SureSelect XT HS2 RNA Target Enrichment using the Magnis NGS Prep System

Protocol

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

Version C0, August 2025

Notices

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CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Guide…

This guide provides instructions for automated preparation of SureSelect XT HS2 RNA target-enriched Illumina paired-end multiplexed sequencing libraries using the Magnis NGS Prep System.

The SureSelect XT HS2 system is used to prepare dual-indexed library samples with molecular barcodes prior to target enrichment to allow high-sensitivity sequencing on the Illumina platform.

What's New in Version C0

- Support for Magnis SureSelect XT HS2 Clinical Research Exome V4 kits (see Table 1 on page 10 and Table 15 on page 59)
- Updates to Table 3 on page 11 and Table 4 on page 11 to show current availability of Agilent's automated electrophoresis platforms
- Support for run setup using various Magnis firmware versions. For instruments running firmware v1.4 or earlier, see page 26. For instruments running firmware v1.5 or later, see page 27. Firmware v1.5 includes a new *Application* menu on the *Enter Run Info* screens
- Labware positioning precaution added to page 29
- Final library sample volume added to page 42, with *Troubleshooting* information on page 72
- Updates to downstream sequencing support information on page 53 to page 56 and page 62. Tween 20 removed from Table 5 on page 12.
- Link to the SureSelect XT HS2 Index Sequence Resource spreadsheet added to page 55, page 62 and page 65

What's New in Version B0

- Support for Magnis protocol *SSEL-RNA-XTHS2-EPIS-ILM,* run using Magnis SureSelect XT HS2 Reagent Kit PN G9750D, which is supplied with empty probe input strips (EPIS). See page 47 to page 49 for detailed probe input strip filling and run setup instructions for this protocol. See page 23, page 25 and page 37 for related run setup updates, and see page 71 for related troubleshooting information. For information on kit PN G9750D, see Table 1 on page 10 and Table 15 on page 59.
- Updates to kit reagent and plasticware preparation instructions on page 20 to page 23
- Minor updates to chiller strip tube loading instructions on page 34 to clarify requirement to leave foil covers intact for all strips loaded for the run
- Minor updates to page 42 including addition of final library solvent composition
- New *Disposal* section on page 43
- Updates to downstream sequencing support information (see page 54 to page 55)
- Updates to Notices section

Content

1 Before You Begin

This chapter contains information for you to read and understand before you start.

Overview of the Workflow

The workflow for SureSelect XT HS2 RNA target enrichment using the Magnis NGS Prep System is summarized in Figure 1. RNA samples, pre-plated reagents and labware are loaded on the instrument. Once loaded, the Magnis NGS Prep System performs all SureSelect XT HS2 RNA library preparation and target enrichment liquid handling and incubation steps. After the Magnis NGS Prep System run is complete, the target-enriched libraries are ready to be pooled for multiplexed NGS sample preparation and sequence analysis using Illumina sequencers.

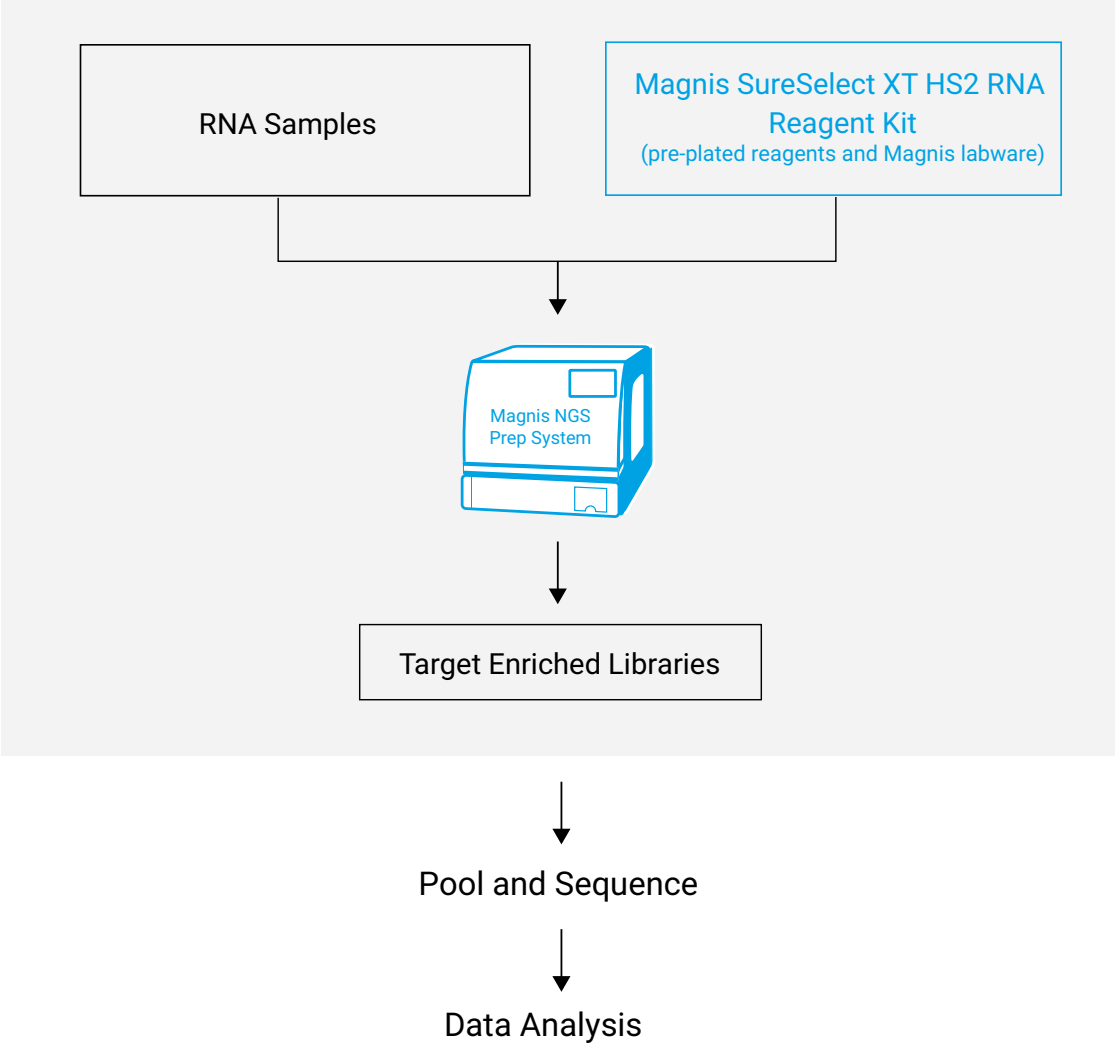


Figure 1 Overall Magnis NGS Prep System NGS sample preparation workflow.

Safety Notes

CAUTION Wear appropriate personal protective equipment (PPE) when working in the laboratory.

Danger of Ultraviolet (UV) Light Exposure

The Magnis instrument door and side panels are not UV-transparent, therefore exposure to UV light is minimal. However, the following precautions are still needed.

- During decontamination of the instrument deck with UV light, do not look directly or indirectly at the UV light source.
- Always perform decontamination with the instrument door closed and locked. The instrument door is programmed to remain locked while the UV light is on.
- Replacement UV tubes must be provided by Agilent and must be installed by an Agilent engineer or Agilent authorized service provider.

Danger of Burns

- During protocol runs, the thermal block and other components of the thermal cycler module quickly attain temperatures of greater than 50°C. To ensure safe operation, the instrument door must remain closed during runs. The instrument is programmed to keep the door locked while protocol runs are in progress.
- Use only Agilent materials (plates, adhesive seals, foils, mats) intended for use on the Magnis NGS Prep System. These materials are sufficiently temperature-stable (up to 120°C).

Materials Required

Required Materials for SureSelect XT HS2 RNA Magnis Runs

Table 1 Supported Reagent Kits (select one)

Description	96 Reactions*	32 Reactions†
Magnis SureSelect XT HS2 RNA Reagent Kit:	Agilent	Agilent
with Tier 1 (1–499 kb) Probe	p/n G9751D	p/n G9751C
with Tier 2 (0.5–2.9 Mb) Probe	p/n G9752D	p/n G9752C
with Tier 3 (3–5.9 Mb) Probe	p/n G9753D	p/n G9753C
with Tier 4 (6–11.9 Mb) Probe	p/n G9754D	p/n G9754C
with Tier 5 (12–24 Mb) Probe	p/n G9755D	p/n G9755C
with 24–50 Mb Probe	p/n G9756D	p/n G9756C
with Human All Exon V7 Probe	p/n G9773D	p/n G9773C
with Human All Exon V8 Probe	p/n G9774D	p/n G9774C
with SureSelect Clinical Research Exome V4 (CRE V4) Probe	p/n G9775D	p/n G9775C
with SureSelect Cancer CGP RNA Probe	p/n G9777D	p/n G9777C
with empty Magnis Probe Input Strips‡	p/n G9750D	Not offered

For a list of kit contents, see page 59 to page 61.

* 96-reaction kits are formatted for 12 runs containing 8 samples per run.

† 32-reaction kits are formatted for 4 runs containing 8 samples per run.

‡ Probe must be purchased separately. See page 48 for information on filling the empty Magnis Probe Input Strip for the run.

Table 2 Required Equipment

Description	Vendor and part number
Magnis NGS Prep System*	Agilent p/n G9710A (firmware version 1.4 or greater)
Robotic Pipetting Tips (Sterile, Filtered, 250 µL)	Agilent p/n G9477G
Hygrometer	Traceable Temperature/Humidity Data Logger, Cole-Parmer p/n 18004-13 or equivalent
Vortex mixer	Vortex Genie-2, VWR p/n 58815-234 or equivalent
Microcentrifuge	Eppendorf microcentrifuge model 5417C or equivalent†
Swinging bucket centrifuge	Eppendorf centrifuge model 5804 with A-2-DWP rotor or equivalent‡
Pipettes (10-, 20-, and 200-µL capacity)	Rainin Pipet-Lite Pipettes or equivalent
Sterile, nuclease-free aerosol barrier pipette tips	General laboratory supplies vendor
Freezers (2) set to –20°C and –80°C	General laboratory supplies vendor
Refrigerator set to +4°C	General laboratory supplies vendor
Ice bucket	General laboratory supplies vendor
Powder-free gloves	General laboratory supplies vendor

* The Magnis SureSelect XT HS2 RNA Reagent Kits and the protocols detailed in this publication are also compatible with the MagnisDx NGS Prep System (p/n K1007A).

† Centrifuge rotor must accommodate the strip tubes supplied with Magnis SureSelect XT HS2 RNA Reagent Kits.

‡ Centrifuge rotor must accommodate the deep-well plates supplied with Magnis SureSelect XT HS2 RNA Reagent Kits. Refrigeration system is not required.

Required Materials for RNA Sample Preparation and Library Analysis

Table 3 Required Materials for RNA Sample Preparation and Analysis

Description	Vendor and part number
1X Low TE Buffer (10 mM Tris-HCl, pH 7.5-8.0, 0.1 mM EDTA)	Thermo Fisher Scientific p/n 12090-015, or equivalent
Small-volume spectrophotometer	NanoDrop 2000, Thermo Fisher Scientific p/n ND-2000 or equivalent
RNA Analysis System and Consumables:*	
Agilent 4150/4200 TapeStation	Agilent p/n G2992AA/Agilent p/n G2991AA
TapeStation-compatible 8-well tube strips	Agilent p/n 401428
8-well tube strip caps	Agilent p/n 401425
RNA ScreenTape/Sample Buffer/Ladder	Agilent p/n 5067-5576/5067-5577/5067-5578
High Sensitivity RNA ScreenTape/Sample Buffer/Ladder	Agilent p/n 5067-5579/5067-5580/5067-5581
OR	
Agilent 5200/5300/5400 Fragment Analyzer Instrument	Agilent p/n M5310AA/M5311AA/M5312AA
RNA Kit (15NT)	p/n DNF-471-0500
HS RNA Kit (15NT)	p/n DNF-472-0500

* If available in your laboratory, the Agilent 2100 Bioanalyzer and RNA 6000 Pico Kit (p/n 5067-1513) or RNA 6000 Nano Kit (p/n 5067-1511) may also be used for RNA sample analysis.

Table 4 Required Materials for cDNA Library Analysis

Description	Vendor and part number
DNA Analysis System and Consumables:*	
Agilent 4150/4200 TapeStation	Agilent p/n G2991AA/G2992AA
96-well sample plates	Agilent p/n 5042-8502
96-well plate foil seals	Agilent p/n 5067-5154
8-well tube strips	Agilent p/n 401428
8-well tube strip caps	Agilent p/n 401425
High Sensitivity D1000 ScreenTape	Agilent p/n 5067-5584
High Sensitivity D1000 Reagents	Agilent p/n 5067-5585
OR	
Agilent 5200/5300/5400 Fragment Analyzer Instrument	Agilent p/n M5310AA/M5311AA/M5312AA
HS NGS Fragment Kit	p/n DNF-474-0500

* If available in your laboratory, the Agilent 2100 Bioanalyzer and High Sensitivity DNA Kit (p/n 5067-2646) may also be used for library DNA analysis.

Optional Materials

Table 5 Supplier Information for optional materials in protocols

Description	Purpose	Vendor and part number
Dilute bleach (10%) wipes	Surface-cleaning of instrument deck (see page 18)*	Hype-Wipe Bleach Towelettes (VWR p/n 16200-218), or equivalent
Alcohol (70%) wipes	Surface-cleaning of instrument deck (see page 18)*, periodic glove decontamination during run setup (see page 17)	VWR Pre-Moistened Clean Wipes (VWR p/n 21910-110), or equivalent
Dry, lint-free, scratch-free wipers	Surface-cleaning of the barcode scanner window	Kimwipes wipers (VWR p/n 21905-026), or equivalent
Adhesive seal applicator	Application of foil seals to filled Magnis Sample Input Strips with reduced contamination risk (see page 22)	Thermo Fisher Scientific p/n AB1391, or equivalent
D1000 ScreenTape and D1000 Reagents	Analysis of optional pre-capture library QC samples using Agilent 4200/4150 TapeStation system (see page 42)	Agilent p/n 5067-5582 and p/n 5067-5583

* Agilent recommends use of the Magnis instrument UV-mediated decontamination programs for routine instrument decontamination. If solvent-based cleaning is required, see the instrument User Guide for complete surface cleaning instructions. Allowed solvents must be applied to a solid cloth support before use. Do not spray water, bleach, alcohol or other liquids inside the instrument. Remove any excess liquid from wipes or towelettes before use to prevent introduction of liquids into instrument components.

2 Sequencing Library Preparation using the Magnis NGS Prep System

This chapter contains instructions for SureSelect XT HS2 target-enriched RNA sequencing library preparation using the Magnis NGS Prep System. For an overview of the workflow, see Figure 1 on page 8.

Detailed instructions are provided here for setting up the Magnis NGS Prep System instrument and assay components for a run, then running a Magnis instrument protocol for automated NGS library sample preparation.

For each sample to be sequenced, an individual dual-indexed and molecular-barcoded library is prepared. Libraries prepared using the protocols described here are ready for sequencing using the Illumina paired-read system.

Critical Sample Tracking Information

Accurate sample tracking is critical to the interpretation of your sequencing results. Before beginning a run, make sure you read and understand the sample tracking information in this section, including 1) sample number orientation in the Magnis Sample Input Strip wells and 2) how to enter sample identities in the Magnis software during run setup.

Sample orientation in the Magnis Sample Input Strip wells

Magnis NGS Prep System runs use the sample orientation shown in Figure 2, below, with Sample 1 loaded in the well farthest from the barcode in the provided Magnis Sample Input Strips. Samples must be loaded in the Magnis Sample Input Strip wells in this orientation during run setup on page 21.

Before setting up the run, assign each sample to a specific sample number 1 through 8 and record the sample number assignments. Methods for entering sample assignments for a run into the Magnis software are described on page 15 to page 16.

CAUTION Do not add any writing or labels that may obscure the barcode on the Magnis Sample Input Strip.

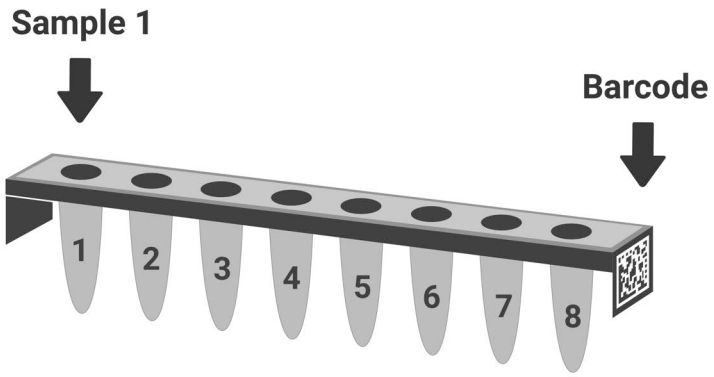


Figure 2 Required orientation of sample numbers 1 through 8 in the Magnis Sample Input Strip.

Assignment of samples to well positions in the Magnis software

The identity of each sample in the run must be specified in the Magnis instrument software using one of the two methods described below. The specific sample IDs to be included in a run are entered in the Magnis system during run setup as detailed in section "Step 4. Enter Sample Info" on page 38. Make sure you understand the sample positioning and tracking information below before you begin run setup.

Each Sample ID must contain 1–30 characters and must be unique within the run. Sample IDs may be reused in different runs.

Sample assignment Method 1: Import of sample assignments using a .csv file

1 Create a .csv (comma separated value) file containing the ordered sample names. The sample name data may be entered in table format using a spreadsheet application, such as Microsoft Excel software, and then saved in .csv format.

a Enter the header text **sample_id** in cell A1, as shown in Figure 3.

b Enter the name of each sample in cells A2 through A9 (see Figure 3, left panel). The sample input file must contain 8 unique sample IDs. If any sample wells are left empty for the run, you must enter placeholder text in the corresponding positions (see Figure 3, right panel).

8 samples in run

	A	B
1	sample_id	
2	HD18060701	
3	HD18060702	
4	HD18060703	
5	HD18060704	
6	HD18060705	
7	HD18060706	
8	HD18060707	
9	HD18060708	
10		

6 samples in run with 2 placeholder Sample IDs

	A	B
1	sample_id	
2	HD18060701	
3	HD18060702	
4	HD18060703	
5	HD18060704	
6	HD18060705	
7	HD18060706	
8	empty1	
9	empty2	
10		

Figure 3 Example .csv file content (shown in spreadsheet format) for uploading sample assignments

2 Save the file in .csv format.

3 Download the .csv file onto a unencrypted USB drive.

4 When setting up the run, on the *Enter Sample Info* screen, press the sample upload button shown below, then follow the protocol setup wizard prompts to transfer the Sample IDs from the USB drive.

Sample assignment Method 2: Manual sample assignment using the Magnis instrument touchscreen

1 Record the identity of each sample number for the run using appropriate hardcopy or softcopy record keeping procedures before dispensing samples into the Magnis Sample Input Strip wells.

2 When setting up the run, follow the Magnis touchscreen prompts to enter the Sample ID for each sample well position using the *Enter Sample Info* screen shown below. The Magnis system automatically assigns a default Sample ID for each sample position. To change the Sample ID, first select a specific sample position on the touchscreen, then use the **Edit Sample ID** tool on the right to enter the desired Sample ID text. Press **Change** to save the entered Sample ID text for the sample.

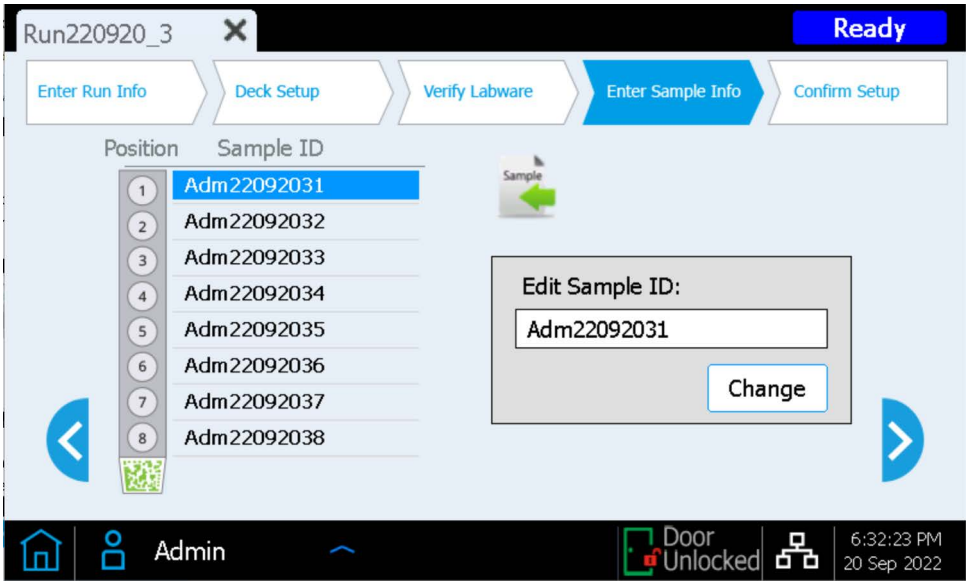


Figure 4 Magnis touchscreen interface used for manual sample assignment during a run.

Preparing Your RNA Samples for the Run

The library preparation protocol is compatible both with intact total RNA prepared from fresh or fresh-frozen samples and with RNA prepared from FFPE samples (qualified prior to use as *Good FFPE* or *Poor FFPE* according to the measured RNA integrity in the sample).

Runs to process either intact or good-quality FFPE RNA require 10 ng, 50 ng, 100 ng or 200 ng of input RNA and runs to process poor-quality FFPE RNA require 50 ng, 100 ng or 200 ng of input RNA. For optimal sequencing results, use the maximum amount of input RNA available within this range. All samples in the same run must be provided in the same quantity.

Before setting up the Magnis run, RNA samples must be prepared, quantified and qualified as described in "Appendix 1: RNA Sample Preparation Guidelines" on page 44. Some RNA sample preparation steps, especially qualification of FFPE-derived samples, may need to be completed up to a day prior to initiating the Magnis run steps.

CAUTION

It is critical to prevent contamination of the RNA samples with ribonucleases and foreign nucleic acids during sample preparation and throughout the library preparation procedure.

Wear gloves through all RNA sample preparation and all Magnis reagent preparation and loading steps (page 20 to page 23 and page 29 to page 34). Change gloves, or decontaminate gloves using a 70% ethanol wipe, frequently during the procedure, especially after contact with potentially contaminated surfaces such as touchscreens or keyboards.

Avoid touching the foil seals of sample strips, reagent strips, and reagent plates, even using gloved hands. Any contaminants deposited on strip or plate foil seals can be introduced into samples during the Magnis liquid-handling steps.

Preparing the Magnis Instrument and the Reagents for the Run

Step 1. Prepare the instrument for running a protocol

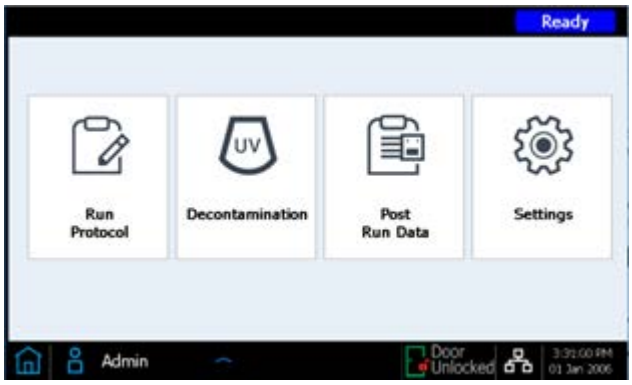
NOTE Instructions below include an instrument-mediated decontamination procedure that uses ultra-violet (UV) light to decontaminate the instrument deck. Other decontamination procedures (for example, using a 10% bleach solution) may be employed in addition to or as alternative to the automated UV decontamination procedure. See the Magnis instrument User Guide for complete surface decontamination and cleaning instructions.

1. Before you begin, use a hygrometer to measure the ambient humidity near the Magnis instrument. Verify that the non-condensing humidity is in the acceptable range of 30% to 70%.
2. Verify that the instrument deck is cleared of all labware from previous runs and of any other stray materials. Any materials present on the instrument deck during run setup can interfere with the instrument start-up and run setup processes.
3. Turn on the instrument by pressing the power button on the front of the device. Close the instrument door.

   The instrument turns on, the LED indicator lights inside the instrument illuminate, and the software launches on the touchscreen.

   Stand by as the system performs a series of start up activities, which may require several minutes.
4. Verify that the instrument is running firmware version 1.4 or later using the steps below.

CAUTION The SureSelect XT HS2 RNA library preparation protocol must be run using Magnis firmware version 1.4 or later. Use of earlier firmware versions will result in erroneous run conditions leading to poor yield or poor sequencing performance.



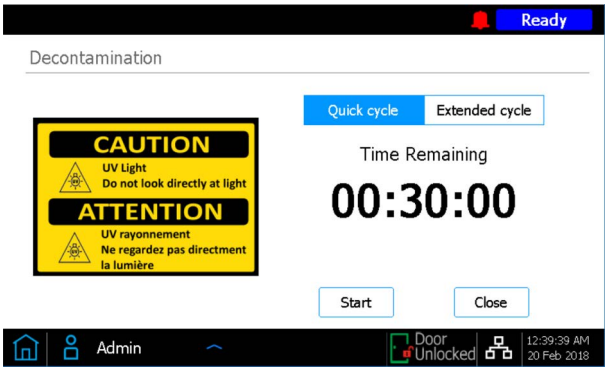
   a. From the Home screen, press **Settings**.
   b. Navigate to **System Settings** then **Instrument Settings.**
   c. Verify that the *Firmware Version* field shows version 1.4 or later.

   If the instrument is running firmware earlier than version 1.4, the firmware must be updated prior to the run. See page 70 for firmware update information.

5 Agilent recommends running the UV decontamination *Quick cycle* procedure (requires 30 minutes) prior to every run, using the steps below.

a From the Home screen, press **Decontamination**.

b From the Decontamination screen, press **Quick cycle**, then press **Start**. The duration of the *Quick cycle* decontamination procedure is 30 minutes. The LED indicator lights are off during the UV decontamination procedure, with the instrument's UV light tube emitting UV light during this interval.



WARNING **Do not look directly at the UV light while decontamination is in progress.**

NOTE During the 30-minute decontamination process, begin the reagent preparation steps detailed on page 20.

6 Once the decontamination cycle is complete, the instrument's LED indicator lights will emit blue light. Return to the Home screen using the touchscreen display for access to run setup steps.

Step 2. Prepare the SureSelect XT HS2 RNA reagents and plasticware

The reagent and plasticware components used in each Magnis SureSelect XT HS2 RNA run are listed in Table 6. Review the "Plate and Strip Tube Handling Instructions" section below before starting the preparative steps listed in the table.

Table 6 Magnis SureSelect XT HS2 RNA run consumables (RT=Room Temperature)

Quantity	Component	Storage condition	Preparative steps
1	Magnis SureSelect RNA Beads/Buffers Plate ILM	+4°C	See step 1 on page 21. Held at RT for 30 min prior to run.
1	Magnis SureSelect XT HS2 Reagent Plate ILM	–20°C	See step 2 on page 21. Thawed at RT for 15–30 min prior to further processing.
1	Empty Magnis Sample Input Strip (red) and replacement foil seal from same package	RT	Ready to fill. See step 3 on page 21 for filling instructions.
1	Index strip (black) from the Magnis SureSelect XT HS2 Index Primer Pairs ILM plate	–20°C	See step 4 on page 22. Thawed on ice briefly prior to further processing.
1	RNA Reagent strip (white) from the Magnis SureSelect RNA Reagent Strips ILM plate	–20°C	See step 5 on page 23. Thawed on ice briefly prior to further processing.
1	Probe strip (white) from the Magnis SureSelect Probe Plate, Pre-filled Single Well Format*	–80°C	See step 6 on page 23. Thawed on ice briefly prior to further processing.
1	Magnis Empty Consumables box containing: Magnis Tip Waste Bin, Magnis Deep-Well HSM Plate, Magnis Thermal Cycler Seal, Magnis 96-Well PCR Plate, Magnis Library Output Strip (green), Magnis QC Strip (blue), Magnis Foil Seals	RT	Ready to use.
4	Boxes Robotic Pipetting Tips (purchased separately from the Magnis Reagent Kit)	RT	Ready to use.

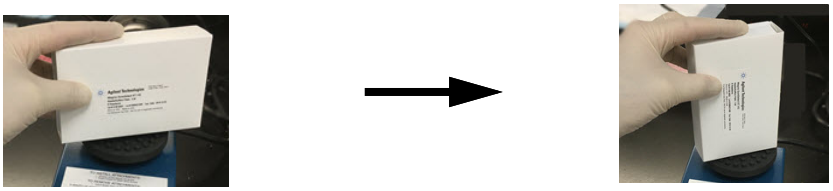
* Runs using protocol *SSEL-RNA-XTHS2-EPIS-ILM* require one (1) empty Magnis Probe Input Strip (white), stored at RT. The empty probe strip must be filled before use in the run using the instructions provided on page 48.

Plate and Strip Tube Handling Instructions

Familiarize yourself with the important labware handling instructions below before you start the component preparation steps on page 21 to page 23.

- Magnis Sample Input Strips (red strips provided in plate format, p/n 5190-9882 or 5191-5676), along with all input RNA sample preparation and dilution reagents, should be stored and used only in pre-PCR areas of the laboratory.
- The adhesive seals and foils covering the kit plates and strip tubes must be left in place during run setup and execution. Avoid touching or damaging the foil and adhesive covers during run setup. The sample input strip foil cover is pierced during run setup, and the wells must be re-sealed with a fresh foil seal strip provided in the same package. Take care to avoid contamination or other damage to the replacement foil seals.
- Filled reagent plates (both Magnis SureSelect RNA Beads/Buffers Plate ILM and Magnis SureSelect XT HS2 Reagent Plates) are provided in white cardboard sleeves. Leave the filled plates in the sleeves during all of the preparation steps described below. To visually inspect the plate wells, carefully slide the reagent plate **only partially** out of the sleeve to avoid bending or damaging the foil or adhesive cover. Improper re-insertion of the plate into the sleeve may compromise the plate integrity.

- Vortex the filled reagent plates after thawing using the following procedure, illustrated in the pictures below. Use a vortex equipped with a broad, rubber-coated platform (as shown), and use a high-speed setting throughout the procedure. Hold the sleeved plate in a vertical position (on its side) instead of horizontally while vortexing. Begin by pressing one long side of the plate on the vortex head and mix for 10 seconds.Then rotate the plate 90° and press short side of the plate on the vortex head for an additional 10 seconds. Continue the rotation/10 second mixing sequence until completed on all four sides of the plate.



- If a kit component appears damaged during unpacking or run setup (e.g. foil or adhesive cover is pierced or plasticware is broken), do not use the component; contact Agilent Technical Support for assistance.

Sample and Reagent Setup Steps

1 Prepare the **Magnis SureSelect RNA Beads/Buffers Plate** for the run using the steps below:

   a Transfer one Magnis SureSelect RNA Beads/Buffers Plate from storage at +4°C to room temperature (RT), keeping the plate in the white cardboard sleeve. Allow the sleeved plate to equilibrate to RT for at least 30 minutes before use in the run.

   b Vortex the sleeved plate, with the plate positioned vertically as detailed in the handling instructions section above.

   c Spin the sleeved plate in a centrifuge set at 250 × *g* for 3 seconds to collect the liquid without pelleting the beads (begin timing once centrifuge achieves full speed). Do not exceed the recommended spin speed and duration to prevent pelleting the beads.

   d Keep the sleeved plate at RT for use in same-day run.

2 Prepare the **Magnis SureSelect XT HS2 Reagent Plate** using the steps below:

   a Transfer one Reagent Plate from storage at –20°C to RT, keeping the plate in the white cardboard sleeve. Allow the reagents to thaw at RT for 15 to 30 minutes. Slide the plate partially out of the sleeve and visually confirm that the reagents are completely thawed.

   b Once the well contents are thawed, vortex the sleeved plate with the plate positioned vertically as detailed in the handling instructions section above.

   c Spin the sleeved plate in a centrifuge set at 250 × *g* for 1 minute (begin timing once centrifuge achieves full speed). Check bottoms of the plate wells for any bubbles, and if bubbles are present, repeat the spin step until all bubbles are released.

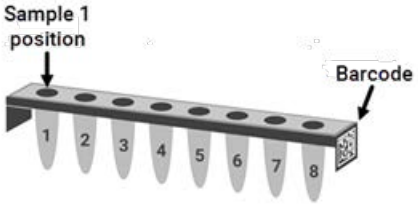
   d Keep the sleeved plate on ice for use in same-day run.

3 Prepare the **Magnis Sample Input Strip** using the steps below. Ensure that all RNA samples for the run were prepared using methods appropriate for the sample type as detailed on page 44 to page 46.

   a Obtain the Magnis Sample Input Strips kit from storage at RT. Remove one empty red Sample Input Strip (with “S” inscribed on end of strip) from the plate support, leaving the foil cover in place. Set aside one fresh foil seal strip and attached backing for re-sealing in step c.

b Place 10 µL of RNA in each sample well, piercing the foil seal with the pipette tip just before dispensing the liquid. All wells of the strip must contain the same amount of RNA (10 ng, 50 ng, 100 ng or 200 ng in 10 µL sample volume).

Make sure to load samples in the correct sample well position, with Sample 1 in the well farthest from the barcode, as shown in the figure below.



c Once all samples have been placed in the Magnis Sample Input Strip wells, re-seal the strip tube with a fresh foil seal, taking care to avoid obscuring the strip tube barcode with the foil seal. Ensure that the seal is applied firmly and evenly, without excessive overhangs or creases that could obstruct strip tube seating when loading in the instrument.

NOTE

Take care to avoid contamination with ribonucleases and foreign nucleic acids during application of the foil seal. Consider using a seal applicator (cleaned before each use) for this step. See Table 5 on page 12 for applicator recommendation. Avoid touching the foil seal during sample strip processing and loading, even using gloved hands.

d Visually check the sealed sample wells to verify that no bubbles are present. Remove any bubbles by spinning the prepared sample strip in a centrifuge set at 250 × *g* for 5 seconds or until all bubbles are released from the RNA solution.

e Keep the sealed sample strip tube on ice until use on page 34.

4 Prepare the **index strip tube** using the steps below:

a Determine the appropriate set of indexes to use for the run. The provided index strips are inscribed with *D1* through *D24* on the strip tube end opposite the barcode to indicate the specific set of dual-index pairs contained in the wells (see page 63 for index maps).

NOTE

Ensure that each sample to be multiplexed is indexed using a unique index pair.

- If samples from different Magnis NGS library preparation runs will be multiplexed for NGS, each run must use a different-numbered index strip.
- Agilent's SureSelect XT HS2 index pairs use a uniform numbering system across all formats. For example, index pairs 1-96 provided in Magnis XT HS2 index strips *D1* to *D12* (black strip tubes) are equivalent to index pairs 1-96 provided in orange plates in the SureSelect XT HS2 Reagent Kits formatted for manual processing. Do not combine samples indexed with the same-numbered index pair from different kit formats for multiplex sequencing.

b Obtain the Magnis SureSelect XT HS2 Index Plate from storage at −20°C. Remove the appropriate black index strip (labeled *D1* through *D24*) from the plate support, leaving the foil cover in place. Place the removed strip on ice to thaw and return the plate with remaining index strips to storage at −20°C.

c Once the index strip well contents are thawed, vortex the strip at high speed for 5 seconds.

d Spin the index strip in a centrifuge set at 250 × *g* for 5 seconds. Check the strip wells to verify that the liquid is collected in the bottom of the wells and that no bubbles are present. Remove any bubbles by repeating the spin step until all bubbles are released.

e Keep the index strip tube on ice until use on page 34.

5 Prepare the **RNA Reagent strip tube** using the steps below:

a Obtain the *Magnis SureSelect RNA Reagent Strips ILM* plate from storage at –20°C. Remove one white RNA Reagent strip (with "R" inscribed on end of strip) from the plate support, leaving the foil cover in place. Place the removed strip on ice to thaw and return the plate with remaining reagent strips to storage at –20°C.

b Once the strip well contents are thawed, vortex the strip at high speed for 5 seconds.

c Spin the reagent strip in a centrifuge set at 250 × *g* for 5 seconds. Check the strip wells to verify that the liquid is collected in the bottom of the wells and that no bubbles are present. Remove any bubbles by repeating the spin step until all bubbles are released.

d Keep the reagent strip on ice until use on page 34.

6 Prepare the **probe strip tube** using the steps below for kits supplied with a pre-filled probe plate (*Magnis SureSelect Probe Plate, Pre-filled Single Well Format*). Note that the full volume of probe solution is provided in well A, with wells B through H provided empty.

If your kit instead includes empty probe strips (kit p/n G9750D) for run-time probe filling, skip the instructions below and prepare the probe strip using the instructions on page 48.

a Obtain the Probe Plate from storage at –80°C. Remove one white probe strip (with "P" inscribed on strip end) from the plate support, leaving the foil cover in place. Thaw the probe strip on ice and return the plate with remaining probe strips to storage at –80°C.

CAUTION The probe strips do not include human-readable labels showing the specific probe design identity. Use appropriate care to track and maintain probe strip identity once a probe strip is removed from the plate packaging. Do not open multiple boxes and remove probe strip tubes for different probe designs at the same time.

b Once the probe is thawed in the strip well, vortex the strip at high speed for 5 seconds.

c Spin the probe strip in a centrifuge set at 250 × *g* for 5 seconds. Visually inspect well A to verify that the liquid is collected in the bottom of the well and that no bubbles are present. Remove any bubbles by repeating the spin step until all bubbles are released.

d Keep the probe strip on ice until use on page 34.

7 Obtain one **Magnis Empty Consumables** box from storage at RT for use during deck setup.

Proceed to "Running the Library Preparation Protocol" on page 24.

Running the Library Preparation Protocol

When the Magnis instrument and all reagents have been prepared for the SureSelect XT HS2 RNA run, follow the prompts provided on the instrument touchscreen to load the labware on the instrument and run the library preparation protocol. The steps are summarized in Figure 5.

The Magnis instrument touchscreen provides prompts for entering the run information, loading the deck, verifying that all labware is present and has the required properties, entering sample information, and confirming the protocol setup. During these setup steps, the instrument's on-deck LED indicator lights emit white light. Additional information about each of these prompted steps is provided for new users on page 25 to page 39.

During the protocol run, the system performs fragmentation, library preparation and target enrichment on your RNA samples to generate target-enriched cDNA libraries that are ready for sequencing. During the run, the LED indicator emits green light.

When the run is complete the system touchscreen guides you through the steps for removing the final sequencing library samples and QC samples (if included) from the instrument. After the LED indicator emits blue light, the instrument door can be opened and samples removed for further processing. Guidelines for processing the final target-enriched libraries for DNA sequencing are provided in "Appendix 3: Guidelines for Post-run cDNA Library Processing for NGS" on page 50.

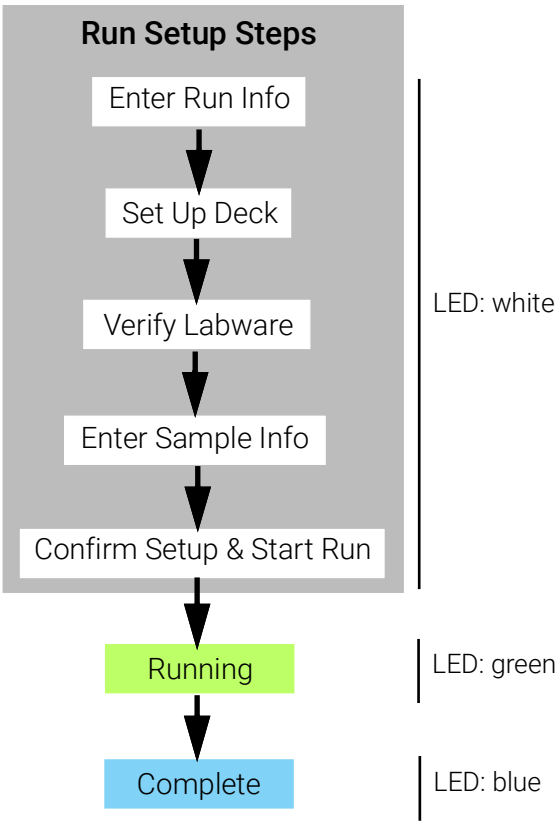


Figure 5 Overview of steps for Magnis NGS Prep System run setup and completion. The color of the light emitted by the instrument's LED indicator lights during these steps is shown at right.

Step 1. Initiate the protocol and Enter Run Info

CAUTION Before starting the run, verify that the Magnis instrument is running firmware version 1.4 or later. See page 70 for firmware verification and update instructions.

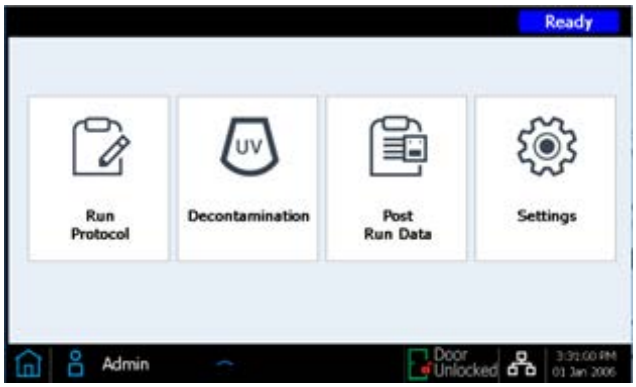
The run setup steps in this section include selection of the specific Magnis protocol to run. Table 7 describes the Magnis protocols supported by this publication. A different set of protocols may be available for use on your instrument and visible on your touchscreen. See page 71 for more information on Magnis protocol availability.

Table 7 Usage information for Magnis protocols supported in this publication

Protocol Name	Reagent Kit Format Supported
SSEL-RNA-XTHS2-ILM	Use to process Magnis SureSelect XT HS2 RNA Reagent Kits supplied with **pre-filled probe input strips** (kits with probe plate labeled *Magnis SureSelect Probe Plate, Pre-filled Single Well Format*)
SSEL-RNA-XTHS2-EPIS-ILM	Use to process Magnis SureSelect XT HS2 RNA Reagent Kits supplied with **empty probe input strips (EPIS)**. The probe input strip must be filled prior to the run as detailed on page 48.

1 From the touchscreen Home screen, press **Run Protocol.**

The system locks the instrument door and performs an Instrument Health Check (IHC), which may require several minutes. If the display reports an IHC issue, see page 70 and page 71 for remediation guidelines.

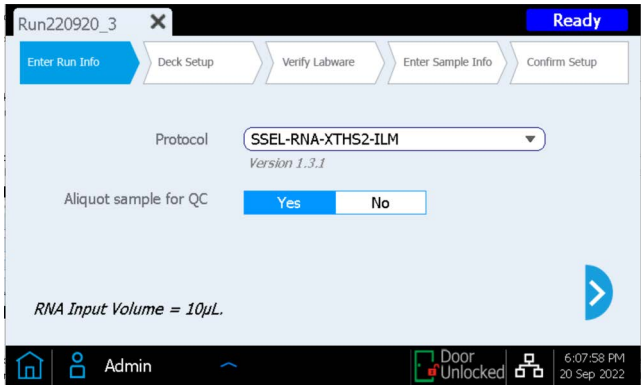


2 Follow the prompts provided on the *Enter Run Info* screens.

If you need more information about using the *Enter Run Info* screens, see the appropriate section below for the Magnis firmware version running on your instrument. For instruments running firmware version 1.5 or later, go to page 27.

***Enter Run Info* instructions for Magnis firmware v1.4 and earlier:**

1 On the first screen, specify the protocol name and whether to include the optional QC sample collection step in the run.



a Expand the **Protocol** menu by pressing the drop-down arrow, and scroll to then select the protocol appropriate for your reagent kit format (see Table 7 on page 25).

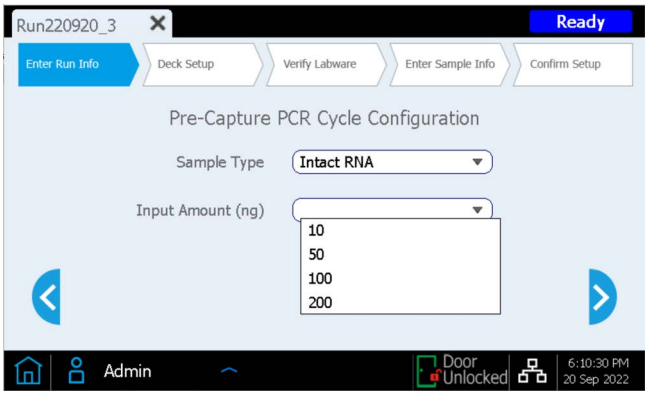
b If you want the instrument to take an aliquot (3 µL) of each pre-capture library sample for optional post-run QC analysis press **Yes** next to **Aliquot sample for QC**. (The pre-capture QC samples are only available for analysis when the full run is complete.) If you are making this selection, be sure to load the blue QC Strip during deck setup on page 34.

Or, press **No** to skip the optional QC aliquot collection step.

c Press the forward arrow to advance to the next *Enter Run Info* screen.

2 On the second screen, select the appropriate **Sample Type** (*Intact RNA, Good FFPE RNA* or *Poor FFPE RNA*) and the RNA **Input Amount** (*10 ng, 50 ng, 100 ng*, or *200 ng*) for the samples being processed in the run.

When **Sample Type** of *Poor FFPE RNA* is selected (for use with samples with DV200 of 20–50% as described on page 46), **Input Amount** options are limited to *50 ng, 100 ng*, or *200 ng* RNA.



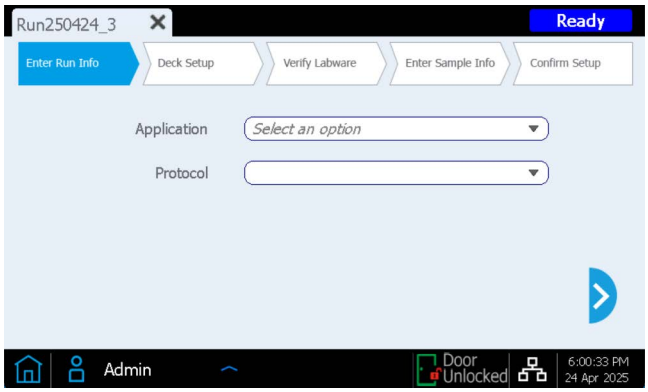
The sample type and input amount settings are used to determine the correct RNA fragmentation and pre-capture PCR cycling conditions for the run. The PCR cycle number to be used during the run is reported on the *Confirm Setup* screen (see page 39).

3 Press the forward arrow to advance to the *Deck Setup* screens and proceed to page 29 for deck setup instructions.

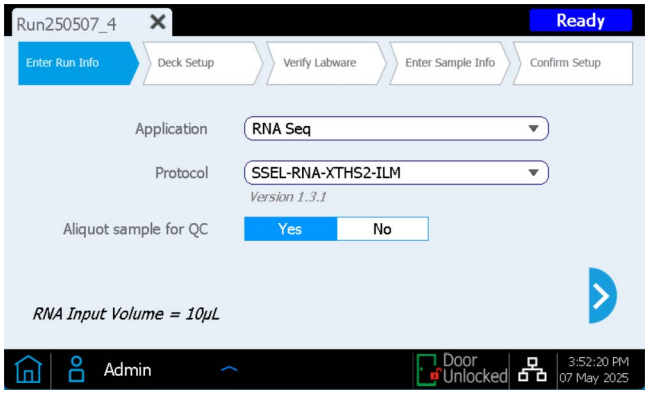
Enter Run Info instructions for Magnis firmware v1.5 and later:

1 On the first screen, expand the **Application** menu by pressing the drop-down arrow and select *RNA Seq*.

Expand the **Protocol** menu and scroll to then select the protocol appropriate for your reagent kit format (see Table 7 on page 25). Press the forward arrow to advance to the next *Enter Run Info* screen.



2 Specify whether to include the optional QC sample collection step in the run using the **Yes/No** buttons shown below.



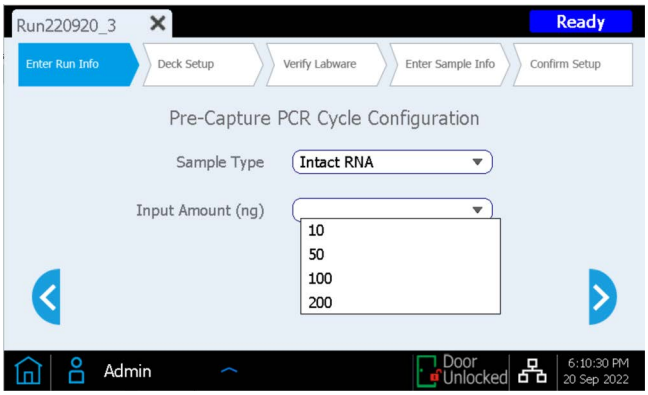
If you want the instrument to take an aliquot (3 µL) of each pre-capture library sample for optional post-run QC analysis press **Yes** next to **Aliquot sample for QC**. (The pre-capture QC samples are only available for analysis when the full run is complete.) If you are making this selection, be sure to load the blue QC Strip during deck setup on page 34.

Or, press **No** to skip the optional QC aliquot collection step.

3 Press the forward arrow to advance to the next *Enter Run Info* screen.

4 Select the appropriate **Sample Type** (*Intact RNA, Good FFPE RNA* or *Poor FFPE RNA*) and the RNA **Input Amount** (*10 ng, 50 ng, 100 ng*, or *200 ng*) for the samples being processed in the run.

When **Sample Type** of *Poor FFPE RNA* is selected (for use with samples with DV200 of 20–50% as described on page 46), **Input Amount** options are limited to *50 ng, 100 ng*, or *200 ng* RNA.



The sample type and input amount settings are used to determine the correct RNA fragmentation and pre-capture PCR cycling conditions for the run. The PCR cycle number to be used during the run is reported on the *Confirm Setup* screen (see page 39).

5 Press the forward arrow to advance to the *Deck Setup* screens and proceed to page 29 for deck setup instructions.

Step 2. Set up the deck

The Magnis touchscreen interface guides you through the deck setup steps. Additional information is provided for new users on page 30 to page 35. The image below shows a fully set-up deck for orientation to the Magnis deck positions and the run labware.

While completing the deck setup steps specified on the touchscreen display, pay special attention to the critical details below to ensure an error-free run:

- Make sure that all labware is positioned flat on the designated deck platform or fully seated in the appropriate labware holder. Refer to the detailed labware loading instructions and positioning precautions provided for each labware component on page 30 to page 34. Improperly positioned labware can cause low or no final library yield for some or all samples.
- The run requires four (4) tip boxes. Make sure all tip boxes are completely full and are seated flat on the platforms. Verify that each tip box is placed within the raised-tab frame of its platform position and that the boxes do not become unseated during lid removal.
- Load strip tubes in the chiller module in left-to-right order to facilitate proper seating of the strip tubes.
- Make sure all labware is positioned with barcode facing you (front of instrument).

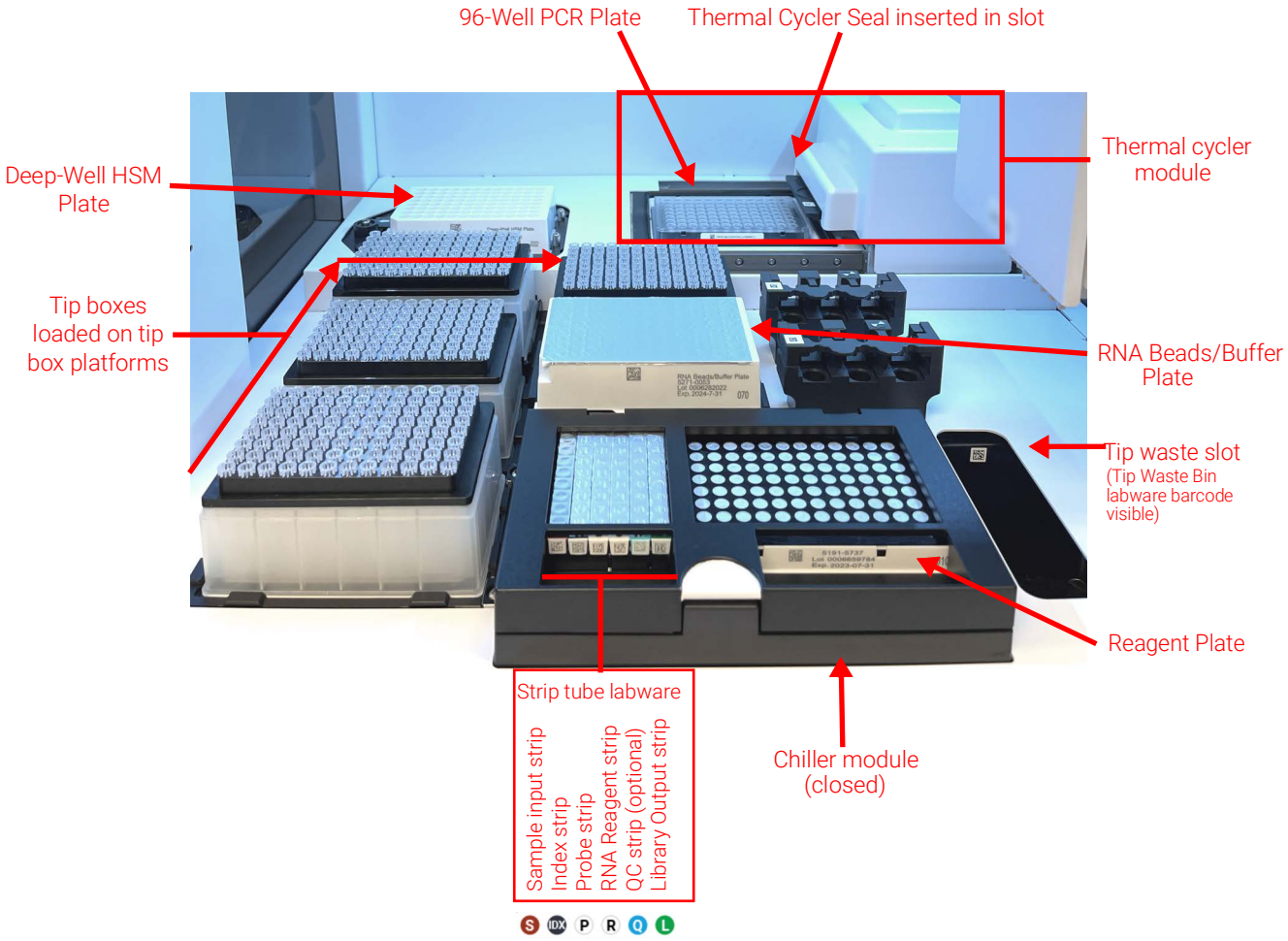
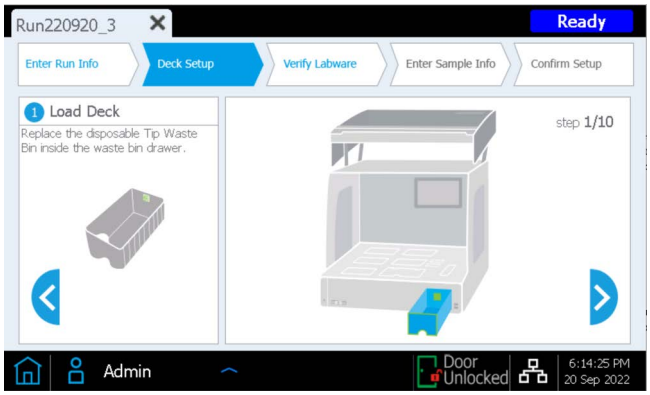


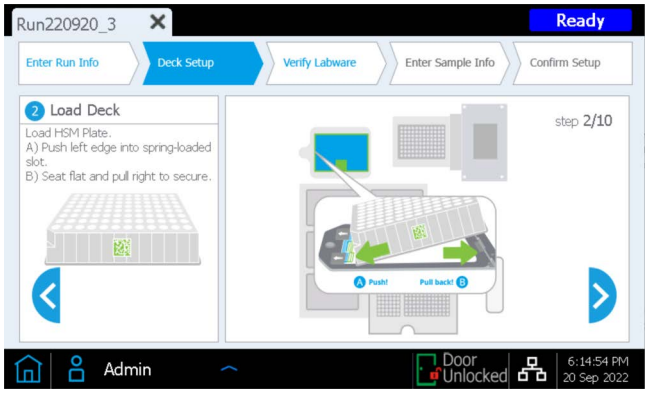
Figure 6 Instrument deck loaded for run. Colors of the strip tube labware are shown in circles below the strip legend.

The *Deck Setup* steps prompted by the Magnis touchscreen interface are detailed below. For each deck loading step, the deck position to be loaded is shaded in blue on the touchscreen display. Once each step is completed, press the forward arrow to advance to the next screen.

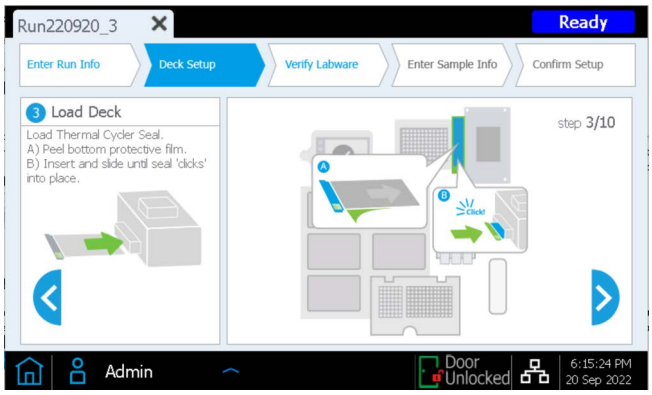
1 Remove the disposable Magnis Tip Waste Bin from the Magnis Empty Consumables package. Place the disposable bin in the waste bin drawer, with the barcode facing you, as shown on the touchscreen. Close the waste drawer.



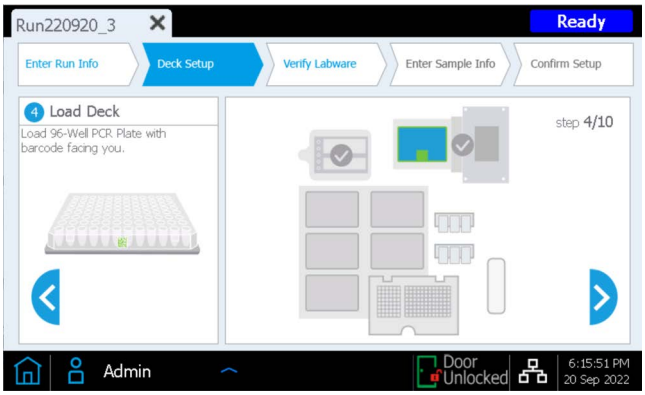
2 Remove the Magnis Deep-Well HSM Plate from the Magnis Empty Consumables package. Install the plate in the deck position shown on the touchscreen, with the barcode facing you. To load the plate, first insert the left edge of the plate into the spring-loaded slot, then lower the right edge of the plate down until the plate is flat on the platform. Once flat, shift the plate slightly to the right (as aided by the spring mechanism) and ensure that the plate is fully seated and secured inside the holder on the platform.



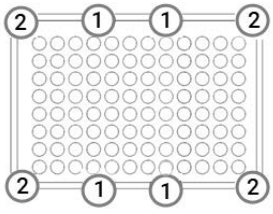
3 Remove the Magnis Thermal Cycler Seal from the Magnis Empty Consumables package. Peel the protective film from the foam pad below the metal plate, starting with the yellow tab. After the full sheet of film has been removed, insert the Thermal Cycler Seal into the slot at the position shown on the touchscreen, with the barcode facing up. Continue sliding the Thermal Cycler Seal into the slot until it clicks into place.



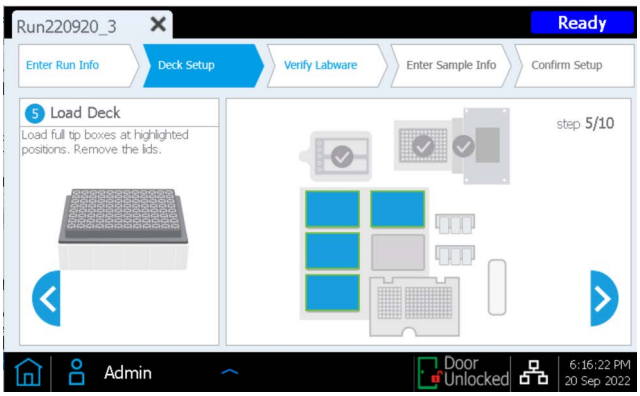
4 Remove the Magnis 96-Well PCR Plate from the Magnis Empty Consumables package. Load the plate in the deck position shown on the touchscreen by inserting the plate wells into the thermal cycler block wells, with the plate barcode facing you.



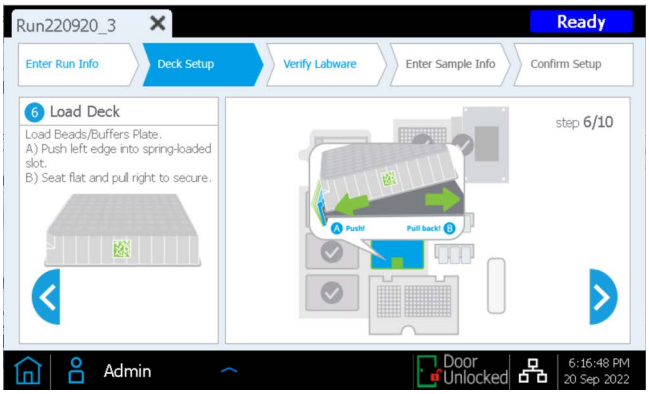
To ensure that the plate is fully seated in the block, first secure the center of the plate in the block wells by pressing evenly at plate positions marked **1** in figure below. Then press evenly on all four corners of the plate (positions marked **2** in figure below).



5 Load a fresh, full tip box at each of the four (4) deck positions indicated on the touchscreen. **Remove lids** from the boxes. After lid removal, verify that each tip box remains sitting flat and within the raised-tab frame of its platform position.

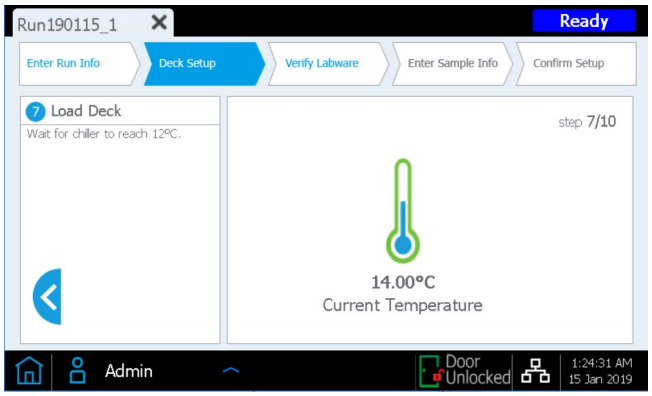


6 Obtain the Magnis SureSelect RNA Beads/Buffers Plate that was prepared on page 21. Remove the white cardboard sleeve, then load the plate in the deck position shown on the touchscreen, with the barcode facing you. To load the plate, first insert the left edge of the plate into the spring-loaded slot, then lower the right edge of the plate down until the plate is flat on the platform. Once flat, shift the plate slightly to the right (as aided by the spring mechanism) and ensure that the plate is fully seated and secured inside the holder on the platform.



7 The instrument's chiller module must reach the correct temperature (typically 12°C) before it can be loaded for the run in step 8 below. Until the chiller reaches loading temperature, the touchscreen display appears as below, allowing you to check the status of the chiller.

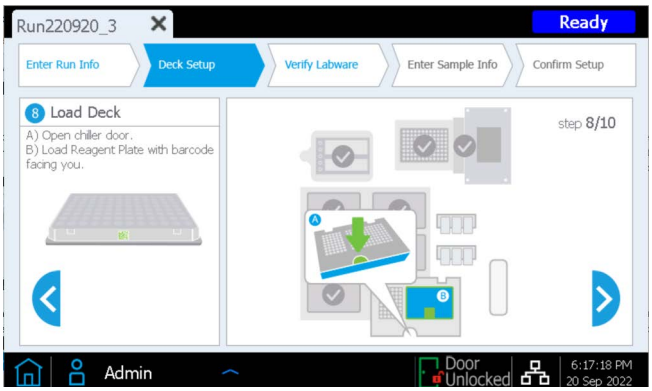
This screen may not appear during your run, if the chiller has already reached the required temperature.



8 Load the chiller module as described below.

a Open the chiller door by pressing on the half-circle button indicated with a green arrow on the touchscreen.

b Obtain the Reagent Plate that was prepared on page 21. Remove the white cardboard sleeve and check bottoms of wells for any bubbles. If bubbles are present, remove by spinning the plate as directed on page 21. Load the plate in the chiller module in position shown on the touchscreen, with the barcode facing you. Press down firmly, applying pressure evenly across the plate. Make sure the reagent plate is securely seated in the chilled plate holder.



9 Load the strip tubes for the run in the indicated positions of the chiller in the left-to-right order listed below. Before loading each strip, check bottoms of wells for any bubbles. If bubbles are present, remove by spinning the strip as directed on page 22. Ensure that each strip is properly seated by pressing firmly and evenly on the strip tube edges during loading. Avoid touching or damaging the foil covers. **Make sure to orient each strip tube with the barcode facing you.**

a Load the **red sample strip tube containing input RNA samples** (prepared on page 21 and held on ice) into the strip tube holder position labeled with **S**. Leave the foil cover intact.

b Load the **black strip tube containing indexed primers** (prepared on page 22 and held on ice) into the strip tube holder position labeled with **IDX**. Leave the foil cover intact.

c Load the **white strip tube containing probe solution** (prepared on page 23 and held on ice) into the strip tube holder position labeled with **P**. Leave the foil cover intact.

d Load the **white RNA Reagent strip tube** (prepared on page 23 and held on ice) into the strip tube holder position labeled with **R**. Leave the foil cover intact.

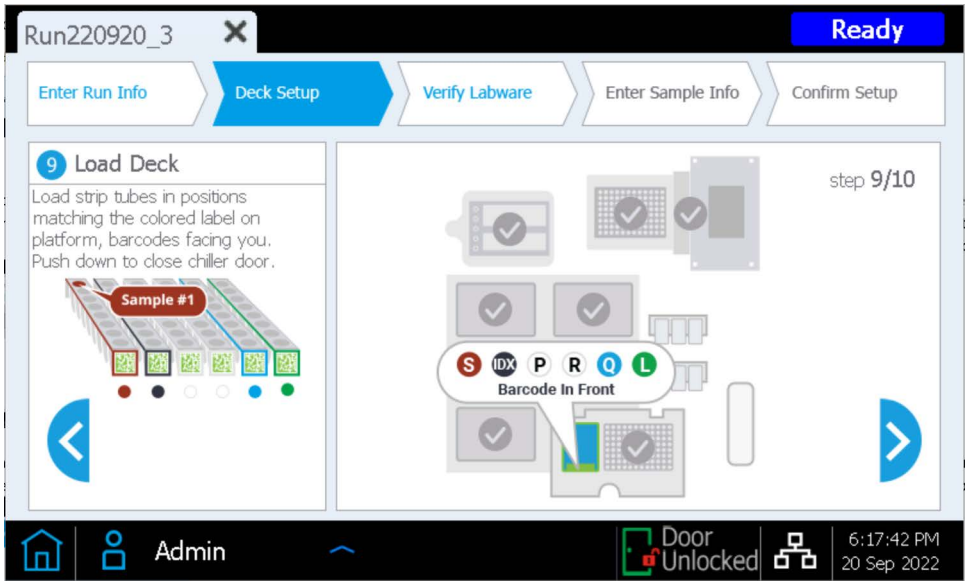
e Obtain the Magnis Library Output Strip, QC Strip, and Foil Seals pack from the Magnis Empty Consumables package.

If the run will include collection of aliquots of the pre-capture library samples for QC, load the **empty blue QC strip** (with "Q" inscribed on end of strip) into the strip tube holder position labeled with **Q**. Leave the foil cover intact.

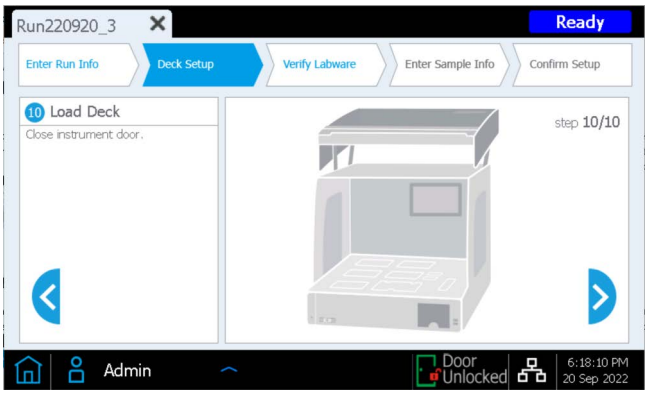
Load the **empty green library output strip** (with "L" inscribed on end of strip) into the strip tube holder position labeled with **L**. Leave the foil cover intact.

Keep the fresh Foil Seals supplied in the package ready for use at the end of the run.

f Once strip tubes are loaded at the **S, IDX, P, R, Q** (when included) and **L** positions, close the chiller door. (Make sure door is fully closed, as indicated by an audible clicking sound).



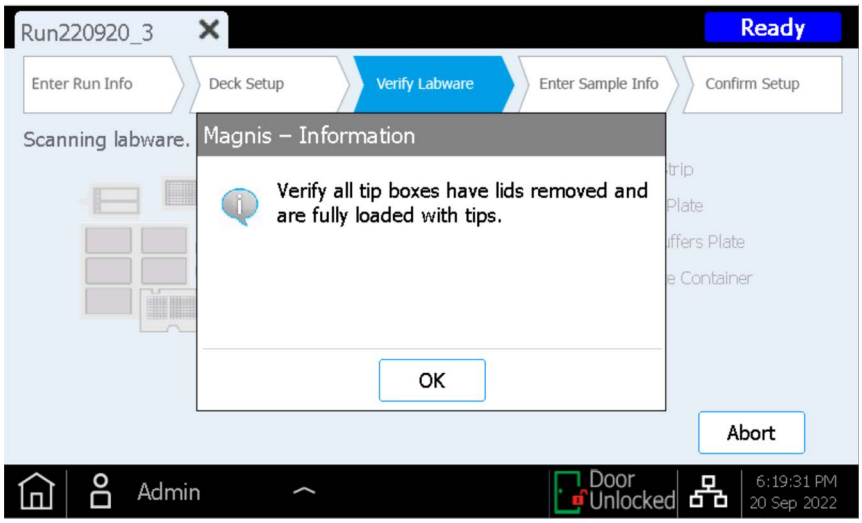
10 Close the instrument door.



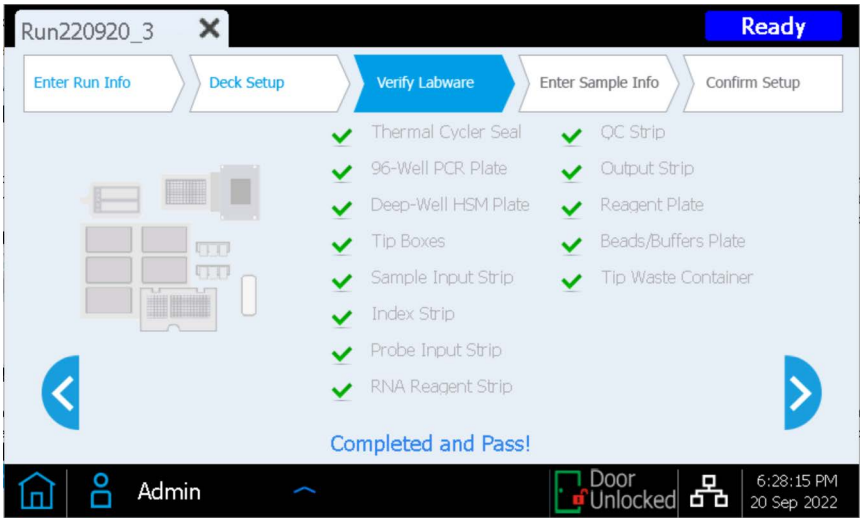
Step 3. Verify Labware

Once all *Deck Setup* steps are complete, the instrument performs the *Verify Labware* phase of the run, in which the instrument scans the barcode on each of the labware components present on the deck.

Before starting the automated labware verification, you need to verify that lids have been removed from all tip boxes and that all tip boxes are full, as indicated in the prompt below. Once the tip box status has been verified, press **OK** to begin the instrument's automated labware verification routine.



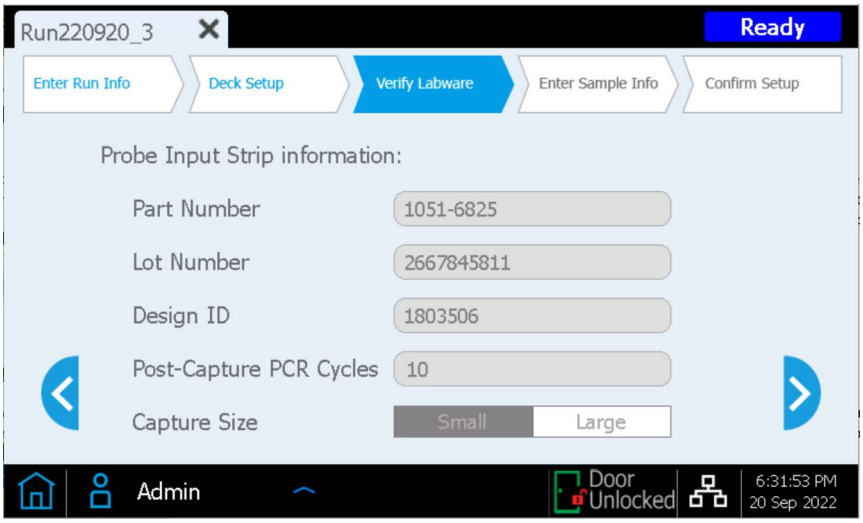
During the barcode scan, the instrument verifies that all components required for the run type are present, in the correct position and orientation, and are not expired. Results of the verification are displayed on the Magnis touchscreen. Press the forward arrow to proceed.



If the *Verify Labware* screen reports an issue with one or more run components, see troubleshooting information on page 71 for remediation guidelines.

The final *Verify Labware* screen allows you to review details for the Probe Input Strip.

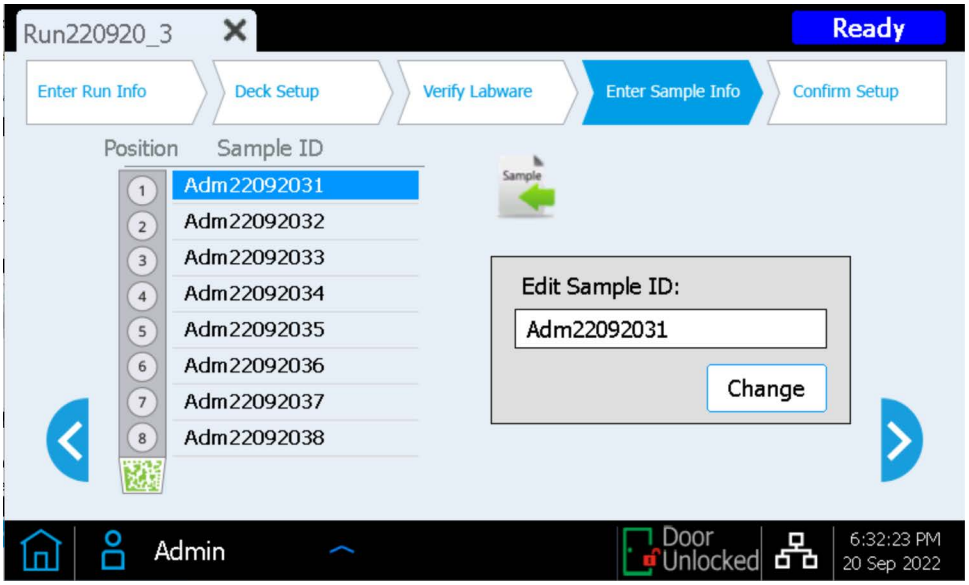
For runs that include pre-dispensed probes the identity of the probe solution is automatically conveyed to the Magnis software by the strip barcode, and the probe properties are reported for your review as shown below. Press the forward arrow to proceed.



For *SSEL-RNA-XTHS2-EPIS-ILM* runs using run-time filled probe strips, you must enter the probe-related properties on this screen manually. See the instructions on page 49. Once all fields are populated, press the forward arrow to proceed.

Step 4. Enter Sample Info

Use this screen to assign each well position to a specific sample in the Magnis software. The Magnis software automatically assigns a default Sample ID for each sample position. The default Sample IDs can be replaced with a chosen sample name/Sample ID using either of the two methods below.



Method 1: Import of sample assignments using a .csv file

1. Create a .csv (comma separated value) file containing the desired Sample IDs for the run in the correct order and download the .csv file onto an unencrypted USB drive, as detailed on page 15.
2. On the *Enter Sample Info* screen shown above, press the sample upload button, then follow the protocol setup wizard prompts to transfer the Sample IDs from the USB drive.

Method 2: Manual run-time sample assignment

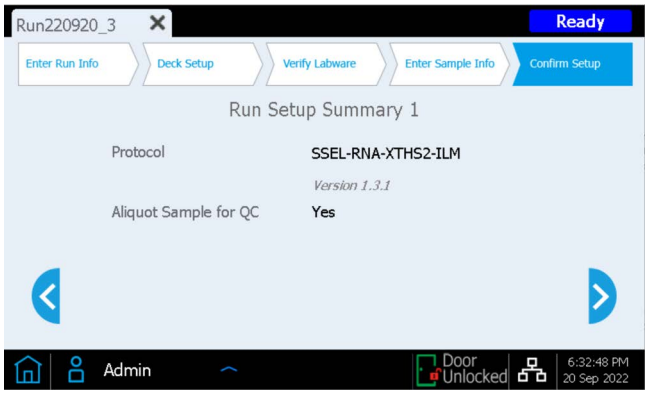
1. Select a specific sample position on the touchscreen.
2. Use the **Edit Sample ID** tool on the right to enter the desired Sample ID text. Press **Change** to save the entered Sample ID text for the sample position.

Step 5. Confirm Setup and start the run

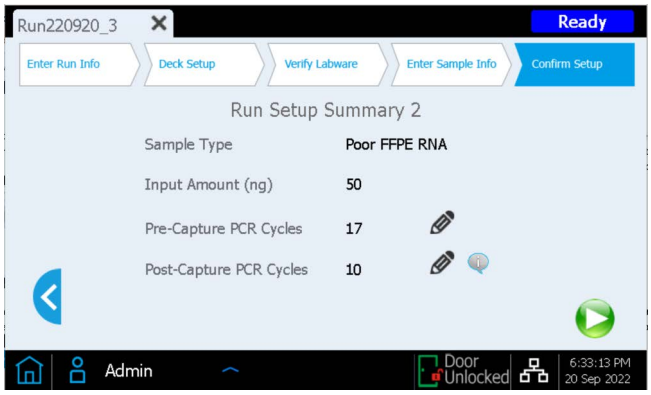
Use this set of screens to confirm the run setup details before initiating a run. Certain run parameters can be changed by pressing the pencil button adjacent to the parameter value.

NOTE Some parameters can only be changed when an *Advanced* access level user is logged in, and the pencil icon may not appear as shown in the screens below when a *Standard* access level user is logged in.

1 Verify the run settings displayed on the first *Run Setup Summary* screen. Once entries are confirmed or corrected, press the forward arrow to proceed to the final setup screen.



2 The second screen displays run details related to the characteristics of the RNA samples and probe used for the run. The pre-capture and post-capture PCR cycle numbers that will be used in the run (based on typical optimal conditions for the input RNA and probe used in the run) are displayed.

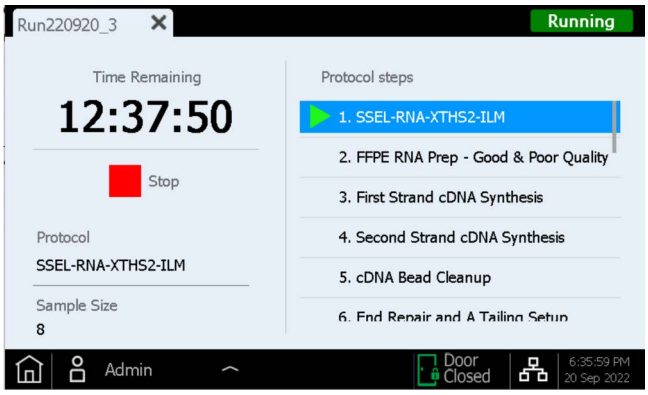


3 After confirming the run setup details, press the Start button to begin the run.

Once the run starts, the LED indicator lights are green, and the touchscreen displays the status of the run, including an estimate of the time remaining prior to run completion. Runs take approximately 12–13 hours.

Runs may be done overnight for convenience. Once the protocol is complete, the prepared libraries are automatically held at 12°C. Collect the libraries from the instrument within 72 hours.

If needed, press the red square **Stop** button to abort the run. A warning message opens asking you to confirm that you want to abort the run. Once you stop a run, the run cannot be resumed, and the labware used in that run cannot be reloaded for a future run.

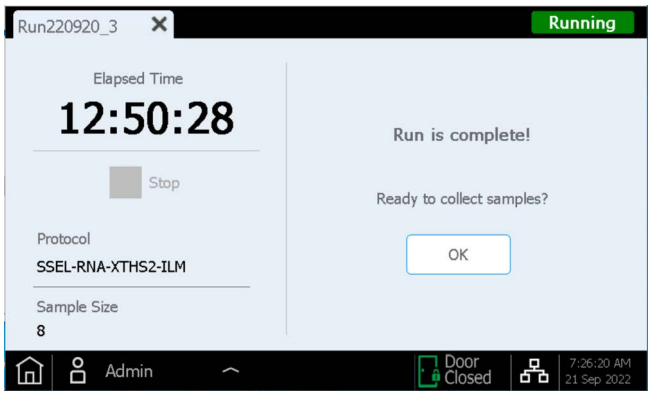


NOTE

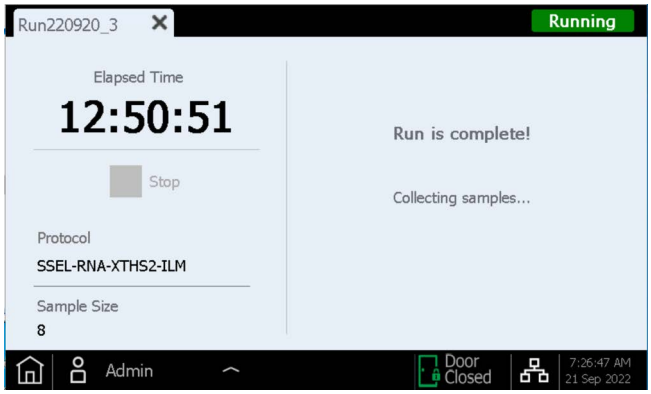
The *Running* screen must remain open through the duration of the run, and the screen close (**×**) button and other navigation buttons are inactive while the run is in progress. You cannot use the touchscreen to perform other functions during a run.

Step 6. Collect final library samples from the instrument

When the run is complete, the touchscreen displays the prompt below. Press **OK** when you are ready to collect the library samples from the instrument.

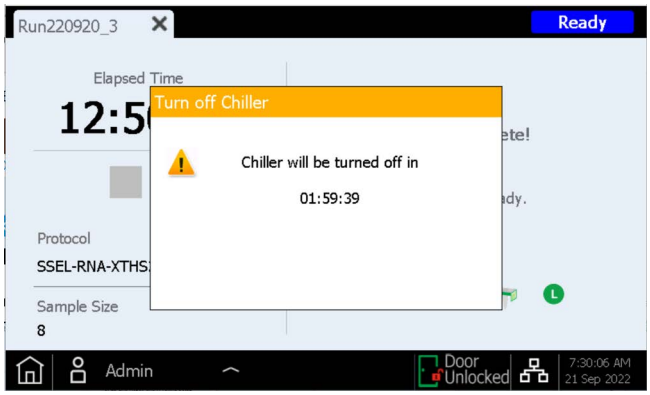


The instrument transfers the prepared library solutions from the PCR plate in the thermal cycler to the green Library Output Strip in the chiller at this time.



Wait for the LED indicator lights to turn blue, indicating that all instrument-mediated sample processing steps are complete, before opening the instrument door.

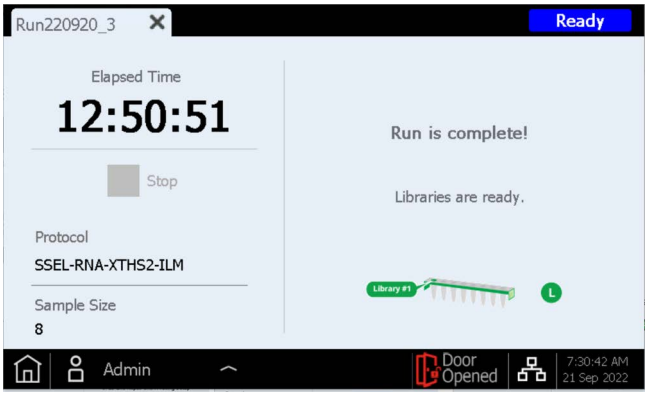
Once samples are placed in the green Library Output Strip in the chiller, the touchscreen display appears as below. The chiller, containing the library samples, is held at 12°C for up to 2 hours, with the remaining cold storage period indicated on the touchscreen dialog as shown below. The chiller is turned off once the instrument door is opened.



Fully open the instrument door (until the LED indicator lights turn white) and collect the final library samples in the green Library Output Strip from the L-position of the chiller module. Re-seal the wells using a fresh foil seal strip (provided in the Library Output and QC strip tube package). The final target-enriched libraries are in ~20–23 µL of Low TE Buffer (10 mM Tris-HCl, pH 7.5-8.0, 0.1 mM EDTA). Place the libraries under suitable storage conditions, according to your research design (see page 52 for storage recommendations).

Guidelines for processing the libraries for DNA sequencing are provided in "Appendix 3: Guidelines for Post-run cDNA Library Processing for NGS" on page 50.

Once the door is opened for library sample collection, the touchscreen appears as shown below.



Close the run screen by pressing the X on the tab to return to the Home screen.

NOTE Closing the screen may take several seconds. Do not repeatedly press the X button.

Processing of the Optional Pre-Capture Library QC Samples

If the optional pre-capture library QC samples were collected for the run, remove the blue QC Strip from the chiller module. Dry the cDNA in the wells by leaving the unsealed QC Strip at RT until the samples are dried. QC samples may be stored in dried condition until the sequencing libraries are analyzed.

NOTE QC samples may appear dried or partially dried at the end of the run, since the QC strips remain unsealed after the 3-µL aliquots are collected during the run. Samples should be completely dried before storage or reconstitution to ensure accurate QC results.

If analysis of the QC samples is required, resuspend the dried samples in 6 µL of nuclease-free water to achieve a concentration suitable for analysis using Agilent's TapeStation system and a D1000 ScreenTape assay, or similar analytical tool. After adding 6 µL of water to each well, incubate at RT for 5–10 minutes then mix well by vortexing to ensure complete resuspension.

Expected Results: Typical pre-capture libraries have a peak of cDNA fragment size between 200 and 700 bp.

QC samples that were dried and resuspended in 6 µL should have a concentration of approximately 30–100 ng/µL depending on input RNA quality and the pre-capture PCR cycle number. The overall pre-capture library yield may be calculated as the amount of cDNA in 1 µL of the reconstituted QC sample x 36 (includes both dilution and sampling adjustments).

Step 7. Clear the instrument after the run

Remove and dispose of all used consumables remaining on the instrument deck:

- Remove the filled tip waste bin from waste bin drawer, then return drawer to closed position
- Remove the used Deep-Well HSM plate from the HSM module
- Remove the used 96-Well PCR Plate and the thermal cycler seal from the PCR module
- Remove all tip boxes, including any partially filled boxes
- Remove the used deep-well Beads/Buffers Plate from the central deck plateholder
- Open the chiller module and remove the used Reagent Plate and the used red, black, and white strip tubes. Make sure that any green Library Output (L) strip tubes and blue QC sample (Q) strip tubes were removed from the chiller and retained for further processing.

NOTE It is critical to remove all labware components and any other stray materials from the instrument deck before initiating a new run. The presence of any materials on the deck when a new run is initiated can cause Instrument Health Check failure for the new run.

If any spilled or leaked materials are observed on the instrument deck, Agilent recommends running the UV decontamination Extended Cycle procedure (see page 19 for more information on UV decontamination). Clean the spill using an alcohol or dilute bleach wipe (see Table 5 on page 12; for complete cleaning instructions see the instrument User Guide).

Disposal

Dispose of unused reagents, waste, and specimens in accordance with country, federal, state and local regulations.

3 Appendix 1: RNA Sample Preparation Guidelines

Before setting up the Magnis SureSelect XT HS2 RNA sequencing library preparation run, RNA samples should be prepared, quantified and qualified using the guidelines in this Appendix.

Magnis library preparation protocols are compatible both with high-quality total RNA prepared from fresh or fresh frozen samples and with lower-quality RNA prepared from FFPE samples. Magnis runs can include 10 ng, 50 ng, 100 ng or 200 ng of input RNA. For optimal sequencing results, use the maximum amount of input RNA available within this range. All samples in the same run must be of the same quality grade and must be provided in the same quantity.

Magnis Sample Input Strips (red strip tubes provided in plate format, p/n 5190-9882 or 5191-5676), along with all input RNA sample preparation and dilution reagents, should be stored and used only in pre-PCR areas of the laboratory.

Step 1. Prepare total RNA from biological samples

Prepare total RNA either from fresh/frozen biological samples or from FFPE tissue sections using a suitable purification system. The library preparation protocol requires 10–200 ng of total RNA in a 10 µL volume of 1X Low TE Buffer.

Store the RNA samples on ice for same-day library preparation, or at –20°C for later processing.

Step 2. Qualify and quantify the RNA samples

1. Use a small-volume spectrophotometer to determine sample absorbance at 260 nm, 280 nm, and 230 nm.
2. Determine the RNA concentration using the 260 nm absorbance value.
3. Calculate 260/280 and 260/230 absorbance ratio values for the sample.

   Values of approximately 1.8 to 2.0 are expected for both ratios. Ratios with significant deviation from 2.0 indicate the presence of organic or inorganic contaminants, which may require further purification or may indicate that the sample is not suitable for use in RNA target enrichment applications.
4. Examine the starting size distribution of RNA in the sample using one of the RNA qualification systems described in Table 8. Select the specific assay appropriate for your sample based on the RNA concentration determined by spectrophotometry.

   Determine the DV200 (percentage of RNA that is >200 nt) using the analysis mode described in Table 8. RNA molecules must be >200 nt for efficient conversion to cDNA library.

Table 8 RNA qualification platforms

Analysis Instrument	RNA Qualification Assay	Analysis to Perform
4200/4150 TapeStation	RNA ScreenTape or High Sensitivity RNA ScreenTape	Region analysis using TapeStation Analysis Software
5200 Fragment Analyzer	RNA Kit (15NT) or HS RNA Kit (15NT)	Analysis using ProSize Data Analysis Software
2100 Bioanalyzer	RNA 6000 Pico Chip or Nano Chip	Smear/Region analysis using 2100 Expert Software

NOTE Qualification of FFPE RNA by RNA Integrity Number (RIN) is not recommended for this application.

RNA input guidelines based on sample qualification results

Intact total RNA from high-quality (fresh/fresh-frozen) samples

Select **Sample Type** of *Intact RNA* during run setup. For optimal sequencing results, use the maximum amount of input RNA available within the 10–200 ng range.

Samples with DV200<50% may not suitable for use in RNA target enrichment applications.

Total RNA from FFPE samples

Grade each FFPE RNA sample based on DV200 results according to Table 9.

Table 9 Classification of FFPE RNA samples based on starting RNA size

Grade	DV200	Recommended input amount	Minimum input amount
Good FFPE RNA	>50%	200 ng	10 ng
Poor FFPE RNA	20% to 50%	200 ng	50 ng
Inapplicable FFPE RNA	<20%	Not recommended for further processing	

For good-quality FFPE RNA samples (DV200>50%) select **Sample Type** of *Good FFPE RNA* during run setup. For optimal sequencing results, use the maximum amount of input RNA available within the 10–200 ng range.

For poor-quality FFPE RNA samples (DV200 20% to 50%) select **Sample Type** of *Poor FFPE RNA* during run setup. For optimal sequencing results, use the maximum amount of input RNA available within the 50–200 ng range.

NOTE

During run setup using the *Enter Run Info* screen, when **Sample Type** of *Poor FFPE RNA* is selected, only **Input Amounts** of *50 ng*, *100 ng* or *200 ng* are available and supported.

Samples with DV200<20% are not suitable for use in this application.

Step 3. Dilute the qualified RNA samples for the run

Prepare each RNA sample for library preparation by diluting the appropriate amount (10 ng, 50 ng, 100 ng or 200 ng) of each total RNA sample in 10 µL volume of 1X Low TE Buffer.

Place samples in wells of a red Magnis Sample Input Strip according to the instructions on page 21. All wells of the strip must contain the same amount of RNA. Keep the sample strip tube on ice until use on page 34.

4 Appendix 2: Use of Run-time Prepared Probe Strips

The instructions in this section are specifically for self-filled probe strip preparation and use in *SSEL-RNA-XTHS2-EPIS-ILM* Magnis protocol runs, set up using Reagent Kit part number G9750D (Magnis SureSelect XT HS2 RNA Reagent Kit provided with empty Probe Input Strips). The *SSEL-RNA-XTHS2-EPIS-ILM* Magnis protocol requires run-time filling of the provided empty probe strips and requires additional probe data entry steps, as described in this section.

Instructions in this section do not apply to kits that include pre-filled probe strips; see page 23 for pre-filled probe strip set up information.

Run-Time Preparation of the Empty Probe Input Strip (EPIS)

The empty Magnis Probe Input Strips (p/n 5190-9883, white strips provided in a plate format) should be stored and filled in a pre-PCR area of the laboratory. Prepare the probe input strip just before use in the *SSEL-RNA-XTHS2-EPIS-ILM* run; do not pre-fill and freeze-thaw the probe input strips used in this protocol.

The 8 wells of the empty Magnis Probe Input Strip may be filled with the same or different probe solutions. All probes used in the same run must, however, have a similar design size to allow use of the same run conditions by the Magnis (see Table 11 on page 49 for compatible probe design size ranges).

1 Obtain one empty white Magnis Probe Input Strip and one fresh foil seal strip (with backing) from kit p/n 5190-9883, stored at RT.

2 Thaw and mix the vial(s) of SureSelect Probe to be used for the run and keep on ice.

3 Refer to Table 10 below to determine the volume of SureSelect Probe solution required for your probe design size.

Table 10 Probe volume requirements

Probe Capture Size	Volume to Pipette per Well	Volume Required for 8-Sample Run	Protocol Selected on *Enter Run Info* Screen
≥3 Mb (**Large** *Capture Size*)*	5 µL	40 µL	*SSEL-RNA-XTHS2-EPIS-ILM*
<3 Mb (**Small** *Capture Size*)*	2 µL	16 µL	

* The **Large** vs. **Small** *Capture Size* designation for the probe(s) used in the run is entered in the Magnis software as described on page 49. All probes used in a run must have the same *Capture Size* designation and must use the same post-capture PCR cycling conditions (see Table 11 on page 49).

4 Fill the wells of the empty Magnis Probe Input Strip with the appropriate volume of SureSelect Probe solution using the steps below:

a Use an empty 200-µL pipette tip to pre-pierce the foil seal of each well of the probe input strip to be filled for the run.

b Using a micropipette qualified to accurately dispense the probe volume listed in Table 10, dispense the indicated amount of SureSelect Probe solution into each well.

Use a 2-µL capacity micropipette and pipette tip when dispensing 2 µL of probe.

Use a 10-µL capacity micropipette and pipette tip when dispensing 5 µL of probe.

NOTE It is important to fill the probe input strip wells using precisely the volumes indicated in Table 10. Use a calibrated pipette qualified to dispense the indicated volume with high accuracy and precision.

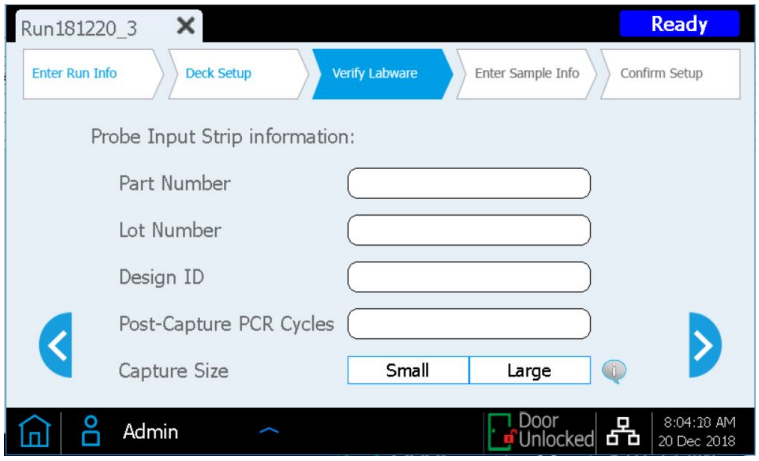
5 After dispensing the probe solution into all wells, re-seal the wells with the fresh foil seal provided in the kit, taking care to avoid obscuring the probe strip barcode with the foil seal. Ensure that the seal is applied firmly and evenly, without excessive overhangs or creases that could obstruct strip tube seating when loading in the instrument.

6 Visually check the probe strip wells to verify that no bubbles are present. Remove any bubbles by spinning the prepared probe strip in a centrifuge set at 250 × *g* for 5 seconds or until all bubbles are released from the probe solution.

7 Keep the probe strip on ice until use in the *SSEL-RNA-XTHS2-EPIS-ILM* run. Load the filled probe strip during the deck setup steps described on page 34. When loading the probe strip, make sure to verify that the strip is properly seated in the chiller module.

Entering Probe Information during Run Setup

For *SSEL-RNA-XTHS2-EPIS-ILM* runs you must enter the **probe**-related properties in the fields shown below during the *Verify Labware* phase of run setup (see page 37).



Enter information in the *Part Number*, *Lot Number* and *Design ID* fields according to the record keeping requirements of your facility. The Design ID and Lot Number for Agilent-supplied SureSelect or ClearSeq probes is provided on the product vial and on the Certificate of Analysis.

Enter the PCR cycle number to be used in the run in the *Post-Capture PCR Cycles* field, according to the size of your probe design(s) and press the appropriate *Capture Size* description for the probe(s) used in the run. See Table 11 below for guidelines. The suggested PCR cycle number is typically optimal for the listed probe design size, but the PCR cycle number may be adjusted to meet the needs of your experimental design. The 8 wells of the Magnis Probe Input Strip may contain different probe solutions, but all probes used in the same run must use the same *Post-Capture PCR Cycles* and *Capture Size* settings.

Table 11 Recommended settings for run-time dispensed probes

SureSelect XT HS Probe Size	Post-Capture PCR Cycles	Capture Size
<200 kb	15	Small
200–749 kb	14	Small
750–2999 kb	13	Small
3–5 Mb	11	Large
>5 Mb	10	Large

Once all fields are populated, press the forward arrow to proceed to the *Enter Sample Info* screen and follow the remaining run setup steps starting on page 38.

5 Appendix 3: Guidelines for Post-run cDNA Library Processing for NGS

After completing the Magnis SureSelect XT HS2 RNA library preparation run, the cDNA sequencing library samples are quantified and qualified, then analyzed by NGS. Guidelines for typical post-run sample processing for NGS are provided in this section; your post-run NGS processing and analysis workflow may vary.

Step 1. Analyze quantity and quality of library DNA samples

Prior to sample pooling for multiplexed sequencing, analyze the quantity and quality of DNA in the individual prepared library samples using an Agilent TapeStation instrument with the High Sensitivity D1000 ScreenTape and associated reagent kit. See Table 4 on page 11 for ordering information. Refer to the Agilent High Sensitivity D1000 Assay Quick Guide for detailed instructions.

NOTE Alternatively, cDNA library samples may be analyzed using the Agilent 5200 Fragment Analyzer and **HS NGS Fragment Kit** or using the Agilent 2100 Bioanalyzer and the Bioanalyzer High Sensitivity DNA Assay. Refer to the linked assay user guides for complete instructions.

1 Prepare the TapeStation assay samples in a fresh tube strip as instructed in the assay Quick Guide. Use 2 µL of each library DNA sample diluted with 2 µL of High Sensitivity D1000 Sample Buffer for the analysis.

CAUTION Make sure that you thoroughly mix the combined DNA and sample buffer on the IKA vortex mixer, as instructed in the assay Quick Guide, for accurate quantitation.

2 Load the High Sensitivity D1000 assay tube strips from step 1, the High Sensitivity D1000 ScreenTape, and loading tips into the TapeStation as instructed in the assay Quick Guide. Start the run.

3 Verify that the electropherogram shows the peak of DNA fragment size positioned between 200 and 700 bp. Sample electropherograms are shown in Figure 7 (library prepared from high-quality RNA) and Figure 8 (library prepared from FFPE RNA).

4 Determine the concentration of each library by integrating under the entire peak.

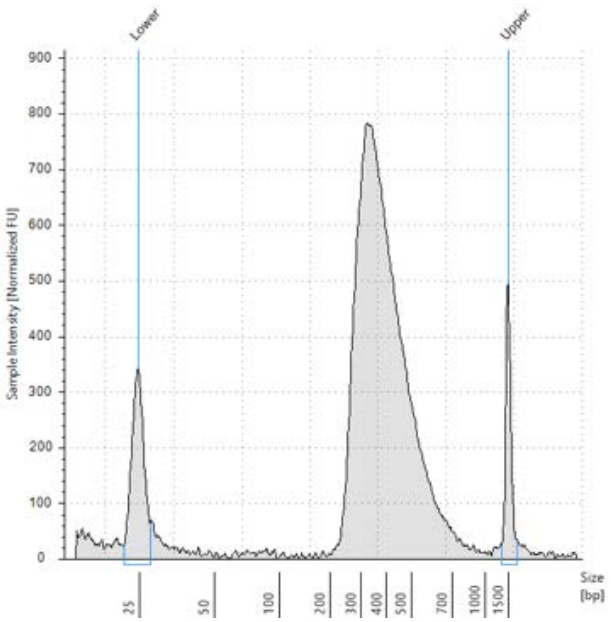


Figure 7 Post-capture library prepared from a high-quality RNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

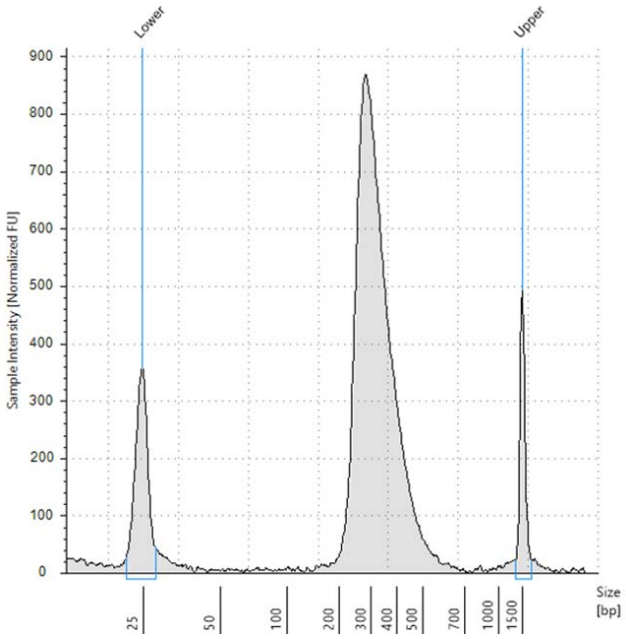


Figure 8 Post-capture library prepared from a typical FFPE RNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

Stopping Point If you do not continue to the next step, store samples at 4°C overnight or at –20°C for prolonged storage.

Step 2. Pool samples for multiplexed sequencing (optional)

The number of indexed libraries that may be multiplexed in a single sequencing lane is determined by the output specifications of the sequencer used, together with the amount of sequencing data required for your research design. Calculate the number of indexes that can be combined per lane, according to the capacity of the sequencer and the amount of sequencing data required per sample.

Combine the libraries such that each index-tagged library is present in equimolar amounts in the pool using one of the following methods. Use the diluent specified by your sequencing provider, such as Low TE, for the dilution steps.

Method 1: Dilute each library sample to be pooled to the same final concentration (typically 4–15 nM, or the concentration of the most dilute sample) then combine equal volumes of all samples to create the final pool.

Method 2: Starting with library samples at different concentrations, add 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. The formula below is provided for determination of the amount of each indexed sample to add to the pool.

$$\text{Volume of Index} = \frac{V(f) \times C(f)}{\# \times C(i)}$$

where *V(f)* is the final desired volume of the pool,

C(f) is the desired final concentration of all the DNA in the pool (typically 4 nM–15 nM or the concentration of the most dilute sample)

is the number of indexes, and

C(i) is the initial concentration of each indexed sample

Table 12 shows an example of the amount of 4 index-tagged samples (of different concentrations) and Low TE needed for a final volume of 20 µL at 10 nM DNA.

Table 12 Example of volume calculation for total volume of 20 µL at 10 nM concentration

Component	V(f)	C(i)	C(f)	#	Volume to use (µL)
Sample 1	20 µL	20 nM	10 nM	4	2.5
Sample 2	20 µL	10 nM	10 nM	4	5
Sample 3	20 µL	17 nM	10 nM	4	2.9
Sample 4	20 µL	25 nM	10 nM	4	2
Low TE					7.6

Store the library pool under the conditions specified by your sequencing provider. Typically, libraries should be stored at –20°C for short-term storage.

Step 3. Prepare the sequencing samples

The final SureSelect XT HS2 library pool is ready for direct sequencing using standard Illumina paired-end primers and chemistry. Each fragment in the prepared library contains one target insert surrounded by sequence motifs required for multiplexed sequencing using Illumina sequencers, as shown in Figure 9.

Figure 9 Content of SureSelect XT HS2 sequencing library. Each fragment contains one target insert (blue) surrounded by the Illumina paired-end sequencing elements (black), unique dual sample indexes (red and green), molecular barcodes (MBCs; brown) and the library PCR primers (yellow).

Proceed to cluster amplification using the appropriate Illumina Paired-End Cluster Generation Kit. Table 13 provides guidelines for use of several instrument and chemistry combinations suitable for this application. For other Illumina NGS platforms, consult Illumina's documentation for kit configuration and seeding concentration guidelines.

Table 13 Illumina Kit Configuration Selection Guidelines

Instrument	Run Type	Read Length	SBS Kit Configuration	Chemistry	Seeding Concentration
MiSeq	All Runs	2 × 100 bp or 2 × 150 bp	300 Cycle Kit	v2	9–10 pM
			600 Cycle Kit	v3	12–16 pM
iSeq 100	All Runs	2 × 100 bp or 2 × 150 bp	300 Cycle Kit	v2	50–150 pM
NextSeq 500/550	All Runs	2 × 100 bp or 2 × 150 bp	300 Cycle Kit	v2.5	1.2–1.5 pM
NextSeq 1000/2000	All Runs	2 × 100 bp or 2 × 150 bp	200 or 300 Cycle Kit	Standard SBS	650–1000 pM
				XLEAP-SBS	650–1000 pM
NovaSeq 6000	Standard Workflow Runs	2 × 100 bp or 2 × 150 bp	200 or 300 Cycle Kit	v1.5	300–600 pM
NovaSeq 6000	Xp Workflow Runs	2 × 100 bp or 2 × 150 bp	200 or 300 Cycle Kit	v1.5	200–400 pM
NovaSeq X	All runs	2 × 100 bp or 2 × 150 bp	200 or 300 Cycle Kit	v1	90–180 pM

Seeding concentration and cluster density may also need to be optimized based on the cDNA fragment size range for the library and on the desired output and data quality. Begin optimization using a seeding concentration in the middle of the range listed in Table 13 or provided by Illumina. Follow Illumina's recommendation for a PhiX control in a low-concentration spike-in for improved sequencing quality control.

Step 4. Sequence the libraries

Set up the sequencing run to generate Read 1 and Read 2 FASTQ files for each sample using the instrument's software in standalone mode or using an Illumina run management tool such as Local Run Manager (LRM), Illumina Experiment Manager (IEM) or BaseSpace. Enter the appropriate **Cycles** or **Read Length** value for your library read length and using 8-bp dual index reads. See Table 14 showing example settings for 2x150 bp sequencing.

Table 14 Run settings for 2x150 bp sequencing

Run Segment	Cycles/Read Length
Read 1	151*
Index 1 (i7)	8
Index 2 (i5)	8
Read 2	151*

* Follow Illumina's recommendation to add one (1) extra cycle to the desired read length.

Follow Illumina's instructions for each platform and setup software option, incorporating the additional setup guidelines below:

- Each of the sample-level indexes (i7 and i5) requires an 8-bp index read. For complete index sequence information, see page 62.
- No custom primers are used for SureSelect XT HS2 library sequencing. Leave all *Custom Primers* options for *Read 1*, *Read 2*, *Index 1* and *Index 2* primers cleared/deselected during run setup.
- Turn off any adaptor trimming tools included in Illumina's run setup and read processing software applications. Adaptors are trimmed in later processing steps to ensure proper processing of the adaptors, including the degenerate molecular barcodes (MBCs) in the adaptor sequences.
- For runs set up using Illumina's LRM, IEM, or BaseSpace applications, refer to Illumina's instructions and support resources for setting up runs with custom library prep kits and index kits in the selected software. For use in these applications, the SureSelect XT HS2 index sequences can be obtained by downloading the SureSelect XT HS2 Index Sequence Resource Excel spreadsheet from Agilent.com. The provided sequences should be converted to .tsv/.csv file format or copied to a Sample Sheet according to Illumina's specifications for each application. If you need assistance with SureSelect XT HS2 run setup in your selected application, contact the SureSelect support team (see page 2) or your local representative.

Step 5. Process the reads

Guidelines are provided below for typical NGS read processing pipeline steps appropriate for SureSelect XT HS2 RNA libraries. Your NGS pipeline may vary.

- Demultiplex using Illumina's bcl2fastq, BCL Convert or DRAGEN software to generate paired end reads based on the dual indexes and remove sequences with incorrectly paired P5 and P7 indexes. Turn off the MBC/UMI trimming options in Illumina's demultiplexing software to allow proper adaptor and MBC processing by downstream software tools.
- The demultiplexed FASTQ data needs to be pre-processed to trim the sequencing adaptors and to extract and use MBC sequences for de-duplication. Agilent's Genomics NextGen Toolkit (AGeNT) software modules, described below, can be used for these pre-processing steps.

NOTE

Read pre-processing steps can also be completed using suitable open-source software tools, such as fgbio. Performance of open-source tools should be verified for appropriate adaptor and MBC sequence processing on both strands. Some non-Agilent adaptor trimmers may fail to remove the MBC sequences from the opposite adaptor, which may affect alignment quality.

- If your sequence analysis pipeline excludes MBCs, you can remove MBCs during the demultiplexing step by trimming or masking the first five bases from each read using the guidelines below.

  If demultiplexing using bcl2fastq, MBCs may be masked by including the base mask **N5Y*,I8,I8,N5Y*** (where * is replaced with the remaining read length after subtracting the 5 masked bases, e.g., use **N5Y146,I8,I8,N5Y146** for 2x150 NGS set up as shown in Table 14 on page 55). The sum of the values following N and Y must match the read length value in the RunInfo.xml file.

  If demultiplexing using BCL Convert, MBCs may be trimmed by including the following string in the sample sheet header: **OverrideCycles,N5Y*;I8;I8;N5Y*** (where * is replaced with read length after trimming, e.g., use **N5Y146;I8;I8;N5Y146** for 2x150 NGS set up as shown in Table 14 on page 55). The sum of the values following N and Y must match the read length value in the RunInfo.xml file.

Using Agilent's AGeNT software for RNA workflows

Agilent's AGeNT is a Java-based toolkit for library read processing steps, designed for users with bioinformatics expertise to enable building internal analysis pipelines. To download this toolkit, visit the AGeNT page at www.agilent.com. Use of the AGeNT read processing tools is outlined briefly below. See the AGeNT Best Practices document for processing steps suitable for XT HS2 RNA libraries.

- Before aligning reads to reference sequences, Illumina adaptor sequences should be trimmed from the reads using the AGeNT Trimmer module, which properly accounts for the degenerate MBCs in the adaptor sequence.
- The trimmed reads should be aligned using a suitable RNA data alignment tool.

- Once alignment is complete, the AGeNT CReaK (Consensus Read Kit) tool can be used in the single-strand consensus mode to generate consensus reads and mark or remove duplicates. (Note that unlike DNA, where both strands are present and the MBCs in the strands can be matched to form a duplex consensus read, analysis of single-stranded RNA is limited to consensus generation using the MBC from one strand.) The resulting BAM files are ready for downstream analysis including gene expression and variant discovery.

Strandedness guidelines

The SureSelect XT HS2 RNA sequencing library preparation method preserves RNA strandedness using dUTP second-strand marking. The sequence of Read 1, which starts at the P5 end, matches the reverse complement of the poly-A RNA transcript strand. Read 2, which starts at the P7 end, matches the poly-A RNA transcript strand. When running analysis of this data to determine strandedness, it is important to include this information. For example, when using the Picard tools (https://broadinstitute.github.io/picard) to calculate RNA sequencing metrics, it is important to include the parameter *STRAND_SPECIFICITY= SECOND_READ_TRANSCRIPTION_STRAND* to correctly calculate the strand specificity metrics.

6 Reference

This chapter contains reference information, including reagent kit contents, index sequences, and troubleshooting information for the SureSelect XT HS2 RNA library preparation runs.

Reagent Kit Contents

Agilent part numbers for the Magnis SureSelect XT HS2 RNA Reagent Kits are summarized in Table 15.

Table 15 Reagent Kit Part Numbers

Included Probe	Magnis SureSelect XT HS2 RNA Reagent Kits	
	96 Reactions	32 Reactions
Custom 1–499 kb	G9751D	G9751C
Custom 0.5–2.9 Mb	G9752D	G9752C
Custom 3–5.9 Mb	G9753D	G9753C
Custom 6–11.9 Mb	G9754D	G9754C
Custom 12–24 Mb	G9755D	G9755C
Custom 24–50 Mb	G9756D	G9756C
Human All Exon V7	G9773D	G9773C
Human All Exon V8	G9774D	G9774C
SureSelect Clinical Research Exome V4 (CRE V4)	G9775D	G9775C
SureSelect Cancer CGP RNA	G9777D	G9777C
None (kit includes empty Probe Input Strips for run-time probe setup)	G9750D	Not offered

Magnis SureSelect XT HS2 RNA Reagent Kits include the component kits listed in Table 16, with the contents of each component kit detailed in Table 17 through Table 23.

Table 16 Component kits provided with Magnis SureSelect XT HS2 RNA Reagent Kits

Component kit name	Storage condition	Component kit p/n	
		96 Reactions	32 Reactions
Magnis SureSelect Probe Plate, Pre-filled Single Well Format	–80°C	p/n varies; see Table 17*	p/n varies; see Table 17
Magnis SureSelect XT HS2 Reagent Plates ILM	–20°C	5191-6831	5191-6830
Magnis SureSelect RNA Reagent Strips ILM	–20°C	5280-0008	5280-0007
Magnis SureSelect XT HS2 Index Primer Pairs ILM	–20°C	5191-6833 (Index Pairs 1-96) **OR** 5191-6835 (Index Pairs 97-192)	5191-6837 (Index Pairs 1-32)
Magnis SureSelect RNA Beads/Buffers Plates ILM	+4°C	5280-0010	5280-0009
Magnis Empty Consumables	Room Temperature	5190-9712	5191-5675
Magnis Sample Input Strips	Room Temperature	5190-9882	5191-5676

* Kit part number G9750D does not include a Magnis Probe Plate. Instead the G9750D kit, configured for run-time probe setup, includes empty Magnis Probe Input Strips for 12 runs (p/n 5190-9883), stored at Room Temperature.

Table 17 Probe Plate part numbers

Reagent Kit p/n	Included Probe design	Probe Plate p/n	Quantity per kit
G9751D (96 Reactions)	Custom 1–499 kb (Tier 1)	5191-6817	1 plate (12 strips)
G9751C (32 Reactions)	Custom 1–499 kb (Tier 1)	5191-6807	1 plate (4 strips)
G9752D (96 Reactions)	Custom 0.5 –2.9 Mb (Tier 2)	5191-6819	1 plate (12 strips)
G9752C (32 Reactions)	Custom 0.5 –2.9 Mb (Tier 2)	5191-6809	1 plate (4 strips)
G9753D (96 Reactions)	Custom 3–5.9 Mb (Tier 3)	5191-6821	1 plate (12 strips)
G9753C (32 Reactions)	Custom 3–5.9 Mb (Tier 3)	5191-6811	1 plate (4 strips)
G9754D (96 Reactions)	Custom 6–11.9 Mb (Tier 4)	5191-6823	1 plate (12 strips)
G9754C (32 Reactions)	Custom 6–11.9 Mb (Tier 4)	5191-6813	1 plate (4 strips)
G9755D (96 Reactions)	Custom 12–24 Mb (Tier 5)	5191-6825	1 plate (12 strips)
G9755C (32 Reactions)	Custom 12–24 Mb (Tier 5)	5191-6815	1 plate (4 strips)
G9756D (96 Reactions)	Custom 24–50 Mb	5191-6846	1 plate (12 strips)
G9756C (32 Reactions)	Custom 24–50 Mb	5191-6845	1 plate (4 strips)
G9773D (96 Reactions)	Human All Exon V7	5191-6827	1 plate (12 strips)
G9773C (32 Reactions)	Human All Exon V7	5191-6826	1 plate (4 strips)
G9774D (96 Reactions)	Human All Exon V8	5191-6974	1 plate (12 strips)
G9774C (32 Reactions)	Human All Exon V8	5191-6973	1 plate (4 strips)
G9775D (96 Reactions)	SureSelect CRE V4	5282-0042	1 plate (12 strips)
G9775C (32 Reactions)	SureSelect CRE V4	5282-0041	1 plate (4 strips)
G9777D (96 Reactions)	SureSelect Cancer CGP RNA	5282-0047	1 plate (12 strips)
G9777C (32 Reactions)	SureSelect Cancer CGP RNA	5282-0046	1 plate (4 strips)

Table 18 Components of Magnis SureSelect XT HS2 Reagent Plates ILM kit

Component provided	Part Number (kit size)	Quantity and format
Magnis SureSelect XT HS2 Reagent Plate ILM	5191-6831 (96 Reactions)	12 plates (use 1 plate per run)
	5191-6830 (32 Reactions)	4 plates (use 1 plate per run)

Table 19 Components of Magnis SureSelect RNA Reagent Strips ILM kit

Component provided	Part Number (kit size)	Quantity and format
Magnis SureSelect RNA Reagent Strips ILM	5280-0008 (96 Reactions)	1 plate (12 strips)
	5280-0007 (32 Reactions)	1 plate (4 strips)

Table 20 Components of Magnis SureSelect XT HS2 Index Primer Pairs ILM kit

Component provided	Part Number (kit size)	Quantity and format
Magnis SureSelect HS2 Index Primer Pairs ILM	5191-6833 (Index Pairs 1-96) **OR** 5191-6835 (Index Pairs 97-192)	1 plate of 12 strips (use 1 strip per run)
	5191-6837 (32 Reactions)	1 plate of 4 strips (use 1 strip per run)

Table 21 Components of Magnis SureSelect RNA Beads/Buffers Plates ILM kit

Component provided	Part Number (kit size)	Quantity and format
Magnis SureSelect RNA Beads/Buffers Plates ILM	5280-0010 (96 Reactions)	12 plates (use 1 plate per run)
	5280-0009 (32 Reactions)	4 plates (use 1 plate per run)

Table 22 Components of Magnis Empty Consumables kit

Components provided	Quantity and format*
Magnis Deep-Well HSM Plate	1 plate
Magnis 96-Well PCR Plate	1 plate
Magnis Library Output Strip	1 green strip tube
Magnis QC Strip	1 blue strip tube
Magnis Foil Seals	1 sheet (6 single-strip tube foil sealing strips)
Magnis Thermal Cycler Seal	1 single-use metal sealing plate
Magnis Tip Waste Bin	1 single-use bin liner

* Parts listed are per single-run box of consumables. Each 96 Reaction kit is supplied with 12 individual boxes (p/n 5190-9712) of consumables for single run, and each 32 Reaction kit is supplied with 4 individual boxes (p/n 5191-5675) of consumables for single run.

Table 23 Components of the Magnis Sample Input Strips kit

Part Number (kit size)	Components provided	Quantity and format
5190-9882 (96 Reactions)	Magnis Sample Input Strips	12 empty red, foil-sealed strips
	Magnis Foil Seals	2 sheets (6 single-strip tube foil sealing strips per sheet)
5191-5676 (32 Reactions)	Magnis Sample Input Strips	4 empty red, foil-sealed strips
	Magnis Foil Seals	1 sheet (6 single-strip tube foil sealing strips per sheet)

Reference Information for SureSelect XT HS2 Indexes

Magnis SureSelect XT HS2 Reagent Kits include the appropriate set of SureSelect XT HS2 Index Primer Pairs in single-use aliquots in the individual wells of index strip tubes, supplied on a plate platform. The primer pairs are provided pre-combined. Each member of the primer pair contains a unique 8-bp P7 or P5 index, resulting in dual-indexed NGS libraries.

Index sequences are provided on page 65 through page 68. Index sequences can also be obtained by downloading the SureSelect XT HS2 Index Sequence Resource Excel spreadsheet from Agilent.com.

NOTE

Clicking the Excel spreadsheet link from Agilent.com automatically downloads the index spreadsheet to the default folder for downloaded files saved by your web browser. Locate the file in the folder and open it in Microsoft Excel or another compatible spreadsheet program. The first tab of the spreadsheet provides instructions for use of the spreadsheet contents.

In Table 28 on page 65 through Table 31 on page 68 and in the downloadable Excel spreadsheet, P7 indexes are shown in forward orientation, applicable to any of the Illumina platforms. P5 indexes are shown in two orientations (forward and reverse complement) for use with different platforms and sequencing run setup and management tools, e.g., Local Run Manager and Instrument Run Setup. A selection of Illumina sequencing platforms and their P5 sequencing orientations are shown in Table 24. Correct representation of the P5 index orientation in sample sheets or during sequencing run setup is crucial to successful demultiplexing. Refer to Illumina support documentation and resources to determine the correct P5 index orientation for your application.

Table 24 P5 index sequencing orientation by Illumina platform

P5 Index Orientation	Platform
Forward	MiSeq
Reverse Complement*	NovaSeq 6000 with v1.5 chemistry NextSeq 500/550/1000/2000 iSeq 100 MiniSeq

* Some run setup and management tools used with these platforms automatically create the reverse complement sequence for the P5 index sequence entered for the run. Be sure to consult Illumina's support documentation for the combination of platform and tools used in your pipeline to determine the correct index orientation to enter during run setup.

Plate Position Information

The plate provided with 32 Reaction kits (p/n 5191-6837) contains one set of four (4) index strips labeled *D1*, *D2*, *D3*, or *D4*, with each of 32 unique dual indexing primer pairs 1-32 provided in a single well. See Table 25 for a plate map.

Table 25 Index map for Magnis SureSelect XT HS2 Index Primer Pairs 1-32, ILM provided with **32 Reaction kits**

Plate Column	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Strip Tube Label	**D1**	**D2**	**D3**	**D4**	**(No index strips provided in plate columns 5–12)**							
Index Pair Numbers	1	9	17	25	—	—	—	—	—	—	—	—
	2	10	18	26	—	—	—	—	—	—	—	—
	3	11	19	27	—	—	—	—	—	—	—	—
	4	12	20	28	—	—	—	—	—	—	—	—
	5	13	21	29	—	—	—	—	—	—	—	—
	6	14	22	30	—	—	—	—	—	—	—	—
	7	15	23	31	—	—	—	—	—	—	—	—
	8	16	24	32	—	—	—	—	—	—	—	—

The plate provided with 96 Reaction kits contains one set of 12 index strips, containing 96 unique dual indexing primer pairs, with each pair in a single well. Kits are supplied either with p/n 5191-6833, containing primer pairs 1-96 in index strips labeled *D1* through *D12*, or with p/n 5191-6835, containing primer pairs 97-192 in index strips labeled *D13* through *D24*. See Table 26 and Table 27 for plate maps.

Table 26 Index map for Magnis SureSelect XT HS2 Index Primer Pairs 1-96, ILM provided with **96 Reaction kits**

Plate Column	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Strip Tube Label	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**
Index Pair Numbers	1	9	17	25	33	41	49	57	65	73	81	89
	2	10	18	26	34	42	50	58	66	74	82	90
	3	11	19	27	35	43	51	59	67	75	83	91
	4	12	20	28	36	44	52	60	68	76	84	92
	5	13	21	29	37	45	53	61	69	77	85	93
	6	14	22	30	38	46	54	62	70	78	86	94
	7	15	23	31	39	47	55	63	71	79	87	95
	8	16	24	32	40	48	56	64	72	80	88	96

Table 27 Index map for Magnis SureSelect XT HS2 Index Primer Pairs 97-192, ILM provided with **96 Reaction kits**

Plate Column	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Strip Tube Label	**D13**	**D14**	**D15**	**D16**	**D17**	**D18**	**D19**	**D20**	**D21**	**D22**	**D23**	**D24**
Index Pair Numbers	97	105	113	121	129	137	145	153	161	169	177	185
	98	106	114	122	130	138	146	154	162	170	178	186
	99	107	115	123	131	139	147	155	163	171	179	187
	100	108	116	124	132	140	148	156	164	172	180	188
	101	109	117	125	133	141	149	157	165	173	181	189
	102	110	118	126	134	142	150	158	166	174	182	190
	103	111	119	127	135	143	151	159	167	175	183	191
	104	112	120	128	136	144	152	160	168	176	184	192

Index Nucleotide Sequences

The nucleotide sequence of each SureSelect XT HS2 index is shown in Table 28 through Table 31. Each index is 8 nt in length, and sequencing runs should be completed using 8-bp index reads (see page 55). Sequences can also be obtained by downloading the SureSelect XT HS2 Index Sequence Resource Excel spreadsheet from Agilent.com.

Table 28 SureSelect XT HS2 Index Primer Pairs 1–48

Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement	Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement
1	D1	CAAGGTGA	ATGGTTAG	CTAACCAT	25	D4	AGATGGAT	TGGCACCA	TGGTGCCA
2	D1	TAGACCAA	CAAGGTGA	TCACCTTG	26	D4	GAATTGTG	AGATGGAT	ATCCATCT
3	D1	AGTCGCGA	TAGACCAA	TTGGTCTA	27	D4	GAGCACTG	GAATTGTG	CACAATTC
4	D1	CGGTAGAG	AGTCGCGA	TCGCGACT	28	D4	GTTGCGGA	GAGCACTG	CAGTGCTC
5	D1	TCAGCATC	AAGGAGCG	CGCTCCTT	29	D4	AATGGAAC	GTTGCGGA	TCCGCAAC
6	D1	AGAAGCAA	TCAGCATC	GATGCTGA	30	D4	TCAGAGGT	AATGGAAC	GTTCCATT
7	D1	GCAGGTTC	AGAAGCAA	TTGCTTCT	31	D4	GCAACAAT	TCAGAGGT	ACCTCTGA
8	D1	AAGTGTCT	GCAGGTTC	GAACCTGC	32	D4	GTCGATCG	GCAACAAT	ATTGTTGC
9	D2	CTACCGAA	AAGTGTCT	AGACACTT	33	D5	ATGGTAGC	GTCGATCG	CGATCGAC
10	D2	TAGAGCTC	CTACCGAA	TTCGGTAG	34	D5	CGCCAATT	ATGGTAGC	GCTACCAT
11	D2	ATGTCAAG	TAGAGCTC	GAGCTCTA	35	D5	GACAATTG	CGCCAATT	AATTGGCG
12	D2	GCATCATA	ATGTCAAG	CTTGACAT	36	D5	ATATTCCG	GACAATTG	CAATTGTC
13	D2	GACTTGAC	GCATCATA	TATGATGC	37	D5	TCTACCTC	ATATTCCG	CGGAATAT
14	D2	CTACAATG	GACTTGAC	GTCAAGTC	38	D5	TCGTCGTG	TCTACCTC	GAGGTAGA
15	D2	TCTCAGCA	CTACAATG	CATTGTAG	39	D5	ATGAGAAC	TCGTCGTG	CACGACGA
16	D2	AGACACAC	TCTCAGCA	TGCTGAGA	40	D5	GTCCTATA	ATGAGAAC	GTTCTCAT
17	D3	CAGGTCTG	AGACACAC	GTGTGTCT	41	D6	AATGACCA	GTCCTATA	TATAGGAC
18	D3	AATACGCG	CAGGTCTG	CAGACCTG	42	D6	CAGACGCT	AATGACCA	TGGTCATT
19	D3	GCACACAT	AATACGCG	CGCGTATT	43	D6	TCGAACTG	CAGACGCT	AGCGTCTG
20	D3	CTTGCATA	GCACACAT	ATGTGTGC	44	D6	CGCTTCCA	TCGAACTG	CAGTTCGA
21	D3	ATCCTCTT	CTTGCATA	TATGCAAG	45	D6	TATTCCTG	CGCTTCCA	TGGAAGCG
22	D3	GCACCTAA	ATCCTCTT	AAGAGGAT	46	D6	CAAGTTAC	TATTCCTG	CAGGAATA
23	D3	TGCTGCTC	GCACCTAA	TTAGGTGC	47	D6	CAGAGCAG	CAAGTTAC	GTAACTTG
24	D3	TGGCACCA	TGCTGCTC	GAGCAGCA	48	D6	CGCGCAAT	CAGAGCAG	CTGCTCTG

Table 29 SureSelect XT HS2 Index Primer Pairs 49–96

Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement	Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement
49	D7	TGAGGAGT	CGCGCAAT	ATTGCGCG	73	D10	AACGCATT	ATAGTGAC	GTCACTAT
50	D7	ATGACGAA	TGAGGAGT	ACTCCTCA	74	D10	CAGTTGCG	AACGCATT	AATGCGTT
51	D7	TACGGCGA	ATGACGAA	TTCGTCAT	75	D10	TGCCTCGA	CAGTTGCG	CGCAACTG
52	D7	AGCGAGTT	TACGGCGA	TCGCCGTA	76	D10	AAGGCTTA	TGCCTCGA	TCGAGGCA
53	D7	TGTATCAC	AGCGAGTT	AACTCGCT	77	D10	GCAATGAA	AAGGCTTA	TAAGCCTT
54	D7	GATCGCCT	TGTATCAC	GTGATACA	78	D10	AAGAACCT	GCAATGAA	TTCATTGC
55	D7	GACTCAAT	GATCGCCT	AGGCGATC	79	D10	CTGTGCCT	AAGAACCT	AGGTTCTT
56	D7	CAGCTTGC	GACTCAAT	ATTGAGTC	80	D10	TACGTAGC	CTGTGCCT	AGGCACAG
57	D8	AGCTGAAG	CAGCTTGC	GCAAGCTG	81	D11	AAGTGGAC	TACGTAGC	GCTACGTA
58	D8	ATTCCGTG	AGCTGAAG	CTTCAGCT	82	D11	CAACCGTG	AAGTGGAC	GTCCACTT
59	D8	TATGCCGC	ATTCCGTG	CACGGAAT	83	D11	CTGTTGTT	CAACCGTG	CACGGTTG
60	D8	TCAGCTCA	TATGCCGC	GCGGCATA	84	D11	GCACGATG	CTGTTGTT	AACAACAG
61	D8	AACTGCAA	TCAGCTCA	TGAGCTGA	85	D11	GTACGGAC	GCACGATG	CATCGTGC
62	D8	ATTAGGAG	AACTGCAA	TTGCAGTT	86	D11	CTCCAAGC	GTACGGAC	GTCCGTAC
63	D8	CAGCAATA	ATTAGGAG	CTCCTAAT	87	D11	TAGTCTGA	CTCCAAGC	GCTTGGAG
64	D8	GCCAAGCT	CAGCAATA	TATTGCTG	88	D11	TTCGCCGT	TAGTCTGA	TCAGACTA
65	D9	TCCGTTAA	GCCAAGCT	AGCTTGGC	89	D12	GAACTAAG	ATACGAAG	CTTCGTAT
66	D9	GTGCAACG	TCCGTTAA	TTAACGGA	90	D12	AAGCCATC	GAGATTCA	TGAATCTC
67	D9	AGTAACGC	GTGCAACG	CGTTGCAC	91	D12	AACTCTTG	AAGCCATC	GATGGCTT
68	D9	CATAGCCA	AGTAACGC	GCGTTACT	92	D12	GTAGTCAT	AACTCTTG	CAAGAGTT
69	D9	CACTAGTA	CATAGCCA	TGGCTATG	93	D12	CTCGCTAG	GTAGTCAT	ATGACTAC
70	D9	TTAGTGCG	CACTAGTA	TACTAGTG	94	D12	AGTCTTCA	CAGTATCA	TGATACTG
71	D9	TCGATACA	TTAGTGCG	CGCACTAA	95	D12	TCAAGCTA	CTTCGTAC	GTACGAAG
72	D9	ATAGTGAC	TCGATACA	TGTATCGA	96	D12	CTTATCCT	TCAAGCTA	TAGCTTGA

Table 30 SureSelect XT HS2 Index Primer Pairs 97–144

Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement	Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement
97	D13	TCATCCTT	CTTATCCT	AGGATAAG	121	D16	CAGGCAGA	AGACGCCT	AGGCGTCT
98	D13	AACACTCT	TCATCCTT	AAGGATGA	122	D16	TCCGCGAT	CAGGCAGA	TCTGCCTG
99	D13	CACCTAGA	AACACTCT	AGAGTGTT	123	D16	CTCGTACG	TCCGCGAT	ATCGCGGA
100	D13	AGTTCATG	CACCTAGA	TCTAGGTG	124	D16	CACACATA	CTCGTACG	CGTACGAG
101	D13	GTTGGTGT	AGTTCATG	CATGAACT	125	D16	CGTCAAGA	CACACATA	TATGTGTG
102	D13	GCTACGCA	GTTGGTGT	ACACCAAC	126	D16	TTCGCGCA	CGTCAAGA	TCTTGACG
103	D13	TCAACTGC	GCTACGCA	TGCGTAGC	127	D16	CGACTACG	TTCGCGCA	TGCGCGAA
104	D13	AAGCGAAT	TCAACTGC	GCAGTTGA	128	D16	GAAGGTAT	CGACTACG	CGTAGTCG
105	D14	GTGTTACA	AAGCGAAT	ATTCGCTT	129	D17	TTGGCATG	GAAGGTAT	ATACCTTC
106	D14	CAAGCCAT	GTGTTACA	TGTAACAC	130	D17	CGAATTCA	TTGGCATG	CATGCCAA
107	D14	CTCTCGTG	CAAGCCAT	ATGGCTTG	131	D17	TTAGTTGC	CGAATTCA	TGAATTCG
108	D14	TCGACAAC	CTCTCGTG	CACGAGAG	132	D17	GATGCCAA	TTAGTTGC	GCAACTAA
109	D14	TCGATGTT	TCGACAAC	GTTGTCGA	133	D17	AGTTGCCG	GATGCCAA	TTGGCATC
110	D14	CAAGGAAG	TCGATGTT	AACATCGA	134	D17	GTCCACCT	AGTTGCCG	CGGCAACT
111	D14	ATTGATGC	AGAGAATC	GATTCTCT	135	D17	ATCAAGGT	GTCCACCT	AGGTGGAC
112	D14	TCGCAGAT	TTGATGGC	GCCATCAA	136	D17	GAACCAGA	ATCAAGGT	ACCTTGAT
113	D15	GCAGAGAC	TCGCAGAT	ATCTGCGA	137	D18	CATGTTCT	GAACCAGA	TCTGGTTC
114	D15	CTGCGAGA	GCAGAGAC	GTCTCTGC	138	D18	TCACTGTG	CATGTTCT	AGAACATG
115	D15	CAACCAAC	CTGCGAGA	TCTCGCAG	139	D18	ATTGAGCT	TCACTGTG	CACAGTGA
116	D15	ATCATGCG	CAACCAAC	GTTGGTTG	140	D18	GATAGAGA	ATTGAGCT	AGCTCAAT
117	D15	TCTGAGTC	ATCATGCG	CGCATGAT	141	D18	TCTAGAGC	GATAGAGA	TCTCTATC
118	D15	TCGCCTGT	TCTGAGTC	GACTCAGA	142	D18	GAATCGCA	TCTAGAGC	GCTCTAGA
119	D15	GCGCAATT	TCGCCTGT	ACAGGCGA	143	D18	CTTCACGT	GAATCGCA	TGCGATTC
120	D15	AGACGCCT	GCGCAATT	AATTGCGC	144	D18	CTCCGGTT	CTTCACGT	ACGTGAAG

Table 31 SureSelect XT HS2 Index Primer Pairs 145–192

Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement	Primer Pair #	Index Strip	P7 Index Forward	P5 Index Forward	P5 Index Reverse Complement
145	D19	TGTGACTA	CTCCGGTT	AACCGGAG	169	D22	CGCTCAGA	CTAACAAG	CTTGTTAG
146	D19	GCTTCCAG	TGTGACTA	TAGTCACA	170	D22	TAACGACA	CGCTCAGA	TCTGAGCG
147	D19	CATCCTGT	GCTTCCAG	CTGGAAGC	171	D22	CATACTTG	TAACGACA	TGTCGTTA
148	D19	GTAATACG	CATCCTGT	ACAGGATG	172	D22	AGATACGA	CATACTTG	CAAGTATG
149	D19	GCCAACAA	GTAATACG	CGTATTAC	173	D22	AATCCGAC	AGATACGA	TCGTATCT
150	D19	CATGACAC	GCCAACAA	TTGTTGGC	174	D22	TGAAGTAC	AATCCGAC	GTCGGATT
151	D19	TGCAATGC	CATGACAC	GTGTCATG	175	D22	CGAATCAT	TGAAGTAC	GTACTTCA
152	D19	CACATTCG	TGCAATGC	GCATTGCA	176	D22	TGATTGGC	CGAATCAT	ATGATTCG
153	D20	CAATCCGA	CACATTCG	CGAATGTG	177	D23	TCGAAGGA	TGATTGGC	GCCAATCA
154	D20	CATCGACG	CAATCCGA	TCGGATTG	178	D23	CAGTCATT	TCGAAGGA	TCCTTCGA
155	D20	GTGCGCTT	CATCGACG	CGTCGATG	179	D23	CGCGAACA	CAGTCATT	AATGACTG
156	D20	ATAGCGTT	GTGCGCTT	AAGCGCAC	180	D23	TACGGTTG	CGCGAACA	TGTTCGCG
157	D20	GAGTAAGA	ATAGCGTT	AACGCTAT	181	D23	AGAACCGT	TACGGTTG	CAACCGTA
158	D20	CTGACACA	GAGTAAGA	TCTTACTC	182	D23	AGGTGCTT	AGAACCGT	ACGGTTCT
159	D20	ATACGTGT	CTGACACA	TGTGTCAG	183	D23	ATCGCAAC	AGGTGCTT	AAGCACCT
160	D20	GACCGAGT	ATACGTGT	ACACGTAT	184	D23	GCCTCTCA	ATCGCAAC	GTTGCGAT
161	D21	GCAGTTAG	GACCGAGT	ACTCGGTC	185	D24	TCGCGTCA	GCCTCTCA	TGAGAGGC
162	D21	CGTTCGTC	GCAGTTAG	CTAACTGC	186	D24	GAGTGCGT	TCGCGTCA	TGACGCGA
163	D21	CGTTAACG	CGTTCGTC	GACGAACG	187	D24	CGAACACT	GCATAAGT	ACTTATGC
164	D21	TCGAGCAT	CGTTAACG	CGTTAACG	188	D24	TAAGAGTG	AGAAGACG	CGTCTTCT
165	D21	GCCGTAAC	TCGAGCAT	ATGCTCGA	189	D24	TGGATTGA	TAAGAGTG	CACTCTTA
166	D21	GAGCTGTA	GCCGTAAC	GTTACGGC	190	D24	AGGACATA	TGGATTGA	TCAATCCA
167	D21	AGGAAGAT	GAGCTGTA	TACAGCTC	191	D24	GACATCCT	AGGACATA	TATGTCCT
168	D21	CTAACAAG	AGGAAGAT	ATCTTCCT	192	D24	GAAGCCTC	GACATCCT	AGGATGTC

Post-Run Tracking of Index Identity

The specific Index Strip used for a Magnis Prep System run is reported in the **Post-Run Data**, accessible from the touchscreen Home screen. From the **Post-Run Data** screen, open the **Labware Info** tab, and under *Labware*, locate the *Index Strip* row to view various properties of the index strip used for the run. The Index Strip number, reported as a 1–24 value, can be viewed by scrolling to the right-most part of the screen, and looking in the *Index Strip* column. The equivalent Index Strip number of 1–24 can also be found in the run log file.

The specific SureSelect XT HS2 dual indexing primer pairs associated with each Index Strip number 1–24 are shown in Table 32.

Table 32 Use of Index Strip numbers from Post-Run Data for Index tracking

Index Strip **Number from Post-Run Data Screen or Log**	**Index Strip Tube Label (Inscription)**	**Dual Index Primer Pair by Sample Number in Run**							
		Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
1	D1	1	2	3	4	5	6	7	8
2	D2	9	10	11	12	13	14	15	16
3	D3	17	18	19	20	21	22	23	24
4	D4	25	26	27	28	29	30	31	32
5	D5	33	34	35	36	37	38	39	40
6	D6	41	42	43	44	45	46	47	48
7	D7	49	50	51	52	53	54	55	56
8	D8	57	58	59	60	61	62	63	64
9	D9	65	66	67	68	69	70	71	72
10	D10	73	74	75	76	77	78	79	80
11	D11	81	82	83	84	85	86	87	88
12	D12	89	90	91	92	93	94	95	96
13	D13	97	98	99	100	101	102	103	104
14	D14	105	106	107	108	109	110	111	112
15	D15	113	114	115	116	117	118	119	120
16	D16	121	122	123	124	125	126	127	128
17	D17	129	130	131	132	133	134	135	136
18	D18	137	138	139	140	141	142	143	144
19	D19	145	146	147	148	149	150	151	152
20	D20	153	154	155	156	157	158	159	160
21	D21	161	162	163	164	165	166	167	168
22	D22	169	170	171	172	173	174	175	176
23	D23	177	178	179	180	181	182	183	184
24	D24	185	186	187	188	189	190	191	192

Troubleshooting Guide

Troubleshooting guidelines are included below for running the automated SureSelect XT HS2 RNA NGS Library Preparation protocols on the Magnis NGS Prep System and for the upstream sample preparation and downstream library analysis steps. For general Magnis instrument troubleshooting, see the instrument User Guide, publication K1007-90000.

If touchscreen appears unresponsive

✓Reboot the system to reset touchscreen functionality.

If instrument is running firmware version prior to 1.4 and update is required for RNA protocol

The Magnis instrument must be running firmware version 1.4 or later before initiating the *SSEL-RNA-XTHS2-ILM* protocol. To verify the firmware version: From the **Home** screen, press **Settings** > **System Settings** > **Instrument Settings**, then verify that the *Firmware Version* field shows version 1.4 or later. If a firmware update is required, use one of the methods below.

✓If the instrument is internet-connected, users with an *Advanced* user account can install new firmware versions directly using the touchscreen.

- From the *Home* screen, open the **Settings** screen and navigate to **System Settings** then **Firmware Update**.
- From the *Firmware Update* screen, press **Online Update** then select firmware version 1.4 (or later) from the list of available versions and press the **Update** button.

✓If the instrument is not internet-connected, *Advanced* users can install new firmware from a USB drive. Using an internet-connected PC, visit the Magnis Software Download page and download version 1.4 (or later) firmware to a USB drive. Follow the firmware installation instructions provided on this webpage.

If the instrument LED indicator lights turn red and touchscreen displays error message "*Teach points are shifted. Please perform auto teaching from the Settings screen*."

✓This error message appears when the Instrument Health Check (IHC) does not pass the teachpoint verification, indicating that the teachpoint markers on the instrument deck may be obscured or that the instrument needs to perform a teachpoint Auto Teaching routine before setting up a run. Complete the steps below to ready the instrument for a run:

- Verify that all Magnis deck positions are cleared of kit consumables and other debris. The presence of any materials on the instrument deck can prevent the successful detection of the teach point markers.
- Clean the barcode scanner window using the cleaning instructions in the instrument User Guide. Debris or fingerprints on the scanner can obscure the teach points, causing verification failure.
- Reboot the system. After login, the instrument will perform another IHC. If this health check is successful, you can resume the setup process without performing Auto Teaching. If the IHC is unsuccessful, complete Auto Teaching using the steps below.
- From the Home screen, open the **Settings** screen and press **Auto Teaching**. Follow the instructions on the touchscreen display. The Auto Teaching process requires approximately 30 minutes, and requires that an operator be present for placement of labware on the instrument.
- Once Auto Teaching is complete, you can begin run setup by pressing **Run Protocol** from the Home screen.

If the instrument LED indicator lights turn red and touchscreen displays an Instrument Health Check (IHC) failure message

✓Agilent recommends restarting the instrument after IHC failure using the steps below:

- From the error dialog, press **Cancel** to decline initiation of diagnostic testing.
- Press the error icon at the bottom of the screen and record the error code for potential use in troubleshooting with Agilent Support.
- Turn off the instrument by pressing the power button on the front of the instrument.
- Verify that all Magnis deck positions are cleared of kit consumables and other debris. The presence of any materials on the instrument deck can interfere with the IHC upon restart.
- Turn on the instrument by pressing the power button on the front of the instrument.
- After login, the instrument will perform another IHC. If this health check is successful, you can begin or restart run setup by pressing **Run Protocol** from the Home screen.

If the IHC fails again after the instrument is restarted, contact Agilent Worldwide Technical Support for assistance.

If a protocol is missing from the Protocol menu on the *Enter Run Info* screen

✓The Magnis run protocols visible on the touchscreen *Enter Run Info* screen and available to run on your instrument may vary, depending on date of instrument purchase, protocol availability date, and whether any post-purchase updates have been made on your instrument. If you need a protocol that is not currently available on your instrument, visit the Magnis protocol download page at Agilent.com for more information.

If seating of strip tubes in chiller module is difficult

✓To facilitate proper seating of strip tubes in the chiller module, load the strips in left-to-right order (filled sample strip, index strip, probe strip, RNA reagent strip, empty QC strip and empty library strip).

✓Improperly-placed foil seals can obstruct strip tube positioning and seating when loading the chiller. When re-sealing the sample input strip or a self-filled probe strip with a foil seal, take care to apply the seal firmly and evenly, without excessive overhangs or creases.

If the *Verify Labware* screen reports an issue with one or more labware components after scanning the labware barcodes

✓If all or most of the labware failed verification, then the barcode scanner window may require cleaning. See the instrument User Guide for cleaning instructions. Once cleaning is completed, repeat the *Verify Labware* step.

✓If only one or a few labware components failed verification, then press the error icon at the bottom of the screen and expand the information for the failed position to view the reason for the failure.

- **If the barcode scanner failed to scan a particular labware component**

  Verify that the labware is present at the required deck position and oriented correctly, with the barcode facing the front of the instrument. Review pages page 30 to page 35 for complete deck loading steps. Correct the omission or positioning error(s) and then repeat the *Verify Labware* step. If the failed labware components are present and correctly positioned, then visually inspect the barcode to verify integrity. For successful scanning, barcodes must be free of scratches, smudges, condensation, obstruction by foil seals, and writing or other marks on the plasticware. If barcode damage or obstruction is suspected, adjust or replace the labware component and repeat the *Verify Labware* step.

- **If the scanned labware is past its expiration date**

  Replace any expired components with unexpired components then repeat the *Verify Labware* step. The expiration date can be found on the Certificate of Analysis provided with each component kit containing pre-filled reagents. Components provided as empty plasticware do not have an expiration date.

- **If scanned labware components are identified as *wrong labware***

  When scanned labware (e.g., the Beads/Buffers Plate or the Probe Input Strip) is identified as *wrong labware*, it is important to verify that the correct protocol was selected for the format of the Reagent Kit loaded on the instrument. Check the format of the Reagent Kit loaded for the run, then use the table below to verify that the correct protocol was selected during run setup. If an incorrect protocol was selected, return to the *Enter Run Info* screen by pressing the backward arrow on the touchscreen and select the correct protocol from the menu, expanding the protocol menu if required. After selecting the correct protocol, use the forward arrow keys to advance back to the *Verify Labware* screen, then repeat the *Verify Labware* step.

Reagent Kit	Correct Processing Protocol
Magnis SureSelect XT HS2 **RNA** Reagent Kits supplied with Magnis SureSelect **RNA** Beads/Buffers Plates ILM and **pre-filled probe input strips**	*SSEL-RNA-XTHS2-ILM*
Magnis SureSelect HS2 **RNA** Reagent Kits supplied with Magnis SureSelect **RNA** Beads/Buffers Plates ILM and **empty probe input strips** (filled at run time)	*SSEL-RNA-XTHS2-EPIS-ILM*
Magnis SureSelect HS2 **DNA** Reagent Kits supplied with Magnis SureSelect XT HS Beads/Buffers Plates ILM and pre-filled or empty probe input strips	*SSEL-DNA-XTHS2-ILM* or *SSEL-DNA-XTHS2-EPIS-ILM*

- **If labware was identified as the *wrong labware*, with correct protocol selected**

  Replace the misplaced labware with the correct labware and repeat the *Verify Labware* step.

If an unattached micropipettor tip is sitting on the instrument deck during run

✓ Occasionally, when the instrument ejects used tips into the waste container, a tip may bounce out and land on the instrument deck. With a gloved hand, move the tip to the waste container or dispose of it as you would when emptying the waste container.

If the touchscreen *Turn off Chiller* dialog obscures the run screen after the instrument door is opened and libraries are collected at end of run

✓ If the instrument door is opened at the end of the run before the LED indicator lights turn blue (indicating completion of all instrument run steps) or if the instrument door is only partially opened at the end of the run, the *Turn off Chiller* dialog may be retained on the run screen, obscuring the screen content. In future runs, wait for the LED indicator lights to turn blue, indicating that the instrument has reached a post-run idle state, before opening the instrument door. Open the door fully (until LED indicator lights turn white) before collecting your samples.

If the touchscreen *Time Remaining* display does not read 0:00 immediately before proceeding to completed run/sample collection screens

✓ The **Time Remaining** value displayed on the touchscreen is only an estimate of time left in the run. The counter may adjust the remaining time estimate during the run and may display time greater than 0:00 when the system is ready to begin sample collection. This is not indicative of an issue with the run or the instrument.

If volume of final library sample is lower than expected

✓ At the end of the run, the final library samples (20–23 µL) are transferred to wells of the green Library Output Strip and held in the chiller at 12°C until removed from the instrument. The duration and the relative humidity conditions of the post-run chiller hold can affect the volume recovered from the Library Output Strip.

If yield of post-capture library is low

✓ Verify that the input RNA sample meets the guidelines for quality and concentration range specified in "Appendix 1: RNA Sample Preparation Guidelines"

✓ Verify that the run was set up for the appropriate input RNA concentration and quality. Settings may be checked on the **Run Setup** tab of the **Post Run Data** screen for the run.

✓ Verify that the instrument is running firmware version 1.4 or later, as described on page 18. Running the *SSEL-RNA-XTHS2-ILM* protocol using instrument firmware versions prior to 1.4 results in erroneous run conditions leading to poor yield or poor sequencing performance.

✓ Ensure that runs are completed in humidity conditions of 30% to 70% (non-condensing). Operating the system at humidity levels outside of this range can impact performance and result in lower or zero library yield.

✓ Very low or zero yield for one or more samples in the run may indicate an issue with the pipette tips used in the run. While loading tips on the instrument, verify that all tip boxes are completely filled and that all tip boxes are seated flat and within the raised-tab frames of the platforms. Make sure that the tip boxes are not disturbed and unseated while removing the tip box lids.

✓ Very low or zero yield for one or more samples in the run may also result from an issue with plasticware positioning or seating for the run. Review and adhere to the plasticware positioning details provided on page 30 to page 34. In particular, make sure that all strip tubes are properly seated in the chiller module; this is facilitated by loading strips in left-to-right order (filled sample input strip, index strip, probe strip, RNA reagent strip, empty QC strip and empty library output strip).

✓ PCR cycle number may require optimization. Repeat library preparation and target enrichment for the sample, increasing the post-capture PCR cycle number by 1 to 2 cycles. Only users with *Advanced* access level can change the post-capture PCR cycle number. See page 39 for more information.

If pre-capture PCR optimization needed

✓ When required the pre-capture PCR cycle number used in a run may be adjusted on the *Confirm Setup* screen. See page 39 for more information. Only users with *Advanced* access level can change the cycle number value. Use the guidelines below as a starting point for cycle number self-optimization.

Input RNA Quantity	Recommended Pre-Capture PCR Cycles		
	Intact Input RNA	Good FFPE-derived Input RNA	Poor FFPE-derived Input RNA
10 ng	14	17	Not supported
50 ng	13	16	17
100 ng	12	15	16
200 ng	11	14	15

If post-capture PCR optimization needed

✓ When required the post-capture PCR cycle number used in a run may be adjusted on the *Confirm Setup* screen. See page 39 for more information. Only users with *Advanced* access level can change the cycle number value. Use the guidelines below as a starting point for cycle number self-optimization.

SureSelect XT HS Probe Design Size	Recommended Post-Capture PCR Cycles
<200 kb	15
200–599 kb	14
600–2999 kb	13
3–5 Mb	11
>5 Mb	10

✓ The initial RNA fragment size of FFPE-derived samples may impact the final library fragment size distribution. Libraries prepared from more degraded FFPE samples may include a greater proportion of smaller library fragments and for some FFPE-derived samples final fragment size may not be increased by reducing the duration of fragmentation.

If sequencing reads do not cover the expected genomic regions

✓ The wrong probe design may have been used in the protocol run for target enrichment. Review the sample and probe tracking that was recorded during the run. Repeat the protocol run with the correct probe design, if necessary.

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In This Book

This guide provides instructions for automated preparation of SureSelect XT HS2 RNA target-enriched Illumina paired-end multiplexed sequencing libraries using the Magnis NGS Prep System.

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