- [ ] Add 25 µl of Oligo(dT) Microparticles to each RNA sample well **>** seal wells **>** vortex gently 5 sec **>** brief spin.
- [ ] Place samples in thermal cycler and press play/continue.

Enrichment 1 (Bind > Wash > Elute)

- [ ] At 4 °C Hold step, remove from thermal cycler **>** incubate at RT 5 min.
- [ ] Collect beads using magnet (2–5 min) **>** remove and discard supernatant.
- [ ] Remove from magnet **>** gently add 200 µl Bead Washing Buffer **>** mix by pipetting up and down 10 times.
  Do not introduce bubbles. If bubbles/foam present, spin to remove.
- [ ] Collect beads using magnet (at least 2 min, until solution clear) **>** remove and discard supernatant.

Step 1 Continued

- [ ] Set up thermal cycler program (Table 2). Start and pause program.

Table 2: Thermal cycler program for RNA elution (vol 25 µl)

Step	Temperature	Time
Step 1	80 °C	2 min
Step 2	4 °C	1 min
Step 3	4 °C	Hold

- [ ] ● Remove samples from magnet **>** add 25 µl Bead Elution Buffer.
- [ ] Seal wells **>** vortex gently 5 sec **>** brief spin **>** place in thermal cycler and press play/continue.

Enrichment 2 (Bind > Wash only)

- [ ] ● At 4 °C Hold step, remove from thermal cycler **>** add 25 µl Bead Binding Buffer.
- [ ] Seal wells **>** vortex gently 5 sec **>** brief spin **>** incubate at RT 5 min.
- [ ] Collect beads using magnet (at least 2 min, until solution clear) **>** remove and discard supernatant.
- [ ] Remove from magnet **>** gently add 200 µl Bead Washing Buffer **>** mix by pipetting up and down 10 times.
  Do not introduce bubbles. If bubbles/foam present, spin to remove.
- [ ] Collect beads using magnet (at least 2 min, until solution clear) **>** remove and discard supernatant.
- [ ] Remove samples from magnet **>** add 10 µl nuclease-free water **>** keep on ice.

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions.

Step 2: Fragment mRNA

10 min

- ☐ Set up thermal cycler program (Table 3). Start and pause program.

Table 3: Thermal cycler program for RNA fragmentation (vol 20 µl)

Step	Temperature	Time
Step 1	94 °C	4 min
Step 2	4 °C	1 min
Step 3	4 °C	Hold

- ☐ ● Add 10 µl 2X Priming Buffer to each bead-bound RNA sample.
  2X Priming Buffer includes both cDNA primers and heat-driven fragmentation agents.
- ☐ Seal wells **>** vortex at high-speed **>** brief spin>place in thermal cycler and press play/continue.
- ☐ At 4 °C Hold step, remove from thermal cycler **>** transfer to magnetic stand at RT to collect beads.
- ☐ Watch for bead suspensions to clear **> transfer each supernatant (~20 µl) to fresh well > keep on ice.**
  Minimize processing time for this step to avoid re-binding mRNA to Oligo(dT) beads.

Step 3: Synthesize first-strand cDNA

55 min

- ☐ Set up thermal cycler program (Table 4). Start and pause program.

Table 4: Thermal cycler program for first-strand synthesis (vol 28 µl)

Step	Temperature	Time
Step 1	25 °C	10 min
Step 2	37 °C	40 min
Step 3	4 °C	Hold

- ☐ Vortex thawed First Strand Master Mix at high speed for 5 sec.
  Reagent is viscous and must be vortexed.
- ☐ ● Add 8.5 µl First Strand Master Mix to RNA samples. Mix **>** brief spin.
- ☐ Place in thermal cycler and press play/continue.
- ☐ At 4 °C Hold step, remove from thermal cycler **>** keep on ice.

Step 4: Synthesize second-strand cDNA

65 min

- ☐ Set up thermal cycler program (Table 5). Start and pause program.

Table 5: Thermal cycler program for second-strand synthesis (vol 58 µl)

Step	Temperature	Time
Step 1	16 °C	60 min
Step 2	4 °C	Hold

- ☐ Vortex thawed Second Strand Enzyme Mix and Second Strand Oligo Mix at high speed for 5 sec.
  Reagent is viscous and must be vortexed.
- ☐ ● Add 25 µl Second Strand Enzyme Mix to sample wells **>** keep on ice.
- ☐ ● Add 5 µl Second Strand Oligo Mix to sample wells **>** keep on ice.
- ☐ Mix **>** brief spin **>** place in thermal cycler and press play/continue.

Prep Ahead: Put AMPure XP beads at RT for use in next step (30 min equilibration)

Step 5: Clean-up cDNA

30 min

- ☐ Get AMPure XP beads held at RT, vortex until homogeneous.
- ☐ Add 105 µl of AMPure XP beads to each cDNA sample well. Mix **>** incubate at RT 5 min.
- ☐ Collect beads using magnet (2–5 min) **>** remove and discard supernatant.
- ☐ Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash.
  Washes completed: ☐ 1 ☐ 2
- ☐ After second wash removed, spin briefly **>** remove residual ethanol.
- ☐ Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- ☐ Elute by adding 52 µl nuclease-free water to each sample well. Seal wells **>** vortex **>** brief spin. Incubate at RT 2 min.
- ☐ Collect beads using magnet (~5 min).
- ☐ Transfer 50 µl cleared supernatant to fresh well **>** keep on ice.

Stopping point

cDNA Library Preparation

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions.

Step 1: Prepare ligation master mix

5 min

- [ ] Vortex thawed Ligation Buffer at high speed for 15 sec.
  Reagent is viscous and must be vortexed.
- [ ] Prepare Ligation master mix (Lig MM, see Table 6). Mix **>** spin **> keep at RT** during end repair/A-tailing steps.

Table 6: Lig MM reagent volumes (including excess for supported run sizes)

Reagent		16 Rxn Kits		96 Rxn Kits	
	Per Rxn	8 Rxn	16 Rxn	24 Rxn	96 Rxn
Ligation Buffer	23 µl	207 µl	414 µl	598 µl	2300 µl
T4 DNA Ligase	2 µl	18 µl	36 µl	52 µl	200 µl
Total	25 µl	225 µl	450 µl	650 µl	2500 µl

Step 2: End repair and dA-tail fragments

40 min

- [ ] Set up thermal cycler program (Table 7). Start and pause program.

Table 7: Thermal cycler program for End repair/A-tailing (vol 70 µl)

Step	Temperature	Time
Step 1	20 °C	15 min
Step 2	72 °C	15 min
Step 3	4 °C	Hold

- [ ] Vortex thawed End Repair-A Tailing Buffer at high speed for 15 sec.
  Reagent is viscous and must be vortexed.
- [ ] Prepare End repair/A-tailing master mix (ER-AT MM, see Table 8). Mix **>** spin **>** keep on ice.

Table 8: ER-AT MM reagent volumes (including excess for supported run sizes)

Reagent		16 Rxn Kits		96 Rxn Kits	
	Per Rxn	8 Rxn	16 Rxn	24 Rxn	96 Rxn
End Repair-A Tailing Buffer	16 µl	144 µl	288 µl	416 µl	1600 µl
End Repair-A Tailing Enzyme Mix	4 µl	36 µl	72 µl	104 µl	400 µl
Total	20 µl	180 µl	360 µl	520 µl	2000 µl

- [ ] Get 50 µl cDNA samples from ice. Add 20 µl ER-AT MM. Mix **>** spin.
- [ ] Place in thermal cycler and press play/continue.
- [ ] At 4 °C Hold step, remove from cycler **>** keep on ice.

Step 3: Ligate MBC-tagged adaptors

40 min

- [ ] Set up thermal cycler program (Table 9). Start and pause program.

Table 9: Thermal cycler program for Ligation (vol 100 µl)

Step	Temperature	Time
Step 1	20 °C	30 min
Step 2	4 °C	Hold

- [ ] Get 70 µl DNA samples from ice. Add 25 µl Lig MM from Step 1. Mix **>** spin.
- [ ] Add 5 µl XT HS2 RNA Adaptor Oligo Mix. Mix **>** spin.
- [ ] Place in thermal cycler and press play/continue.
- [ ] At 4 °C Hold step, remove from cycler **>** keep on ice.

Prep Ahead: Put AMPure XP beads at RT for use in next step (30 min equilibration)

Note: Reagent vial cap colors are indicated by colored circles adjacent to reagent use instructions. Clear cap is indicated by "c".

Step 4: Clean-up ligation

30 min

Libraries prepared from <100 ng RNA input or from lower-quality RNA samples (RIN 6–8) require two rounds of purification.

For all libraries:

- ☐ Get AMPure XP beads held at RT, vortex until homogeneous.
- ☐ Add 80 µl of AMPure XP beads to each cDNA sample well. Mix **>** incubate at RT 5 min.
- ☐ Collect beads using magnet (5–10 min) **>** remove and discard supernatant.
- ☐ Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash.
  Washes completed: ☐ 1 ☐ 2
- ☐ After second wash solution removed, spin briefly **>** remove residual ethanol.
- ☐ Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- ☐ Proceed to the appropriate instructions below, based on input RNA quality and quantity.

For high-quality (RIN≥8) AND high amount (≥100 ng) libraries:

- ☐ Elute by adding 35 µl nuclease-free water. Seal wells **>** vortex **>** brief spin. Incubate at RT 2 min.
- ☐ Collect beads using magnet (~5 min).
- ☐ Transfer 34 µl cleared supernatant to fresh well **>** keep on ice.

For low-quality (RIN 6–8) OR low amount (<100 ng) libraries:

- ☐ Elute by adding 50 µl nuclease-free water. Seal wells **>** vortex **>** brief spin. Incubate at RT 2 min.
- ☐ Collect beads using magnet (~5 min).
- ☐ Transfer ~50 µl cleared supernatant to fresh well **>** keep at RT.
- ☐ Repeat the clean-up steps as described above using 60 µl of AMPure XP beads. After drying samples at 37 °C, elute in 35 µl nuclease-free water and transfer 34 µl cleared supernatant to fresh well.

Step 5: Amplify and dual index

35 min

- ☐ Set up thermal cycler program (Table 10). Start and pause program.

Table 10: Thermal cycler program for indexing (vol 50 µl)

Step	Number of Cycles	Temperature	Time
Step 1	1	98 °C	2 min
Step 2	☐ 8 for 1000 ng RNA input library ☐ 10 for 250 ng RNA input library ☐ 11 for 100 ng RNA input library ☐ 13 for 50 ng RNA input library ☐ 15 for 10 ng RNA input library	98 °C	30 sec
		60 °C	30 sec
		72 °C	1 min
Step 3	1	72 °C	5 min
Step 4	1	4 °C	Hold

- ☐ Prepare PCR master mix (PCR MM; Table 11). Mix **>** spin **>** keep on ice.

Table 11: PCR MM reagent volumes (including excess for supported run sizes)

Reagent		16 Rxn Kits		96 Rxn Kits	
	Per Rxn	8 Rxn	16 Rxn	24 Rxn	96 Rxn
ⓒ 5X Herculase II Buffer with dNTPs	10 µl	90 µl	180 µl	260 µl	1000 µl
● Herculase II Fusion DNA Polymerase	1 µl	9 µl	18 µl	26 µl	100 µl
Total	11 µl	99 µl	198 µl	286 µl	1100 µl

- ☐ Get 34 µl purified library samples from ice. Add 11 µl PCR MM.
- ☐ Add 5 µl of the appropriate SureSelect XT HS2 Index Primer Pair to each sample well. Vortex **>** spin.
- ☐ Resume thermal cycler program without adding samples. Once cycler reaches 98 °C, add sample plate/strip **>** close lid.

Prep Ahead: Put AMPure XP beads at RT for use in next step (30 min equilibration)

Step 6: Clean-up library

30 min

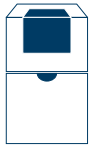
- ☐ Get AMPure XP beads held at RT, vortex until homogeneous.
- ☐ Remove samples from cycler **>** add 50 µl of AMPure XP beads to each amplified DNA well. Mix **>** incubate at RT 5 min.
- ☐ Collect beads using magnet (5 min) **>** remove and discard supernatant.
- ☐ Wash beads 2X with 200 µl freshly-prepared 70% ethanol per wash.
  Washes completed: ☐ 1 ☐ 2
- ☐ After second wash solution removed, spin briefly **>** remove residual ethanol.
- ☐ Dry samples unsealed on the thermal cycler at 37 °C (1–2 min).
- ☐ Elute by adding 15 µl 1X Low TE to each sample well. Seal wells **>** vortex **>** brief spin. Incubate at RT 2 min.
- ☐ Collect beads using magnet (2–3 min).
- ☐ Transfer cleared supernatant to fresh well **>** keep on ice.

Step 7: Quality control

Varies

Assess quality and quantity using one of these platforms:

Agilent 2100 Bioanalyzer System with DNA 1000 Assay



Agilent 4200 or 4150 TapeStation System with D1000 Assay

Agilent 5200, 5300 or 5400 Fragment Analyzer System with NGS Fragment Kit (1–6000 bp)

Stopping point

Step 8: Pool libraries for multiplex NGS

30 min

- ☐ Calculate the number of indexes that can be combined per lane, according to sequencer capacity and amount of sequencing data required per sample.
- ☐ Combine the libraries such that each index-tagged sample is present in equimolar amounts in the pool using one of the following methods:
  - ☐ Dilute each sample to be pooled to the concentration of the most dilute sample using Low TE **>** combine equal volumes of all samples in final pool.
  - ☐ Combine the appropriate volume of each sample to achieve equimolar concentration in the pool, then adjust the pool to the desired final volume using Low TE. See the **assay user guide** for more information on this method.

Run notes

Run Date	
Operator	
Index Pairs	
Reagent Lot Information	– Poly-A Selection Module: – cDNA Module: – Library Prep Kit: – Other Materials:
Sample Information	
Library Pooling Information	
Comments	

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