Agilent

SureSelect$^{XT\ HS}$ Target Enrichment using the Magnis NGS Prep System

Protocol

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Version D0, October 2021

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In This Guide…

This guide provides instructions for automated preparation of SureSelect$^{XT\ HS}$ target-enriched Illumina paired-end multiplexed sequencing libraries using the Magnis NGS Prep System.

The SureSelect$^{XT\ HS}$ system is used to prepare indexed library samples with molecular barcodes prior to target enrichment to allow high-sensitivity sequencing on the Illumina platform.

1 **Before You Begin**

2 **Sequencing Library Preparation using the Magnis NGS Prep System**

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

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What's New in Version D0

- Support for Magnis protocols *SSEL XTHS-EPIS-RevB-ILM* and *LT-SSEL XTHS-EPIS-RevB-ILM*, set up using a Magnis SureSelect XT HS Rev B Reagent Kit (PN G9730D), supplied with empty probe input strips (EPIS). See "Appendix 2: Use of Run-Time Prepared Probe Strips" on page 50 to page 53 for detailed probe input strip setup instructions for this protocol. For information on Reagent Kit PN G9730D, see Table 1 on page 11 and Table 16 on page 67. For information on Magnis protocol and reagent kit format compatibility, see Table 7 on page 26 and troubleshooting information on page 77.
- Support for Magnis SureSelect XT HS Rev B Reagent Kits PN G9736C and G9736D, supplied with custom 24–50 Mb probe plates, and updates to additional part number and naming information for the probe plates supplied with Magnis SureSelect XT HS Rev B Reagent Kits. See Table 1 on page 11, Table 16 and Table 17 on page 67, Table 18 on page 68, and see step 5 on page 24.
- Updates to probe plate descriptions for Magnis SureSelect XT HS Reagent Kits (original format). See Table 25 on page 70.
- Updates to touchscreen interface images on page 27, page 37, page 38, page 39 and page 40.
- Updates to Illumina sequencing kit guidelines in Table 15 on page 59.
- Updates to troubleshooting information on the Magnis touchscreen Time Remaining display (see page 77).

What's New in Version C0

- Support for use of the *LT-SSEL XTHS-RevB-ILM* protocol with Magnis SureSelect XT HS Rev B Reagent Kits. For information on how to select the protocol compatible with your reagent kit format and other workflow parameters during run setup see page 26. Also see *Troubleshooting* on page 77.
- The Magnis SureSelect XT HS Rev B Reagent Kit supplied with empty probe input strips, p/n G9730D, is not currently available; see updates to lists of supported Magnis Reagent Kits in Table 1 on page 11 and Table 16 on page 67. Information on use of the *SSEL XTHS-EPIS-RevB-ILM* protocol for processing runs set up with the G9730D Reagent Kit was removed from Table 7 on page 26 and from "Appendix 2: Use of Run-Time Prepared Probe Strips" on page 50 to page 53. Visit Agilent.com for updates on Magnis Reagent Kit and protocol availability.

What's New in Versions B1 and B0

- Support for Magnis SureSelect XT HS Rev B Reagent Kits, processed using the *SSEL XTHS-RevB-ILM* protocol. The *Rev B* reagent kits include reformatted components (*Magnis SureSelect XT HS Reagent Plates Rev B ILM* and a *Magnis SureSelect XT HS Probe Plate, Pre-filled Single Well Format)* which must be processed using a *RevB* protocol. See page 67 through page 69 for Magnis SureSelect XT HS Rev B Reagent Kit component information. The instructions in this document also support sample processing using the original reagent kit format. See page 70 through page 71 for Magnis SureSelect XT HS Reagent Kit (original format) component information. See Table 1 on page 11 for a summary list of all supported Reagent Kits. For current Reagent Plate and Probe Plate setup instructions see page 23 to page 24. For information on how to select the protocol compatible with your reagent kit format during run setup see page 26.
- Support for custom probes produced with updated design and manufacturing processes beginning August 2020. Probes for all new custom designs are produced using the updated

process. Probes for existing custom designs, created before August 2020, are produced using the legacy manufacturing process. See Table 18 on page 67 for information on probes supplied with Magnis SureSelect XT HS Rev B Reagent Kits. See Table 25 on page 69 for information probes supplied with Magnis SureSelect XT HS Reagent Kits in the original format.

- Support for Magnis SureSelect XT HS Rev B Reagent Kits with Human All Exon V8 Probe (Table 1 on page 11, Table 16 on page 67, and Table 18 on page 67).
- Updates to kit component handling instructions on page 22 to page 23 including Reagent Plate thawing instructions and handling details for foil or adhesive covers and plate sleeves.
- Minor updates to chiller module loading instructions on page 32 to page 33.
- Addition of hygrometer device to list of Required Equipment in Table 2 on page 12 and addition of humidity measurement step to the instrument setup instructions on page 20.
- Updates to ordering information for swinging bucket centrifuge (Table 2 on page 12) and for 1X Low TE Buffer and Qubit Fluorometer (Table 3 on page 13).
- Support for library QC using Agilent 5200 Fragment Analyzer (see footnote to Table 3 on page 13 and *Note* on page 55)
- Updates to "Appendix 2: Use of Run-Time Prepared Probe Strips" on page 50 to page 53. Updates include support for the *SSEL XTHS-EPIS-RevB-ILM* protocol, set up using a Magnis SureSelect XT HS Rev B Reagent Kit supplied with empty probe input strips (EPIS).
- Updates to *Troubleshooting* on page 76 including information on protocol access, strip tube seating and *Verify Labware* indicators of mismatched protocol and labware.
- Update to page 9 to include description of compatible sequencing platforms and updates to Illumina sequencing kit guidelines in Table 15 on page 59.
- Removal of ethylene glycol ordering information from Table 6 on page 14. See page 43 and page 48 for related updates to DNA shearing setup instructions.
- Addition of D1000 ScreenTape assay ordering information to Table 6 on page 14.
- Update to Technical Support contact information (see page 2).

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

Overview of the Workflow

The workflow for SureSelect$^{XT\ HS}$ target enrichment using the Magnis NGS Prep System is summarized in Figure 1. Once the sheared genomic DNA samples and the pre-plated reagents and labware are loaded, the Magnis NGS Prep System performs all SureSelect$^{XT\ HS}$ 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 HiSeq, MiSeq, NextSeq 500 or NovaSeq 6000 sequencers.

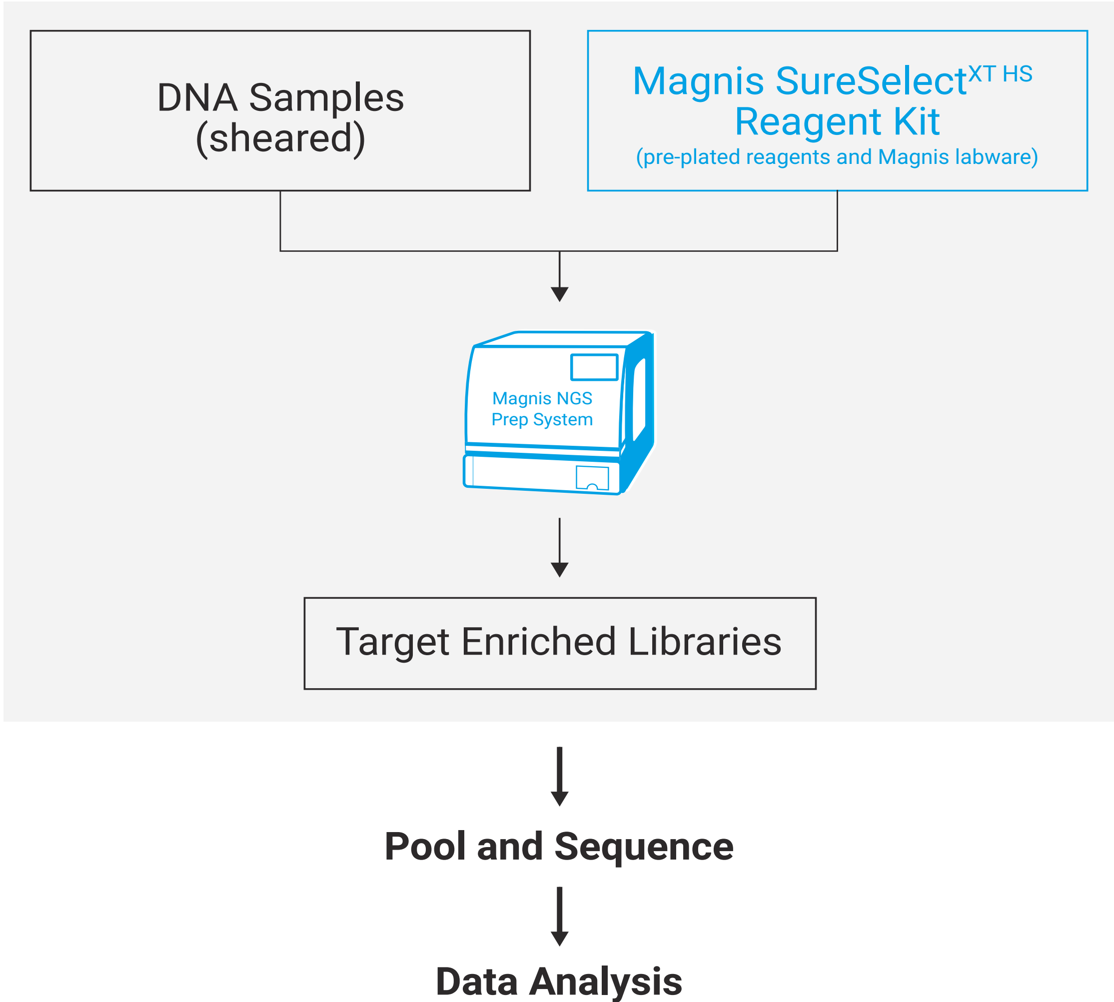

Figure 1 Overall Magnis NGS Prep System NGS sample preparation workflow.

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 HS Magnis Prep System Runs

Table 1 Supported Reagent Kits (select one)

Description	96 Reactions*	32 Reactions†
Magnis SureSelect XT HS Rev B Reagent Kit:	Agilent	Agilent
with Tier 1 (1–499 kb) Probe	p/n G9731D	p/n G9731C
with Tier 2 (0.5–2.9 Mb) Probe	p/n G9732D	p/n G9732C
with Tier 3 (3–5.9 Mb) Probe	p/n G9733D	p/n G9733C
with Tier 4 (6–11.9 Mb) Probe	p/n G9734D	p/n G9734C
with Tier 5 (12–24 Mb) Probe	p/n G9735D	p/n G9735C
with 24–50 Mb Probe	p/n G9736D	p/n G9736C
with Human All Exon V7 Probe	p/n G9771D	p/n G9771C
with Human All Exon V8 Probe	p/n G9772D	p/n G9772C
with empty Magnis Probe Input Strips‡	p/n G9730D	Not offered
For a list of kit contents, see page 67 to page 69.		
OR		
Magnis SureSelect XT HS Reagent Kit (original format):	Agilent	Agilent
with 1–499 kb Probe	p/n G9731B	p/n G9731A
with 0.5–2.9 Mb Probe	p/n G9732B	p/n G9732A
with 3–5.9 Mb Probe	p/n G9733B	p/n G9733A
with 6–11.9 Mb Probe	p/n G9734B	p/n G9734A
with 12–24 Mb Probe	p/n G9735B	p/n G9735A
with Human All Exon V7 Probe	p/n G9771B	p/n G9771A
with empty Magnis Probe Input Strips‡	p/n G9730B	Not offered
For a list of kit contents, see page 70 to page 71.		

* 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 51 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 G9710AA
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 (2-, 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 HS Reagent Kits and the protocols detailed in this publication are also compatible with the Magnis Dx NGS Prep System (p/n K1007AA).

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

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

Required Materials for DNA Sample Preparation and Analysis

Table 3 Required Materials for DNA Sample Preparation and Analysis--All Sample Types

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
Qubit BR dsDNA Assay Kit 100 assays 500 assays	Thermo Fisher Scientific p/n Q32850 p/n Q32853
Qubit Fluorometer	Thermo Fisher Scientific p/n Q33238
Qubit Assay Tubes	Thermo Fisher Scientific p/n Q32856
Covaris Sample Preparation System	Covaris model E220
Covaris microTUBE sample holders	Covaris p/n 520045
DNA Analysis System and Consumables:* Agilent 4150 TapeStation **OR** Agilent 4200 TapeStation **AND** TapeStation-compatible 8-well tube strips 8-well tube strip caps High Sensitivity D1000 ScreenTape High Sensitivity D1000 Reagents	 Agilent p/n G2992AA Agilent p/n G2991AA Agilent p/n 401428 Agilent p/n 401425 Agilent p/n 5067-5584 Agilent p/n 5067-5585

* The Agilent 2100 Bioanalyzer (p/n G2939BA) and High Sensitivity DNA Kit (p/n 5067-2646) or the Agilent 5200 Fragment Analyzer (p/n M5310AA) and HS NGS Fragment Kit (p/n DNF-474-0500) may also be used for library DNA analysis.

Table 4 Required Materials--High-Quality DNA Samples Only

Description	Vendor and part number
High-quality gDNA purification system, for example: QIAamp DNA Mini Kit 50 Samples 250 Samples	 Qiagen p/n 51304 p/n 51306

Table 5 Required Materials--FFPE DNA Samples Only

Description	Vendor and part number
FFPE gDNA purification system, for example: QIAamp DNA FFPE Tissue Kit, 50 Samples Deparaffinization Solution	Qiagen p/n 56404 p/n 19093
FFPE DNA integrity assessment system: Agilent NGS FFPE QC Kit (recommended method) 16 reactions 96 reactions **OR** TapeStation Genomic DNA analysis assay:* Genomic DNA ScreenTape Genomic DNA Reagents	 Agilent p/n G9700A p/n G9700B Agilent p/n 5067-5365 p/n 5067-5366

* Agilent's 4150 TapeStation or 4200 TapeStation, and compatible plasticware, are also required. See Table 3 above for ordering information.

Optional Materials

Table 6 Supplier Information for optional materials in protocols

Description	Purpose	Vendor and part number
Dilute bleach (10%) wipes	Surface-cleaning of instrument deck (see page 20)*	Hype-Wipe Bleach Towelettes (VWR p/n 16200-218), or equivalent
Alcohol (70%) wipes	Surface-cleaning of instrument deck (see page 20)*	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
D1000 ScreenTape and D1000 Reagents	Analysis of optional pre-capture library QC samples using Agilent 4200/4150 TapeStation system (see page 40)	Agilent p/n 5067-5582 and p/n 5067-5583
Tween 20	Sequencing library storage (see page 57)	Sigma-Aldrich p/n P9416-50ML

* 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
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This chapter contains instructions for SureSelect$^{XT\ HS}$ target-enriched DNA sequencing library preparation using the Magnis NGS Prep System. For an overview of the workflow, see Figure 1 on page 9.

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 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 22.

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 17 to page 18.

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

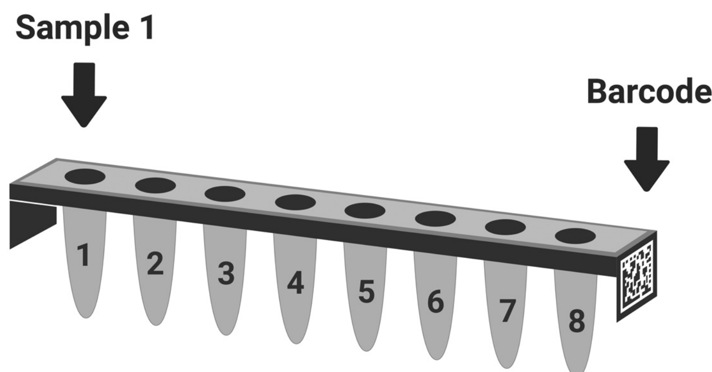


Figure 2 Required orientation of sample numbers 1 through 8 in the Magnis Sample Input Strip.

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 36. 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

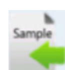
	A	B
1	sample_id	
2	HD18060701	
3	HD18060702	
4	HD18060703	
5	HD18060704	
6	HD18060705	
7	HD18060706	
8	HD18060707	
9	HD18060708	
10		

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 disk.
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 disk.



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.

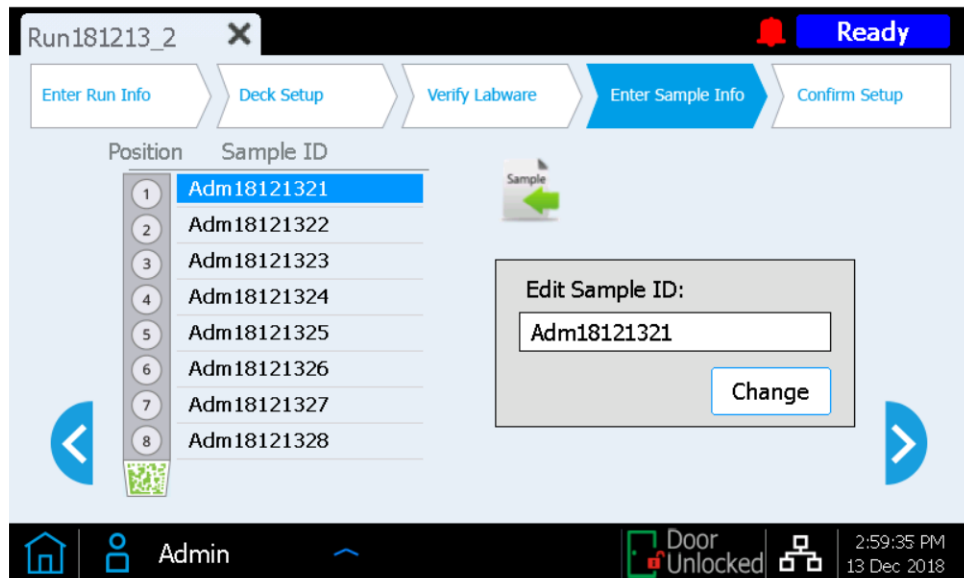


Figure 4 Magnis touchscreen interface used for manual sample assignment during a run.

Preparing Your DNA Samples for the Run

The library preparation protocol is compatible both with high-quality gDNA prepared from fresh or fresh-frozen samples and with lower-quality DNA prepared from FFPE samples. Runs to process either high-quality or FFPE-derived DNA can include 10 ng, 50 ng, 100 ng or 200 ng of input DNA. For optimal sequencing results, use the maximum amount of input DNA available within this range. All samples in the same run must be provided in the same quantity.

Before setting up the Magnis run, DNA samples must be prepared, quantified, qualified, and sheared, using the guidelines and protocols in "Appendix 1: DNA Sample Preparation Guidelines" on page 42. Some parts of the DNA sample preparation protocol, especially qualification of FFPE-derived samples, may need to be completed up to a day prior to initiating the Magnis run steps. DNA samples must, however, be sheared and dispensed into the input DNA sample strip tube immediately before use in the run.

Before starting the Magnis NGS Prep System setup steps on the following page, review the DNA sample preparation steps beginning on page 42 to ensure that the gDNA samples and the Covaris E220 instrument are ready for DNA shearing at run setup time.

NOTE Preparing the Covaris E220 instrument for DNA shearing requires approximately 30–60 minutes for chilling and degassing the water bath. Initiate these conditioning steps (see step 1 on page 43) before you start any of the Magnis NGS Prep System and reagent setup steps on the following pages.

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 system 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.

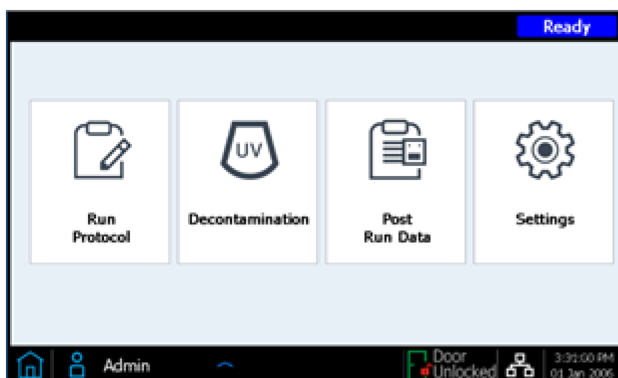
3 Turn on the instrument by pressing the power button on the front of the device. Close the instrument door.

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

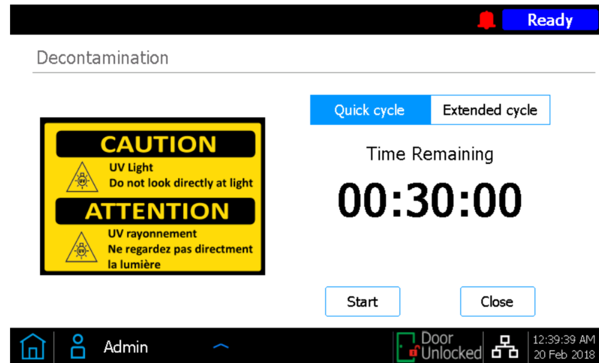
   Stand by as the system performs a series of start up activities, which may require several minutes.

4 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 22.

5 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$^{XT\ HS}$ reagents and plasticware

Plate and Strip Tube Handling Instructions

Familiarize yourself with the important labware handling instructions below before you start setting up the run.

- Magnis Sample Input Strips (red strips provided in plate format, p/n 5190-9882 or 5191-5676), along with all input DNA sample preparation and dilution reagents, should be stored and used only in pre-PCR areas of the laboratory. The empty Magnis Probe Input Strips (p/n 5190-9883, supplied only with kits G9730D and G9730B) should also be stored and filled in a pre-PCR area.
- 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 kit. Take care to avoid contamination or other damage to the replacement foil seals.
- Filled reagent plates (both Magnis SureSelect XT HS Beads/Buffers Plates and Magnis SureSelect XT HS 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 using the following procedure, illustrated in the pictures below. 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 Support for assistance.

Sample and Reagent Setup Steps

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

  a Transfer one Magnis SureSelect XT HS Beads/Buffers Plate from storage at +4°C to 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 HS Reagent Plate Rev B ILM** or **Magnis SureSelect XT HS Reagent Plate ILM** 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** to contain the DNA samples using the steps below.

   a Verify that the Covaris E220 instrument is ready for use in the DNA shearing step, with the water bath chilled to 5°C and degassed as detailed on page 43 or page 48.

   b 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 d.

   c Using the instructions appropriate to your DNA sample type, prepare the Magnis Sample Input Strip for the run. For high-quality DNA samples, follow the instructions on page 43 to page 45. For FFPE DNA samples, follow the instructions on page 46 to page 49.

   The final sample input strips must contain 50 µl of sheared DNA (10 ng, 50 ng, 100 ng or 200 ng) in each sample well, with all wells of the strip containing the same amount of DNA.

   d Once all samples have been placed in the Magnis Sample Input Strip wells, re-seal the strip tube with the fresh foil seal from step b, 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.

   e 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 DNA solution.

   f Keep the sample strip tube on ice until use on page 33.

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 strip tubes are inscribed with *A1*, *A2*, *A3*, or *A4* on the strip tube end opposite the barcode, to indicate the specific set of indexes contained in the wells (see page 72 for complete index information). If samples from different Magnis NGS library preparation runs will be multiplexed for NGS, each run must use a different set of indexes to ensure that all multiplexed samples are tagged with a unique index sequence.

   b Obtain the Magnis SureSelect XT HS Index Plate from storage at –20°C. Remove the appropriate black index strip (labeled *A1*, *A2*, *A3*, or *A4*) 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 33.

5 Prepare the **probe strip tube** using the steps below for kits supplied with either of the pre-filled probe plate formats listed below:

- *Magnis SureSelect Probe Plate, Pre-filled Single Well Format* or *Magnis SureSelect XT HS Probe Plate, Pre-filled Single Well Format* with full volume of probe solution for 8-sample run provided in well A (wells B through H are empty)
- *Magnis SureSelect XT HS Probe Plate*, with volume of probe solution for 8-sample run split between all 8 wells

If your kit does not include pre-filled probe strips (kit p/n G9730D or G9730B) and instead includes empty probe strips for run-time probe preparation, skip the instructions below and instead prepare the probe strip using the instructions on page 51.

a Obtain the Probe Plate from storage at –80°C. Remove one white probe strip (with "P" 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 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 strip well contents are thawed, 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 the strip wells to verify that the liquid is collected in the bottom of the well(s) and that no bubbles are present. Remove any bubbles by repeating the spin step until all bubbles are released.

NOTE The full volume of probe solution for the run is present in a single well for probe strips supplied with Rev B Reagent Kits and is apportioned to all 8 wells for probe strips supplied with Reagent Kits in the original format.

d Keep the probe strip on ice until use on page 33.

6 Obtain one Magnis Empty Consumables box from storage at room temperature (RT) for use during deck setup.

Proceed to "Running the Library Preparation Protocol" on page 25.

Running the Library Preparation Protocol

When the Magnis instrument and all reagents have been prepared for the SureSelect XT HS 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 26 to page 37.

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

When the run is complete, as indicated by emission of blue light from the LED indicator, the system touchscreen prompts you to remove the final sequencing library samples and QC samples (if included) from the instrument. Guidelines for processing the final target-enriched libraries for DNA sequencing are provided in "Appendix 3: Guidelines for Post-run DNA Sample Processing for NGS" on page 54.

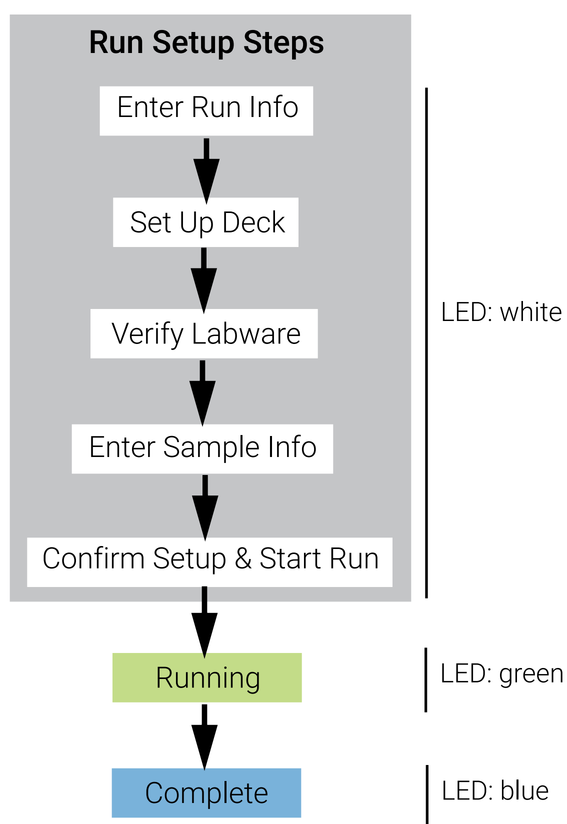
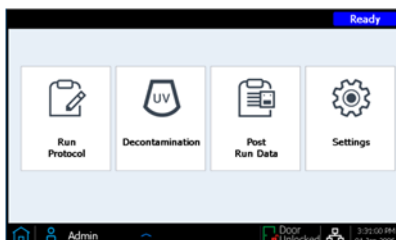


Figure 5 Overview of steps for Magnis NGS Prep System run setup and completion. The color of the light emitted by the instrument's LED indicator lights during these steps is shown at right.

Step 1. Initiate the protocol and Enter Run Info

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 the "Troubleshooting Guide" on page 75 for remediation guidelines.



2 Follow the prompts provided on the *Enter Run Info* screen as detailed below. On the first screen, specify the protocol name and QC collection settings for the run.

a Expand the **Protocol** menu, and select the protocol appropriate for your Reagent Kit format, as described in Table 7. Protocols visible on your touchscreen and available for use on your instrument may vary from the protocols listed in Table 7 (see *Troubleshooting* on page 76 for more information).

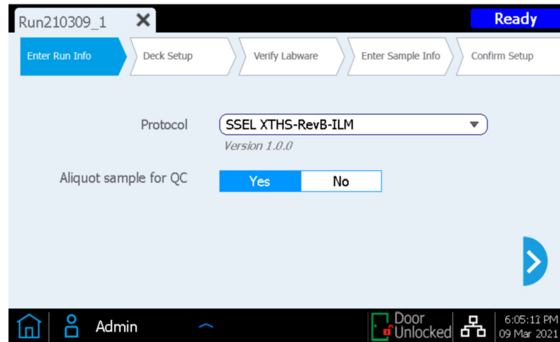
Table 7 Protocol Usage Information

Protocol Name	Compatible Reagent Kit(s)	Use Details
SSEL XTHS-RevB-ILM	Magnis SureSelect XT HS **Rev B** Reagent Kits supplied with **pre-filled probe input strips** (Pre-filled single well format)	This protocol provides optimal hybridization conditions for SureSelect Human All Exon V7 and V8 probes and for most custom SureSelect XT HS probe designs with the SureSelect XT HS Rev B Reagent Kits.
LT-SSEL XTHS-RevB-ILM	Magnis SureSelect XT HS **Rev B** Reagent Kits supplied with **pre-filled probe input strips** (Pre-filled single well format)	This protocol provides hybridization conditions equivalent to the *SureSelectXT HS-Illumina* protocol. Use is recommended for processing samples with Magnis SureSelect XT HS Rev B Reagent Kits while maintaining performance of workflows established using the *SureSelectXT HS-Illumina* protocol or when using custom probes originally designed for use with the SureSelect XT system.
SSEL XTHS-EPIS-RevB-ILM	Magnis SureSelect XT HS **Rev B** Reagent Kit supplied with **empty probe input strips** (PN G9730D)	Use to process Magnis SureSelect XT HS Rev B Reagent Kits supplied with empty probe input strips (EPIS) using hybridization conditions equivalent to the *SSEL XTHS-RevB-ILM* protocol. The probe input strip must be filled prior to the run as detailed on page 51.
LT-SSEL XTHS-EPIS-RevB-ILM	Magnis SureSelect XT HS **Rev B** Reagent Kit supplied with **empty probe input strips** (PN G9730D)	Use to process Magnis SureSelect XT HS Rev B Reagent Kits supplied with empty probe input strips (EPIS) using hybridization conditions equivalent to the *LT-SSEL XTHS-RevB-ILM* protocol. The probe input strip must be filled prior to the run as detailed on page 51.
SureSelectXT HS-Illumina	Magnis SureSelect XT HS Reagent Kits in **original format** supplied with either **pre-filled or empty probe input strips**	Magnis Reagent Kits supplied in the original format must be processed using this protocol. Where applicable, the empty probe input strip must be filled prior to the run as detailed on page 51.

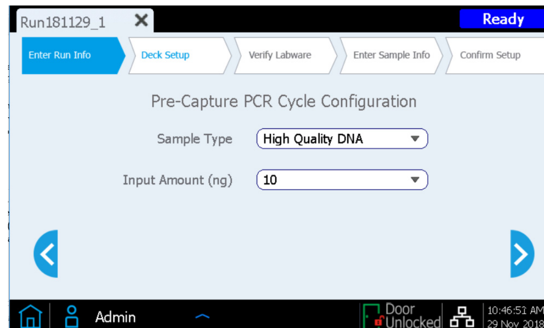
b If you want the instrument to take an aliquot (3 µl) of each pre-capture library sample for optional post-run QC analysis press **Yes** next to **Aliquot sample for QC**. (The pre-capture QC samples are only available for analysis when the full run is complete.) If you are making this selection, be sure to load the blue QC Strip during deck setup on page 33.

Or, clear the checkbox to skip the optional QC aliquot collection step.

c Press the forward arrow to advance to the next screen.



3 On the second screen, select the appropriate **Sample Type** (either *High Quality DNA* or *FFPE DNA*) and the DNA **Input Amount** (*10 ng, 50 ng, 100 ng*, or *200 ng*) for the samples processed in the run. These settings are used to determine the correct PCR cycling conditions for the run. PCR cycle number and other conditions to be used during the run are reported during *Confirm Setup* steps (see page 37).



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 29 to page 33. 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 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.
- Make sure all labware is positioned with barcode facing you (front of instrument).

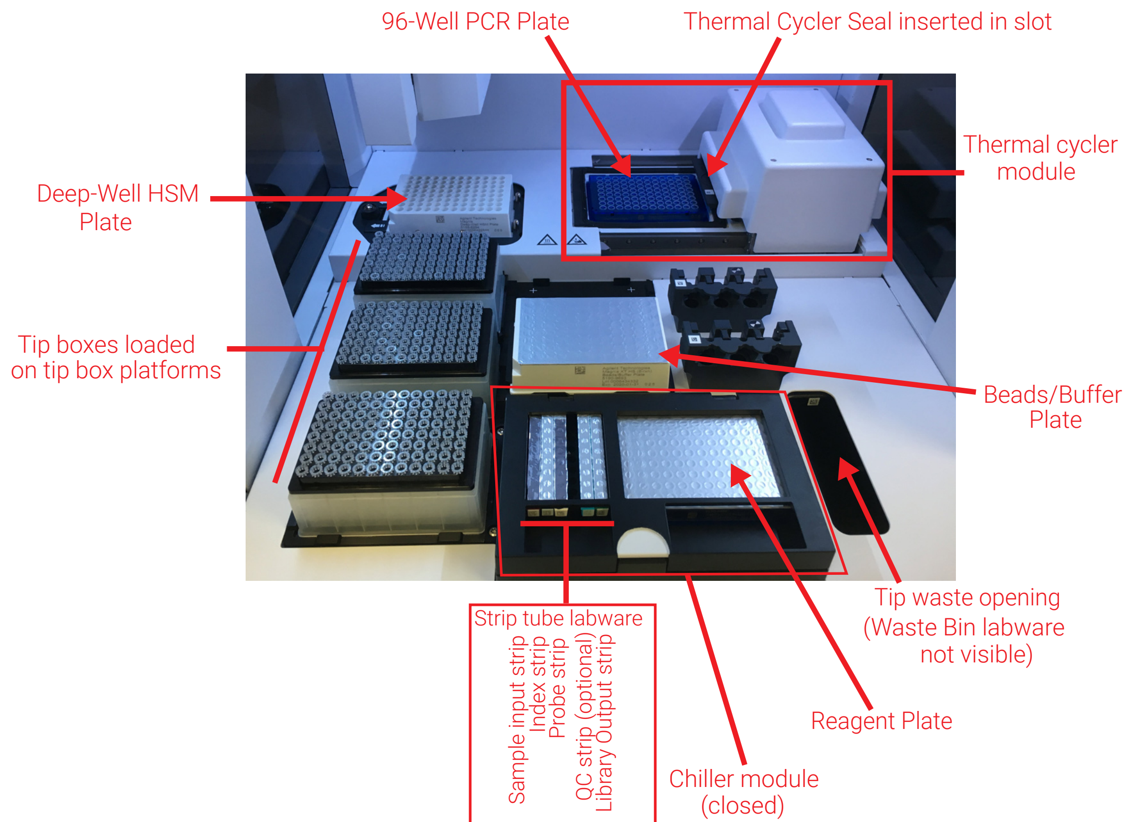
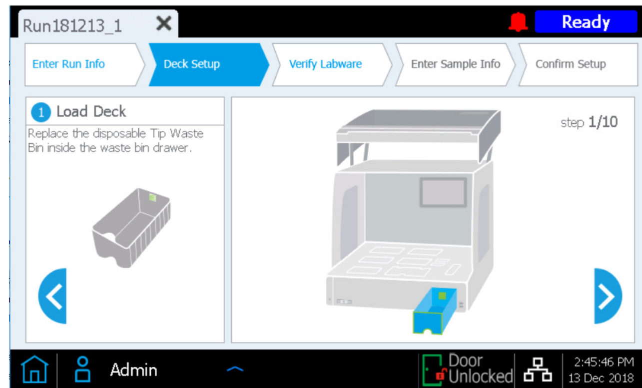


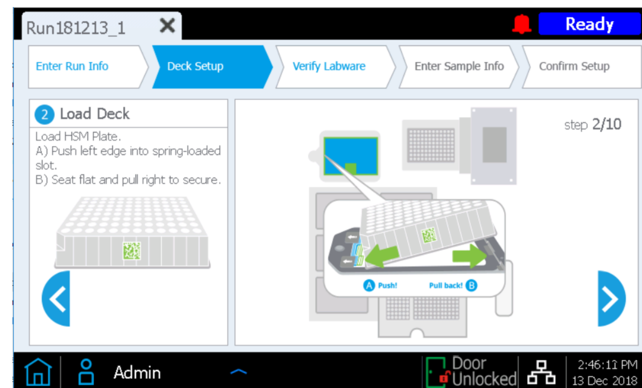
Figure 6 Instrument deck loaded for run

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.

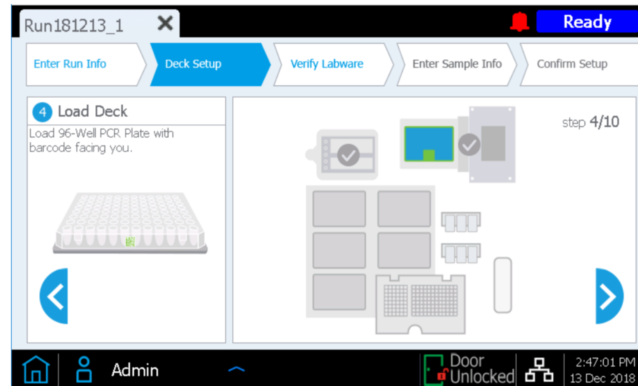


2 Remove the Magnis Deep-Well HSM Plate from the Magnis Empty Consumables package. Install the plate in the deck position shown on the touchscreen, with the barcode facing you. To load the plate, first insert the left edge of the plate into the spring-loaded slot, then lower the right edge of the plate 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.

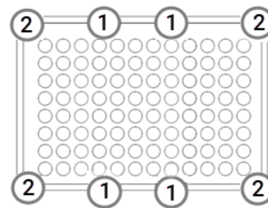


3 Remove the Magnis Thermal Cycler Seal from the Magnis Empty Consumables package. Peel the protective film from the foam pad below the metal plate, starting with the yellow tab. After the full sheet of film has been removed, insert the Thermal Cycler Seal into the slot at the position shown on the touchscreen,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

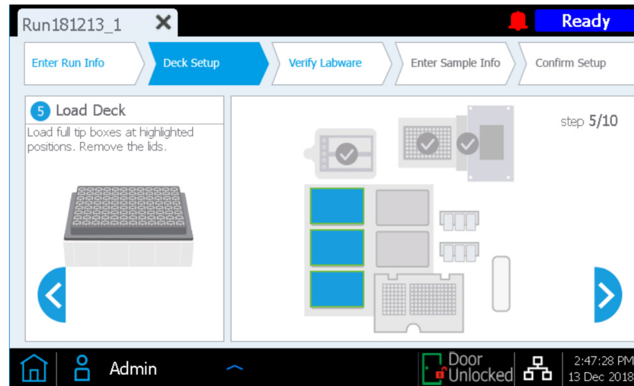
4 Remove the Magnis 96-Well PCR Plate from the Magnis Empty Consumables package. Load the plate in the deck position shown on the touchscreen by inserting the plate wells into the thermal cycler block wells, with the plate barcode facing you.



To ensure that the plate is fully seated in the block, first seat 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).



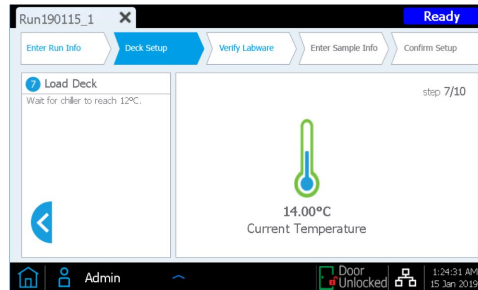
5 Load a fresh, full tip box at each of the deck positions indicated on the touchscreen. **Remove lids** from the boxes. After lid removal, verify that each tip box remains sitting flat and within the raised-tab frame of its platform position.



6 Obtain the Magnis SureSelect XT HS Beads/Buffers Plate that was prepared on page 22. 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.

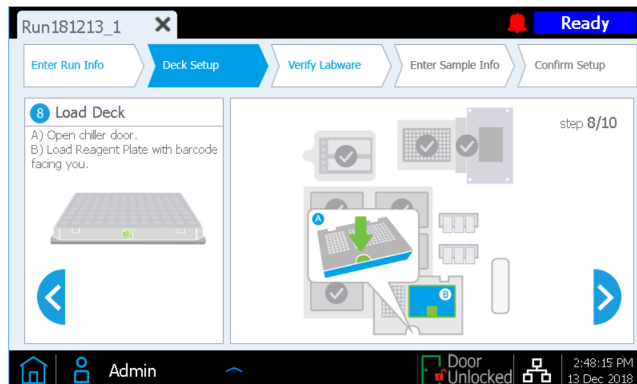
This screen may not appear during your run, if the chiller has already reached the required temperature.



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 23. 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 23. 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 as detailed below, in the order listed. Before loading each strip, check bottoms of wells for any bubbles. If bubbles are present, remove by spinning the strip as directed on page 23. 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 DNA samples** (prepared on page 23 and held on ice) into the strip tube holder position labeled with **S**.

  b Load the **black strip tube containing indexed primers** (prepared on page 23 and held on ice) into the strip tube holder position labeled with **IDX**.

  c Load the **white strip tube containing probe solution** (prepared on page 24 and held on ice) into the strip tube holder position labeled with **P**.

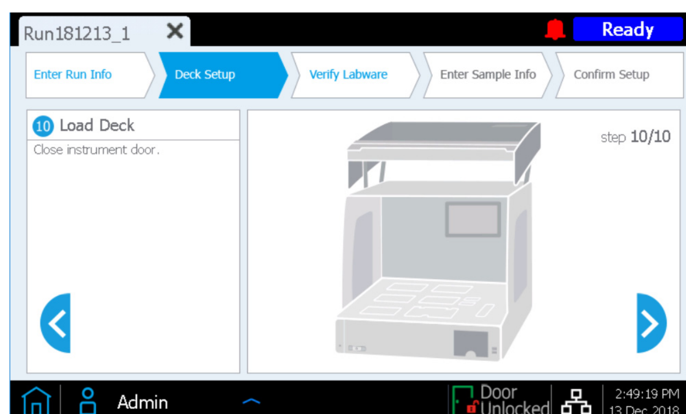
  d Obtain the Magnis Library Output Strip, QC Strip, and Foil Seals pack from the Magnis Empty Consumables package. Load the **empty green library output strip** (with "L" inscribed on end of strip) into the strip tube holder position labeled with **L**. Leave the foil cover intact.

  If the run will include collection of aliquots of the pre-capture library samples for QC (see page 26) 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.

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

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

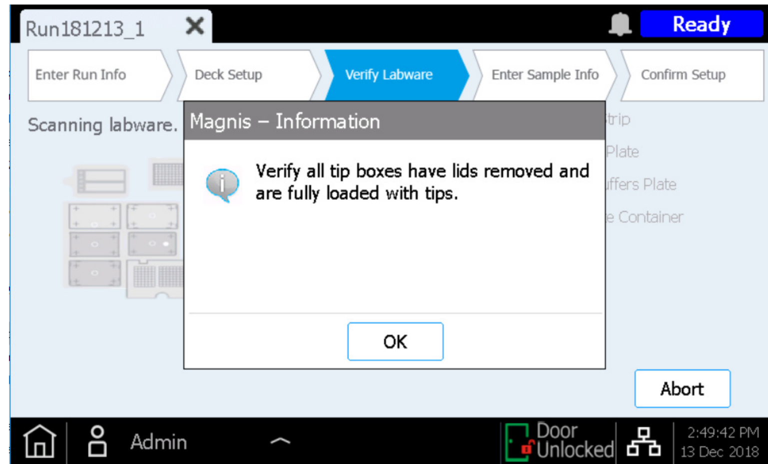
10 Close the instrument door.



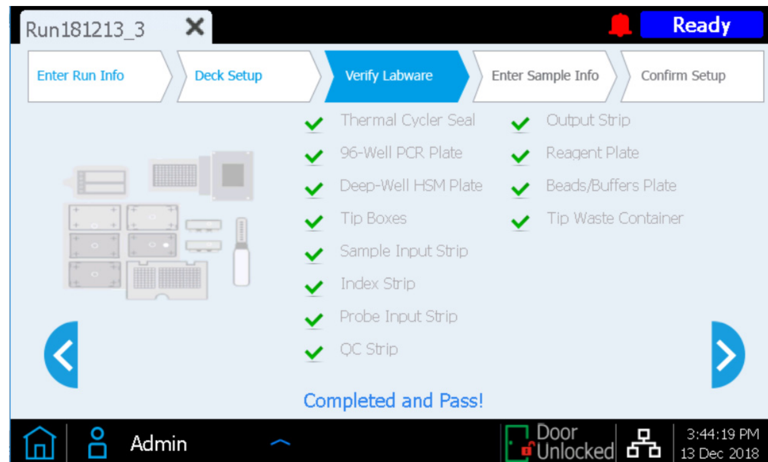
Step 3. Verify Labware

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 the "Troubleshooting Guide" on page 75 for remediation guidelines.

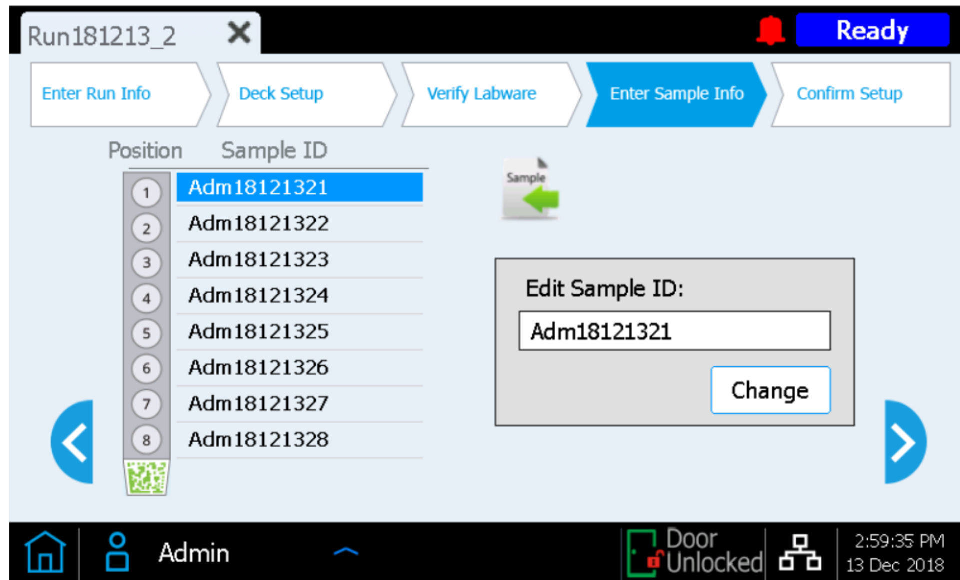
The final *Verify Labware* screen allows you to review details for the Probe Input Strip.

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 runs that include run-time filled probe strips (runs using kit p/n G9730D or G9730B, which include empty probe input strips), you must enter the probe-related properties on this screen manually. See the instructions on page 53. 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 disk, as detailed on page 17.
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 disk.

Method 2: Manual run-time sample assignment

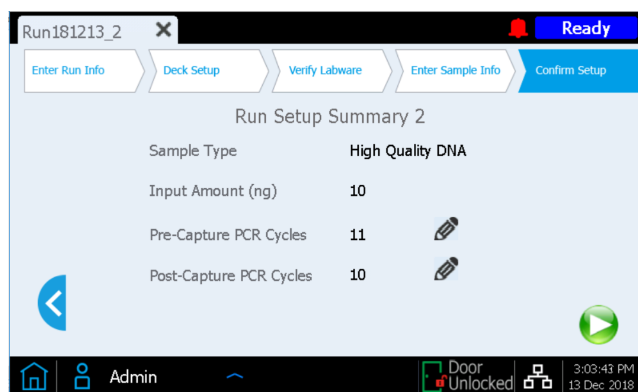
1. Select a specific sample position on the touchscreen.
2. Use the **Edit Sample ID** tool on the right to enter the desired Sample ID text. Press **Change** to save the entered Sample ID text for the sample position.

Step 5. Confirm Setup and start the run

Use this set of screens to confirm the run setup details before initiating a run.

1 On the first screen, verify the general features of the run. Once entries are confirmed to be correct, press the forward arrow to proceed to the final setup screen.

2 The second screen displays run details related to the characteristics of the DNA sample 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 DNA and probe used in the run) are displayed.



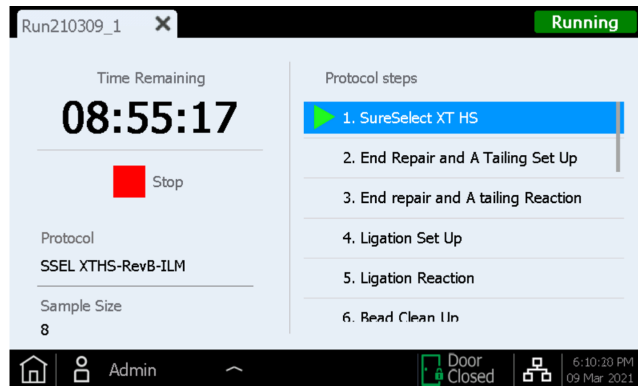
When an *Advanced* access level user is logged in, either cycle number value can be changed by pressing the pencil button. *Standard* access level users are not able to change these run parameters and the pencil button is not visible in the screen shown above.

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 typically take 8.5 to 9 hours and 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.

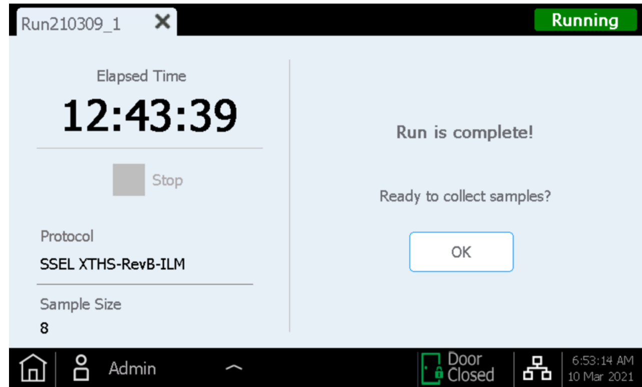
If needed, press the red square **Stop** button to abort the run. A warning message opens asking you to confirm that you want to abort the run. Once you stop a run, the run cannot be resumed, and the labware used in that run cannot be reloaded for a future run.



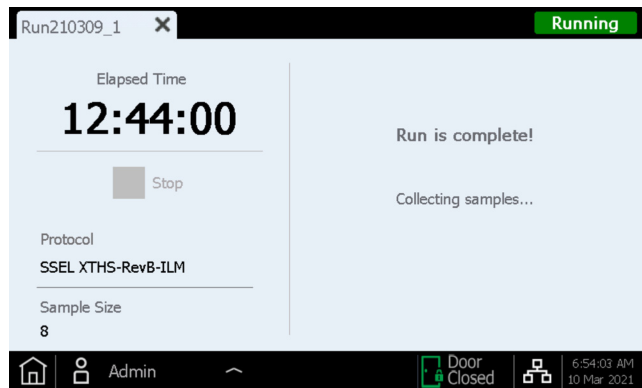
NOTE The *Running* screen must remain open through the duration of the run, and the screen close (**×**) button and other navigation buttons are inactive while the run is in progress. You cannot use the touchscreen to perform other functions during a run.

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.

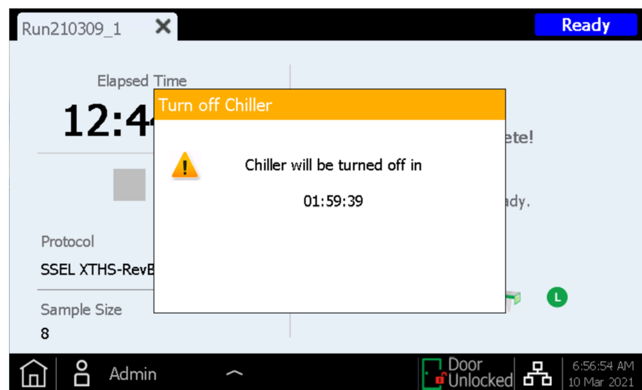


The instrument transfers the prepared library solutions from the PCR plate in the thermal cycler to the green Library Output Strip in the chiller at this time.



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

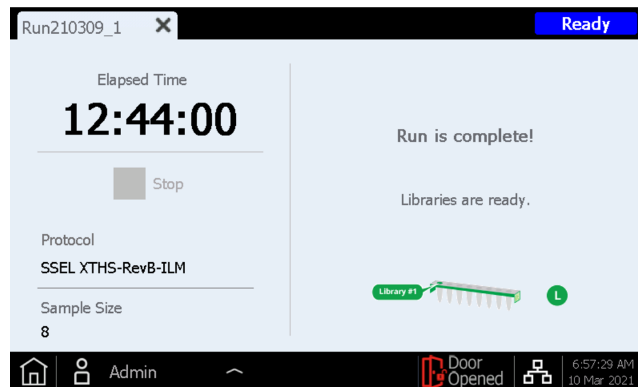
Once samples are placed in the green Library Output Strip in the chiller, the touchscreen display appears as below. The chiller, containing the library samples, is held at 12°C for up to 2 hours, with the remaining cold storage period indicated on the touchscreen dialog as shown below. The chiller is 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), then place the libraries under suitable storage conditions, according to your research design.

Guidelines for processing the final target-enriched libraries for DNA sequencing are provided in "Appendix 3: Guidelines for Post-run DNA Sample Processing for NGS" on page 54.

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 DNA 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 DNA fragment size between 300 and 400 bp for high-quality input DNA or between 200 and 400 bp for FFPE-derived input DNA.

QC samples that were dried and resuspended in 6 µl should have a concentration of approximately 30–100 ng/µl depending on input DNA quality and the pre-capture PCR cycle number. The overall pre-capture library yield may be calculated as the amount of DNA 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 20 for more information on UV decontamination). Clean the spill using the instructions provided in the instrument User Guide.

3
Appendix 1: DNA Sample Preparation Guidelines

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II. Preparation of FFPE-Derived DNA Samples for Magnis Runs 46
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  Step 3. Shear the FFPE DNA samples 48

Before setting up the Magnis SureSelect$^{XT\ HS}$ DNA sequencing library preparation run, DNA samples must be prepared, quantified, qualified, and sheared, using the guidelines and protocols in this section.

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

The library preparation protocol is compatible both with high-quality gDNA prepared from fresh or fresh frozen samples and with lower-quality DNA prepared from FFPE samples. For high-quality gDNA samples, see page 43. For FFPE-derived DNA samples, see page 46.

Magnis Runs can include 10 ng, 50 ng, 100 ng or 200 ng of input DNA. For optimal sequencing results, use the maximum amount of input DNA available within this range.

I. Preparation of High-Quality DNA Samples for Magnis Runs

Magnis SureSelect XT HS runs require 10 ng, 50 ng, 100 ng or 200 ng of input DNA in a volume of 50 µl 1X Low TE Buffer. All samples in the same run must be provided in the same quantity.

NOTE **Do not dilute samples to be sheared using water.** Shearing samples in water reduces the overall library preparation yield and complexity.

Step 1. Prepare, quantify, and qualify the genomic DNA samples

1 Prepare high-quality gDNA from fresh or frozen biological samples using a suitable purification system, such as Qiagen's QIAamp DNA Mini Kit, following the manufacturer's protocol.

NOTE Make sure genomic DNA samples are of high quality with an OD 260/280 ratio ranging from 1.8 to 2.0.

2 Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer's instructions for the instrument and assay kit.

3 Prepare each DNA sample for the library preparation protocol by diluting the appropriate amount (10 ng, 50 ng, 100 ng or 200 ng) of each gDNA sample with 1X Low TE Buffer to a final volume of 50 µl. Vortex well to mix, then spin briefly to collect the liquid. Keep the samples on ice.

Step 2. Shear the DNA

In this step, the 50-µl gDNA samples are sheared using conditions optimized for high-quality DNA. The target DNA fragment size is 150 to 200 bp.

NOTE This protocol has been optimized using a Covaris model E220 instrument and the 130-µl Covaris microTUBE. Consult the manufacturer's recommendations for use of other Covaris instruments or sample holders to achieve the same target DNA fragment size.

1 Set up the Covaris E220 instrument. Refer to the instrument user guide for details.

- **a** Check that the water in the Covaris tank is filled with fresh deionized water to the appropriate fill line level according to the manufacturer's recommendations for the specific instrument model and sample tube or plate in use.
- **b** Check that the water covers the visible glass part of the tube.
- **c** On the instrument control panel, push the Degas button. Degas according to the manufacturer's recommendations, typically 30–60 minutes.
- **d** Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C. Consult the manufacturer's recommendations for addition of coolant fluids to prevent freezing.

2 Complete the DNA shearing steps below for each of the gDNA samples.

a Transfer the 50-µl DNA sample into a Covaris microTUBE, using a tapered pipette tip to slowly transfer the sample through the pre-split septum of the cap.

b Spin the microTUBE for 30 seconds to collect the liquid and to remove any bubbles from the bottom of the tube.

c Secure the microTUBE in the tube holder and shear the DNA with the settings in Table 8.

Table 8 Shear settings for Covaris E-series instrument (SonoLab software v7 or later)

Setting	Value
Duty Factor	10%
Peak Incident Power (PIP)	175
Cycles per Burst	200
Bath Temperature	2° to 8° C
Treatment Time	2 × 120 seconds (two-round shearing, using steps detailed below) • Shear for 120 seconds • Spin the microTUBE for 10 seconds • Vortex the microTUBE at high speed for 5 seconds • Spin the microTUBE for 10 seconds • Shear for additional 120 seconds • Spin the microTUBE for 10 seconds • Vortex the microTUBE at high speed for 5 seconds • Spin the microTUBE for 10 seconds

d Proceed directly to the next step, do not leave the sheared DNA in the Covaris microTUBE for longer than required.

3 Put the Covaris microTUBE containing sheared DNA back into the loading and unloading station. Keeping the microTUBE snap-cap on, insert a pipette tip through the pre-split septum, then slowly remove the sheared DNA.

4 Transfer the 50-µl sheared DNA sample from the Covaris microTUBE into the designated well of the red Magnis Sample Input Strip, piercing the foil seal with the pipette tip just before dispensing the liquid. Keep the samples on ice.

Make sure to load samples in the correct sample well position, with Sample 1 in the well farthest from the barcode, as shown in Figure 7 below.

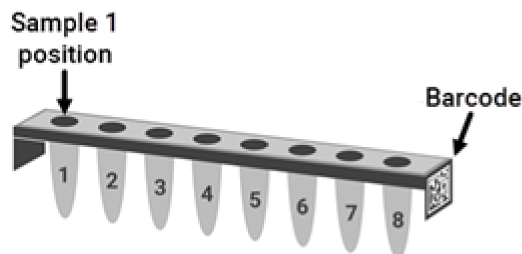

Figure 7 Required orientation of sample numbers 1 through 8 in the Magnis Sample Input Strip.

5 After transferring the DNA sample, spin the microTUBE briefly to collect any residual sample volume. Transfer any additional collected liquid to the same well of the Magnis Sample Input Strip.

NOTE It is important to avoid loss of input DNA at this step, especially for low-abundance DNA samples. Visually inspect the microTUBE to ensure that all of the sample has been transferred. If droplets remain in the microTUBE, repeat step 5.

6 Once all samples are loaded, continue with the sample input strip setup as detailed on page 23 (see part d of step 3).

II. Preparation of FFPE-Derived DNA Samples for Magnis Runs

Magnis SureSelect XT HS runs require 10 ng, 50 ng, 100 ng or 200 ng of input DNA in a volume of 50 µl 1X Low TE Buffer. All samples in the same run must be provided in the same quantity.

NOTE **Do not dilute samples to be sheared using water.** Shearing samples in water reduces the overall library preparation yield and complexity.

Step 1. Prepare genomic DNA from FFPE samples

Preparation and qualification of gDNA from FFPE samples

1 Prepare gDNA from FFPE tissue sections using Qiagen's QIAamp DNA FFPE Tissue Kit and Qiagen's Deparaffinization Solution, following the manufacturer's protocol. Elute the final gDNA samples from the MinElute column in two rounds, using 30 µl Buffer ATE in each round, for a final elution volume of approximately 60 µl.

NOTE If tissue lysis appears incomplete after one hour of digestion with Proteinase K, add an additional 10 µl of Proteinase K and continue incubating at 56°C, with periodic mixing, for up to three hours.

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

2 Assess the quality (DNA integrity) for each FFPE DNA sample using one of the methods below.

Step 2. Qualify and quantify the FFPE DNA samples

Assess the quality (DNA integrity) for each FFPE-derived DNA sample using one of the two methods below. The DNA integrity measured at this step determines the appropriate means of sample quantification needed to include 10 ng, 50 ng, 100 ng or 200 ng of amplifiable gDNA samples in the run.

Method Option 1: Qualification using the Agilent NGS FFPE QC Kit (Recommended Method)

The Agilent NGS FFPE QC Kit provides a qPCR-based assay for DNA sample integrity determination. Results include a ΔΔCq DNA integrity score and the precise quantity of amplifiable DNA in the sample, allowing direct normalization of DNA input for each sample. DNA input recommendations based on ΔΔCq scores for individual samples are summarized in Table 9.

a Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer's instructions for the instrument and assay kit.

b Remove a 1 µl aliquot of the FFPE gDNA sample for analysis using the Agilent NGS FFPE QC Kit to determine the ΔΔCq DNA integrity score. See the kit user manual at www.agilent.com for more information.

c For all samples with ΔΔCq DNA integrity score ≤1, use the Qubit-based gDNA concentration determined in step a, above, to determine volume of input DNA needed for the protocol.

d For all samples with ΔΔCq DNA integrity score >1, use the qPCR-based concentration of amplifiable gDNA, reported by the Agilent NGS FFPE QC Kit results, to determine amounts of input DNA for the protocol.

Table 9 SureSelect XT HS DNA input modifications based on ΔΔCq DNA integrity score

Protocol Parameter	non-FFPE Samples	FFPE Samples	
		ΔΔCq≤1*	ΔΔCq >1
DNA input for Library Preparation	10 ng, 50 ng, 100 ng or 200 ng DNA, based on Qubit Assay	10 ng, 50 ng, 100 ng or 200 ng DNA, based on Qubit Assay	10 ng, 50 ng, 100 ng or 200 ng of amplifiable DNA, based on qPCR quantification

* FFPE samples with ΔΔCq scores ≤1 should be treated like non-FFPE samples for DNA input amount determinations. For samples of this type, make sure to use the DNA concentration determined by the Qubit Assay, instead of the concentration determined by qPCR, to calculate the volume required for 10–200 ng DNA.

3 Prepare each FFPE DNA sample for the library preparation protocol by diluting the appropriate amount (10 ng, 50 ng, 100 ng or 200 ng) of each gDNA sample with 1X Low TE Buffer to a final volume of 50 µl. Vortex well to mix, then spin briefly to collect the liquid. Keep the samples on ice.

Method Option 2: Qualification using Agilent’s Genomic DNA ScreenTape assay DIN score

Agilent’s Genomic DNA ScreenTape assay, used in conjunction with Agilent’s 4200 TapeStation, provides a quantitative electrophoretic assay for DNA sample integrity determination. This assay reports a DNA Integrity Number (DIN) score for each sample which is used to estimate the appropriate normalization of DNA input required for low-integrity DNA samples.

a Use the Qubit BR dsDNA Assay Kit to determine the concentration of each gDNA sample. Follow the manufacturer’s instructions for the instrument and assay kit.

b Remove a 1 µl aliquot of the FFPE gDNA sample and analyze using the Genomic DNA ScreenTape assay. See the user manual at www.agilent.com for more information.

c Using the DIN score reported for each sample in the Genomic DNA ScreenTape assay, consult Table 10 to determine the recommended amount of input DNA for the sample.

Table 10 SureSelect XT HS DNA input modifications based on DNA Integrity Number (DIN) score

Protocol Parameter	non-FFPE Samples	FFPE Samples		
		DIN > 8*	DIN 3–8	DIN<3
DNA input for Library Preparation	10 ng, 50 ng, 100 ng or 200 ng DNA, quantified by Qubit Assay	10 ng, 50 ng, 100 ng or 200 ng DNA, quantified by Qubit Assay	Use 50 ng, 100 ng or 200 ng DNA (use the maximum amount of DNA available, up to 200 ng). Quantify by Qubit Assay to determine volume required for 50 ng, 100 ng or 200 ng input.	Use 100 ng or 200 ng DNA (use the maximum amount of DNA available, up to 200 ng). Quantify by Qubit Assay to determine volume required for 100 ng or 200 ng input.

* FFPE samples with DIN>8 should be treated like non-FFPE samples for DNA input amount determinations.

4 Prepare each FFPE DNA sample for the library preparation protocol by diluting the appropriate amount (10 ng, 50 ng, 100 ng or 200 ng) of each gDNA sample with 1X Low TE Buffer to a final volume of 50 µl. Vortex each sample dilution to mix, then spin briefly to collect the liquid. Keep the samples on ice. samples on ice.

Step 3. Shear the FFPE DNA samples

In this step, the 50-µl gDNA samples are sheared using conditions optimized for FFPE-derived DNA. The target DNA fragment size is 150 to 200 bp.

NOTE This protocol has been optimized using a Covaris model E220 instrument and the 130-µl Covaris microTUBE. Consult the manufacturer's recommendations for use of other Covaris instruments or sample holders to achieve the same target DNA fragment size.

1 Set up the Covaris E220 instrument. Refer to the instrument user guide for details.
   - a Check that the water in the Covaris tank is filled with fresh deionized water to the appropriate fill line level according to the manufacturer's recommendations for the specific instrument model and sample tube or plate in use.
   - b Check that the water covers the visible glass part of the tube.
   - c On the instrument control panel, push the Degas button. Degas according to the manufacturer's recommendations, typically 30–60 minutes.
   - d Set the chiller temperature to between 2°C to 5°C to ensure that the temperature reading in the water bath displays 5°C. Consult the manufacturer's recommendations for addition of coolant fluids to prevent freezing.

2 Complete the DNA shearing steps below for each of the 50-µl gDNA samples (containing 10ng, 50 ng, 100 ng, or 200 ng gDNA in 50 µl of 1X Low TE Buffer).
   - a Transfer the 50-µl DNA sample into a Covaris microTUBE, using a tapered pipette tip to slowly transfer the sample through the pre-split septum of the cap.
   - b Spin the microTUBE for 30 seconds to collect the liquid and to remove any bubbles from the bottom of the tube.
   - c Secure the microTUBE in the tube holder and shear the DNA with the settings in Table 11.

Table 11 Shear settings for Covaris E-series instrument (SonoLab software v7 or later)

Setting	Value
Duty Factor	10%
Peak Incident Power (PIP)	175
Cycles per Burst	200
Bath Temperature	2° to 8° C
Treatment Time	240 seconds

   - d Proceed directly to the next step, do not leave the sheared DNA in the Covaris microTUBE for longer than required.

3 Put the Covaris microTUBE containing sheared DNA back into the loading and unloading station. Keeping the microTUBE snap-cap on, insert a pipette tip through the pre-split septum, then slowly remove the sheared DNA.

4 Transfer the 50-µl sheared DNA sample from the Covaris microTUBE into the designated well of the red Magnis Sample Input Strip, piercing the foil seal with the pipette tip just before dispensing the liquid. Keep the samples on ice.

Make sure to load samples in the correct sample well position, with Sample 1 in the well farthest from the barcode, as shown in Figure 8 below.

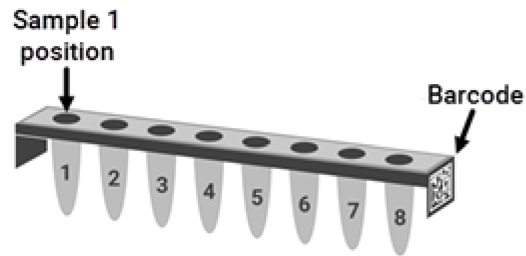

Figure 8 Required orientation of sample numbers 1 through 8 in the Magnis Sample Input Strip.

5 After transferring the DNA sample, spin the microTUBE briefly to collect any residual sample volume. Transfer any additional collected liquid to the same well of the Magnis Sample Input Strip.

NOTE It is important to avoid loss of input DNA at this step, especially for low-abundance DNA samples. Visually inspect the microTUBE to ensure that all of the sample has been transferred. If droplets remain in the microTUBE, repeat step 5.

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6 Once all samples are loaded, continue with the sample input strip setup as detailed on page 23 (see part d of step 3).

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

Run-Time Preparation of the Probe Strip 51
Entering Probe Information in the Magnis Software during Run Setup 53

The instructions in this section are specifically for use with Reagent Kit part number G9730D or G9730B, provided with empty Probe Input Strips (EPIS). Runs performed using these kits require run-time preparation of probe strips and require the additional data entry steps described in this section.

Reagent Kit part numbers G9730D and G9730B are processed using different Magnis run protocols. Probe strip setup steps are similar for both protocols, differing only in the volume of probe required per sample well, as summarized in Table 12 on page 51.

Instructions in this section do not apply to kits that include pre-filled probe strips; see page 24 for pre-filled probe strip set up information.

Run-Time Preparation of the Probe Strip

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 run; do not pre-fill and freeze-thaw the probe input strips used in these protocols.

Refer to Table 12 below to determine the volume of SureSelect Probe required per well for your reagent kit type and probe capture size.

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 13 on page 53 for compatible probe design size ranges).

Table 12 Empty Magnis Probe Input Strip filling requirements

Reagent Kit Name (Part Number)	Protocol Selected on *Enter Run Info* Screen	Probe Capture Size	Volume to Pipette per Well	Volume Required for 8-Sample Run
Magnis SureSelect XT HS **Rev B** Reagent Kit (G9730D)	*SSEL XTHS-EPIS-**RevB**-ILM* OR *LT-SSEL XTHS-EPIS-**RevB**-ILM*	≥3 Mb (**Large** *Capture Size*)*	5 µl	40 µl
		<3 Mb (**Small** *Capture Size*)*	2 µl	16 µl
Magnis SureSelect XT HS Reagent Kit (G9730B)	*SureSelectXT HS-Illumina*	≥3 Mb (**Large** *Capture Size*)*	8 µl	64 µl
		<3 Mb (**Small** *Capture Size*)*	6 µl	48 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 53. 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 13 on page 53).

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. Fill the wells of the empty Magnis Probe Input Strip with the amount of SureSelect Probe solution required for your reagent kit type and probe design size 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 12, 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, 6 µl, or 8 µl of probe.

NOTE It is important to fill the probe input strip wells using precisely the volumes indicated in Table 12. Use a calibrated pipette qualified to dispense the indicated volume with high accuracy and precision.

4. 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.

5 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.

6 Keep the probe strip on ice until use during deck setup on page 33.

When loading the probe strip during deck setup, make sure to verify that the strip is properly seated in the chiller module.

Entering Probe Information in the Magnis Software during Run Setup

For runs that include probe input strips filled at run-time (runs using kit part number G9730D or G9730B), you must enter the probe-related properties in the fields shown below during the *Verify Labware* phase of run setup (see page 35).

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 13 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. While the 8 wells of the Magnis Probe Input Strip may contain different probe solutions, all probes used in the same run must use the same *Post-Capture PCR Cycles* and *Capture Size* settings.

Table 13 Recommended settings for run-time dispensed probes

SureSelect Probe Design Size	Post-Capture PCR Cycles	Capture Size
<200 kb	14	Small
200–749 kb	13	Small
750–2999 kb	12	Small
3–5 Mb	10	Large
>5 Mb	9	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 36.

5 Appendix 3: Guidelines for Post-run DNA Sample Processing for NGS

Step 1. Analyze quantity and quality of library DNA samples 55
Step 2. Pool samples for multiplexed sequencing (optional) 57
Step 3. Prepare the sequencing samples 58
Step 4. Do the sequencing run and analyze the data 60
HiSeq/NextSeq/NovaSeq instrument sequencing run setup guidelines 60
MiSeq instrument sequencing run setup guidelines 62
Sequence analysis resources 65

After completing the Magnis SureSelect$^{XT HS}$ library preparation run, the DNA 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 4200 TapeStation or 4150 TapeStation and the High Sensitivity D1000 ScreenTape and associated reagent kit. See Table 3 on page 13 for ordering information. Refer to the Agilent High Sensitivity D1000 Assay Quick Guide for detailed instructions.

NOTE Alternatively, library DNA samples may be analyzed using the Agilent 2100 Bioanalyzer and the Bioanalyzer High Sensitivity DNA Assay or using the Agilent 5200 Fragment Analyzer and HS NGS Fragment Kit. 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 400 bp. Sample electropherograms are shown in Figure 9 (library prepared from high-quality DNA), Figure 10 (library prepared from medium-quality FFPE DNA), and Figure 11 (library prepared from low-quality FFPE DNA).

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

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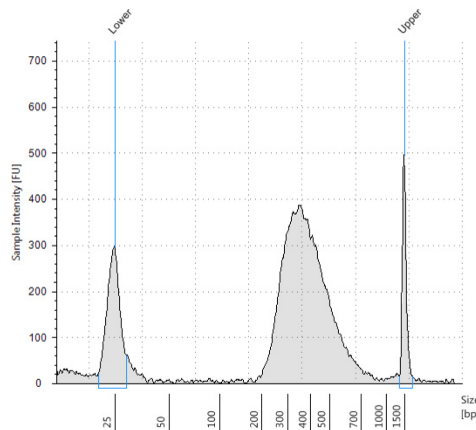


Figure 9 Post-capture library prepared from a high-quality gDNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

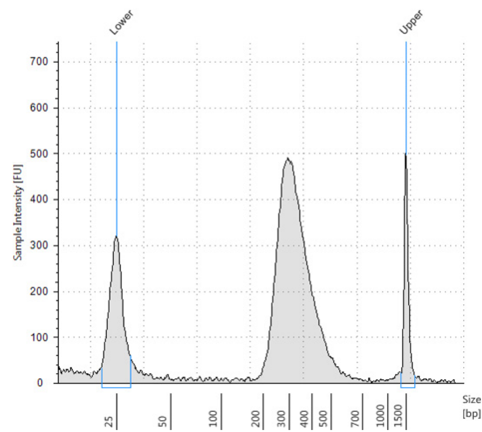


Figure 10 Post-capture library prepared from a typical FFPE gDNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

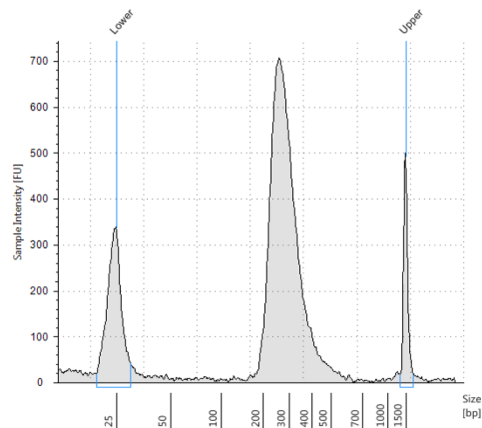


Figure 11 Post-capture library prepared from a low-quality FFPE gDNA sample analyzed using a High Sensitivity D1000 ScreenTape assay.

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 14 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 14 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

If you store the library before sequencing, add Tween 20 to 0.1% v/v and store at -20°C short term, or store under the conditions specified by your sequencing provider.

Step 3. Prepare the sequencing samples

The final SureSelect$^{XT\ HS}$ 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 12.

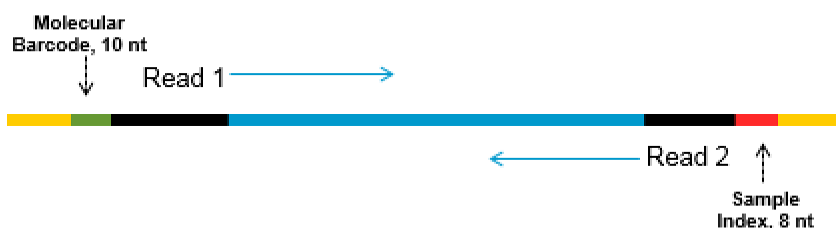


Figure 12 Content of SureSelect XT HS sequencing library. Each fragment contains one target insert (blue) surrounded by the Illumina paired-end sequencing elements (black), the sample index (red), the molecular barcode (green) and the library bridge PCR primers (yellow).

Libraries can be sequenced on Illumina HiSeq, MiSeq, NextSeq or NovaSeq sequencers using the run type and chemistry combinations shown in Table 15.

CAUTION Do not use the HiSeq 2500 instrument in high-output run mode (v4 chemistry) if your analysis pipeline includes molecular barcode (i5) reads. Poor molecular barcode sequence data quality (lower Q scores, with impacts on coverage and sensitivity of variant calls) has been observed when SureSelect$^{XT\ HS}$ libraries are sequenced on the HiSeq 2500 instrument in this mode. See Table 15 for alternative run mode/chemistry options for the HiSeq 2500 instrument. Sequencing performance for Read 1, Read 2, and sample-level index (i7) reads is not affected and this instrument/run mode/chemistry may be used for applications that omit molecular barcode analysis.

Proceed to cluster amplification using the appropriate Illumina Paired-End Cluster Generation Kit. See Table 15 for kit configurations compatible with the recommended read length.

The optimal seeding concentration for SureSelect$^{XT\ HS}$ target-enriched libraries varies according to sequencing instrument, run type, and Illumina kit version. See Table 15 for guidelines. Seeding concentration and cluster density may also need to be optimized based on the DNA 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 listed range.

Follow Illumina’s recommendation for a PhiX control in a low-concentration spike-in for improved sequencing quality control.

Table 15 Illumina Kit Configuration Selection Guidelines

Instrument	Run Type	Read Length	SBS Kit Configuration	Chemistry	Seeding Concentration
HiSeq 2500	Rapid Run	2 × 100 bp	200 Cycle Kit	v2	9–10 pM
HiSeq 2500	High Output*	2 × 100 bp	250 Cycle Kit	v4	12–14 pM
MiSeq	All Runs	2 × 100 bp	300 Cycle Kit	v2	9–10 pM
MiSeq	All Runs	2 × 75 bp	150 Cycle Kit	v3	12–16 pM
NextSeq 500/550	All Runs	2 × 100 bp	300 Cycle Kit	v2.5	1.2–1.5 pM
HiSeq 3000/4000	All Runs	2 × 100 bp	300 Cycle Kit	v1	300–400 pM
NovaSeq 6000	Standard Workflow Runs	2 × 100 bp	300 Cycle Kit	v1.0 or v1.5	300–600 pM
NovaSeq 6000	Xp Workflow Runs	2 × 100 bp	300 Cycle Kit	v1.0 or v1.5	200–400 pM

* Do not use HiSeq 2500 High Output (v4 chemistry) runs if your analysis pipeline includes molecular barcode (i5) reads. Reduced molecular barcode sequence quality and lowered Q scores have been observed in sequences obtained from HiSeq 2500 runs under these conditions. Sequencing performance for Read 1, Read 2, and sample-level index (i7) reads is not affected.

Step 4. Do the sequencing run and analyze the data

Use the guidelines below for SureSelect$^{XT\ HS}$ library sequencing run setup and analysis.

- The sample-level index (i7) requires an 8-bp index read. For complete i7 index sequence information, see Table 34 on page 73.
- The degenerate molecular barcode (i5) requires a 10-bp index read.
- For HiSeq, NextSeq, and NovaSeq instrument runs, set up the run using the instrument's user interface, following the guidelines on page 60.
- For MiSeq instrument runs, set up the run using Illumina Experiment Manager (IEM) using the steps detailed on page 62 to page 65 to generate a custom sample sheet.
- Retrieval of I2 index files containing the molecular barcode (i5) index reads requires offline conversion of .bcl to fastq files. For information on how to do this step, see page 61 for HiSeq, NextSeq, and NovaSeq runs and see page 65 for MiSeq runs.
- Before aligning reads to the reference genome, trim the reads from Illumina adaptor sequences. See page 65 for information on Agilent's SureCall data analysis software, which may be used for this task.

HiSeq/NextSeq/NovaSeq instrument sequencing run setup guidelines

Set up sequencing runs using the instrument control software interface. A sample run setup for the HiSeq instrument using 100 + 100 bp paired-end sequencing is shown below.

If using the NextSeq or NovaSeq instrument, locate the same parameters on the *Run Setup* screen, and populate the **Read Length** fields using the **Cycles** settings shown in HiSeq instrument example above. In the **Custom Primers** section of the NextSeq or NovaSeq instrument *Run Setup* screen, clear (do **not** select) the checkboxes for all primers (*Read 1, Read 2, Index 1* and *Index 2*).

BaseSpace currently does not support the sequencing of molecular barcodes as index reads. Set up NextSeq runs using the stand-alone mode.

Retrieve I2 FASTQ files containing molecular barcodes

Retrieval of I2 index files containing the molecular barcode (i5) index reads requires offline conversion of .bcl to fastq files using one of the two methods below.

Option 1: Use bcl2fastq software with base masking

To generate Index 2 fastq files containing the P5 molecular barcodes using the bcl2fastq software, follow Illumina's instructions for use of the software with the following modifications:

1. Use of a sample sheet is mandatory and not optional. Modify the sample sheet to include only the sample index and not the molecular barcode index by clearing the contents in the **I5_Index_ID** and **index2** columns.
2. Set ***mask-short-adapter-reads*** to value of 0.
3. Use the following base mask: Y*, I8, Y10, Y* (where * should be replaced with the actual read length, with the value entered matching the read length value in the RunInfo.xml file).

CAUTION

When generating fastq files using Illumina's bcl2fastq software, make sure to clear the contents of the **index2** column in the sample sheet as described above. **Do not enter an N_{10} sequence to represent the degenerate molecular barcode**; instead, simply leave the column cells cleared.

The bcl2fastq software does not treat the "N" character as a wildcard when found in sample sheet index sequences, and usage in this context will cause a mismatch for any sequence character other than "N".

Option 2: Use Broad Institute Picard tools

To generate Index 2 fastq files containing the P5 molecular barcodes using the Broad Institute Picard tools, complete the following steps:

1. Use tool **ExtractIlluminaBarcodes** to find the barcodes. A sample set of commands is shown below (commands used by your facility may vary).

```
nohup java -jar picard.jar ExtractIlluminaBarcodes
BASECALLS_DIR=<sequencing_run_directory>/Data/Intensities/BaseCalls/
OUTPUT_DIR=<barcode_output_dir_name> LANE=1 READ_STRUCTURE=<read_structure>
BARCODE_FILE=<barcode_file> METRICS_FILE=<metric_file_name> NUM_PROCESSORS=<n>
```

2. Use tool **IlluminaBaseCallsToFastq** to generate the fastq files based on output of step 1. A sample set of commands is shown below (commands used by your facility may vary).

```
nohup java -jar picard.jar IlluminaBasecallsToFastq
BASECALLS_DIR=<sequencing_run_directory>/Data/Intensities/BaseCalls/ LANE=1
BARCODES_DIR=<barcode_output_dir_name> READ_STRUCTURE=<read_structure>
FLOWCELL_BARCODE=<FCID> MACHINE_NAME=<machine_name> RUN_BARCODE=<run_number>
ADAPTERS_TO_CHECK=PAIRED_END

NUM_PROCESSORS=<n> READ_NAME_FORMAT=CASAVA_1_8 COMPRESS_OUTPUTS=true
MULTIPLEX_PARAMS=<multiplex_params_file> IGNORE_UNEXPECTED_BARCODES=true
TMP_DIR=<temp_directory_location>
```

MiSeq instrument sequencing run setup guidelines

Use the Illumina Experiment Manager (IEM) software to generate a custom Sample Sheet according to the guidelines below. Once a Sample Sheet has been generated, index sequences need to be manually changed to the SureSelect XT HS indexes used for each sample. See Table 34 on page 73 for nucleotide sequences of the SureSelect XT HS system indexes.

Set up a custom Sample Sheet:

1 In the IEM software, create a Sample Sheet for the MiSeq instrument using the following Workflow selections.

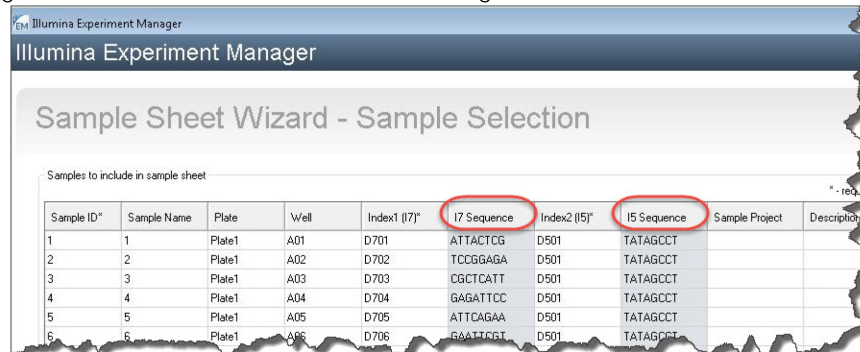
- Under *Category*, select **Other**.
- Under *Application*, select **FASTQ Only**.

2 On the *Workflow Parameters* screen, enter the run information, making sure to specify the key parameters highlighted below. In the *Library Prep Workflow* field, select **TruSeq Nano DNA**. In the *Index Adapters* field, select **TruSeq DNA CD Indexes (96 Indexes)**. If your pipeline uses SureCall for adaptor trimming, then make sure to clear both adaptor-trimming checkboxes under *FASTQ Only Workflow-Specific Settings* (circled below), since these are selected by default.

If **TruSeq Nano DNA** is not available in the *Sample Prep Kit* field, instead select **TruSeq HT**.

3 Using the **Sample Sheet Wizard**, set up a **New Plate**, entering the required information for each sample to be sequenced. In the **I7 Sequence** column, assign each sample to any of the Illumina i7 indexes. The index will be corrected to a SureSelect XT HS index at a later stage.

Likewise, in the **I5 Sequence** column, assign any of the Illumina i5 indexes, to be corrected to the degenerate molecular barcode at a later stage.



4 Finish the sample sheet setup tasks and save the sample sheet file.

Edit the Sample Sheet to include SureSelect XT HS indexes and molecular barcodes

1 Open the Sample Sheet file in a text editor and edit the i7 and i5 index information for each sample in columns 5–8 (highlighted in Figure 13).

- In column 5 under **I7_Index_ID**, enter the name of the SureSelect XT HS index assigned to the sample. In column 6 under **index**, enter the corresponding SureSelect XT HS Index sequence. See Table 34 on page 73 for nucleotide sequences of the SureSelect XT HS indexes.
- In column 7 under **I5_Index_ID**, enter *MBC* for all samples. In column 8 under **index2**, enter text *NNNNNNNNNN* for all samples to represent the degenerate 10-nucleotide molecular barcode tagging each fragment.

NOTE Enter N_{10} text in the **index2** column only when sample sheets are processed using MiSeq Reporter software adjusted to retrieve I2 fastq files containing molecular barcodes, as detailed on page 65. Sample sheets processed offline using Illumina's bcl2fastq software must not contain N_{10} wildcard index sequences. See the *Caution* advisory on page 61 for more information.

[Header]									
IEMFileVer	5								
Experimen	XTHS								
Date	1/22/2018								
Workflow	GenerateFASTQ								
Applicatio	FASTQ Only								
Instrumen	MiSeq								
Assay	TruSeq Nano DNA								
Index Ada	TruSeq DNA CD Indexes (96 Indexes)								
Description									
Chemistry	Amplicon								
[Reads]									
100									
100									
[Settings]									
ReverseCo	0								
[Data]									
Sample_ID	Sample_Name	Sample_Plate	Sample_W	Index_Plate_W	I7_Index_ID	index	I5_Index_I	index2	Sampl
XTHS-S1	XTHS-S1	1	A01	A01	A01	GTCTGTCA	MBC	NNNNNNNNNN	XTHS
XTHS-S2	XTHS-S2	1	B01	B01	B01	TGAAGAGA	MBC	NNNNNNNNNN	XTHS

Figure 13 Sample sheet for use with MiSeq instrument after MiSeq Reporter reconfiguration

2 Save the edited Sample Sheet in an appropriate file location for use in the MiSeq run.

Reconfigure the MiSeq Reporter Software to retrieve I2 FASTQ files

By default, MiSeq Reporter software does not generate fastq files for index reads. To generate fastq I2 index files containing the molecular barcode reads using MiSeq Reporter, adjust the software settings as described below before the first use of the MiSeq instrument for SureSelect XT HS library sequencing. Once changed, this setting is retained for future runs.

To change this setting, open the file **MiSeq Reporter.exe.config**. Under the **<appSettings>** tag, add **<add key="CreateFastqForIndexReads" value="1"/>**. You must restart the instrument for this setting change to take effect.

NOTE

If you are using the same instrument for assays other than SureSelect XT HS library sequencing, the configuration file should be edited to **<add key="CreateFastqForIndexReads" value="0"/>** and the instrument should be restarted before running the other assay.

If you are using the MiSeqDx system, run the instrument in research mode to make changes to MiSeq Reporter settings. If research mode is not available on your instrument, you may need to upgrade the system to include the dual boot configuration to allow settings changes in research mode.

The alternative methods for retrieval of I2 fastq files described on page 61 for HiSeq NextSeq, and NovaSeq runs may also be applied to MiSeq runs.

Sequence analysis resources

Agilent SureCall NGS data analysis software is designed to perform adaptor trimming, alignment of reads, and variant calling of sequencing data generated from SureSelect$^{XT\ HS}$ libraries. To download SureCall free-of-charge and for additional information, including SureCall software tutorials, visit the SureCall page at www.agilent.com.

If using another pipeline for alignment and downstream analysis, Agilent provides the Agilent Genomics NextGen Toolkit (AGeNT), with certain of the Agilent SureCall capabilities in a flexible command-line interface for integration into your bioinformatics pipeline. AGeNT is a Java-based software module that has been designed to provide adaptor and low- quality bases trimming and duplicate read removal for high-sensitivity (HS) and non-HS data. This tool is explicitly designed for users with established in-house bioinformatics experts with the capability to build, integrate, maintain, and troubleshoot internal analysis pipelines. Moreover, the module is designed specifically for users with sufficient computing infrastructure and IT support to troubleshoot all issues unrelated to the execution of the AGeNT algorithms. Because Agilent provides limited support of AGeNT, users with limited bioinformatics expertise should instead use Agilent SureCall software. Agilent does not guarantee the usability of third party tools (open- or closed-source) in upstream/downstream analysis of data in conjunction with AGeNT. For additional information on this tool, visit the AGeNT page at www.agilent.com.

6
Reference

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This chapter contains reference information, including Reagent Kit contents, index sequences, and troubleshooting information for the SureSelect$^{XT\ HS}$ library preparation runs.

Reagent Kit Contents

Agilent part numbers for the Magnis SureSelect XT HS Reagent Kits, including reformatted Rev B kits and original format kits, are summarized in Table 16. Make sure you have verified the format type of your kit before you begin setting up the run; reformatted Rev B kits must be processed using the appropriate *RevB* protocol (selected on the *Enter Run Info* screen during run setup).

Content details for the Magnis SureSelect XT HS Rev B Reagent Kits are provided on page 67 to page 69, and for Magnis SureSelect XT HS Reagent Kits in original format on page 70 to page 71.

Table 16 Reagent Kit Formats and Part Numbers

Included SureSelect Probe	Magnis SureSelect XT HS Rev B Reagent Kits		Magnis SureSelect XT HS Reagent Kits (original format)	
	96 Reactions	32 Reactions	96 Reactions	32 Reactions
Custom 1–499 kb	G9731D	G9731C	G9731B	G9731A
Custom 0.5–2.9 Mb	G9732D	G9732C	G9732B	G9732A
Custom 3–5.9 Mb	G9733D	G9733C	G9733B	G9733A
Custom 6–11.9 Mb	G9734D	G9734C	G9734B	G9734A
Custom 12–24 Mb	G9735D	G9735C	G9735B	G9735A
Custom 24–50 Mb	G9736D	G9736C	Not offered	Not offered
Human All Exon V7	G9771D	G9771C	G9771B	G9771A
Human All Exon V8	G9772D	G9772C	Not offered	Not offered
None (kit includes empty Probe Input Strips for run-time probe setup)	G9730D	Not offered	G9730B	Not offered

Magnis SureSelect XT HS Rev B Reagent Kit Contents

Magnis SureSelect XT HS Rev B Reagent Kits include the component kits listed in Table 17, with the contents of each component kit detailed in Table 18 through Table 23.

Table 17 Component kits provided with Magnis SureSelect XT HS Rev B 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 18	p/n varies; see Table 18
Magnis SureSelect XT HS Reagent Plates Rev B ILM	−20°C	5191-6805	5191-6804
Magnis SureSelect XT HS Index Plate, ILM	−20°C	5190-9880	5191-5673
Magnis SureSelect XT HS Beads/Buffers Plates, ILM	+4°C	5190-9692	5191-5674
Magnis Empty Consumables	Room Temperature	5190-9712	5191-5675
Magnis Sample Input Strips	Room Temperature	5190-9882	5191-5676

* May also be labeled as *Magnis SureSelect XT HS Probe Plate, Pre-filled Single Well Format*.

† Kit part number G9730D does not include a Magnis Probe Plate. Instead the G9730D 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 18 Magnis SureSelect Probe Plate, Pre-filled Single Well Format part numbers

Reagent Kit p/n	Included Probe design	Probe Plate p/n	Quantity per kit
G9731D (96 Reactions)	Custom 1–499 kb (Tier 1)	5191-6817 or 5191-6816*	1 plate (12 strips)
G9731C (32 Reactions)	Custom 1–499 kb (Tier 1)	5191-6807 or 5191-6806*	1 plate (4 strips)
G9732D (96 Reactions)	Custom 0.5 –2.9 Mb (Tier 2)	5191-6819 or 5191-6818*	1 plate (12 strips)
G9732C (32 Reactions)	Custom 0.5 –2.9 Mb (Tier 2)	5191-6809 or 5191-6808*	1 plate (4 strips)
G9733D (96 Reactions)	Custom 3–5.9 Mb (Tier 3)	5191-6821 or 5191-6820*	1 plate (12 strips)
G9733C (32 Reactions)	Custom 3–5.9 Mb (Tier 3)	5191-6811 or 5191-6810*	1 plate (4 strips)
G9734D (96 Reactions)	Custom 6–11.9 Mb (Tier 4)	5191-6823 or 5191-6822*	1 plate (12 strips)
G9734C (32 Reactions)	Custom 6–11.9 Mb (Tier 4)	5191-6813 or 5191-6812*	1 plate (4 strips)
G9735D (96 Reactions)	Custom 12–24 Mb (Tier 5)	5191-6825 or 5191-6824*	1 plate (12 strips)
G9735C (32 Reactions)	Custom 12–24 Mb (Tier 5)	5191-6815 or 5191-6814*	1 plate (4 strips)
G9736D (96 Reactions)	Custom 24–50 Mb	5191-6846	1 plate (12 strips)
G9736C (32 Reactions)	Custom 24–50 Mb	5191-6845	1 plate (4 strips)
G9771D (96 Reactions)	Human All Exon V7	5191-6827	1 plate (12 strips)
G9771C (32 Reactions)	Human All Exon V7	5191-6826	1 plate (4 strips)
G9772D (96 Reactions)	Human All Exon V8	5191-6974	1 plate (12 strips)
G9772C (32 Reactions)	Human All Exon V8	5191-6973	1 plate (4 strips)

* The Probe Plate p/n appropriate for your custom probe design is determined by Agilent when you place an order for a Magnis SureSelect XT HS Rev B Reagent Kit. Both part numbers listed for each reagent kit are fully compatible with the Magnis protocols supported in this publication.

Table 19 Components of Magnis SureSelect XT HS Reagent Plates Rev B ILM kit

Part Number (kit size)	Component provided	Quantity and format
5191-6805 (96 Reactions)	Magnis SureSelect XT HS Reagent Plate Rev B ILM	12 plates (use 1 plate per run)
5191-6804 (32 Reactions)		4 plates (use 1 plate per run)

Table 20 Components of Magnis SureSelect XT HS Index Plate, ILM kit

Part Number (kit size)	Component provided	Quantity and format
5190-9880 (96 Reactions)	Magnis SureSelect XT HS Index Plate, ILM	1 plate of 12 strips (use 1 strip per run)
5191-5673 (32 Reactions)		1 plate of 4 strips (use 1 strip per run)

Table 21 Components of Magnis SureSelect XT HS Beads/Buffers Plates, ILM kit

Part Number (kit size)	Component provided	Quantity and format
5190-9692 (96 Reactions)	Magnis SureSelect XT HS Beads/Buffers Plate, ILM	12 plates (use 1 plate per run)
5191-5674 (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)

Magnis SureSelect XT HS (Original Format) Reagent Kit Contents

Magnis SureSelect XT HS Reagent Kits (original format) include the component kits listed in Table 24, with the contents of each component kit detailed in Table 25 through Table 31.

Table 24 Component kits provided with Magnis SureSelect$^{XT HS}$ Reagent Kits

Component kit name	Storage condition	Component kit p/n	
		96 Reactions	32 Reactions
Magnis SureSelect XT HS Probe Plate*	−80°C*	p/n varies; see Table 25	p/n varies; see Table 25
Magnis SureSelect XT HS Reagent Plates, ILM	−20°C	5190-9688	5191-5672
Magnis SureSelect XT HS Index Plate, ILM	−20°C	5190-9880	5191-5673
Magnis SureSelect XT HS Beads/Buffers Plates, ILM	+4°C	5190-9692	5191-5674
Magnis Empty Consumables	Room Temperature	5190-9712	5191-5675
Magnis Sample Input Strips	Room Temperature	5190-9882	5191-5676

* Kit part number G9730B does not include a Magnis SureSelect XT HS Probe Plate. Instead the G9730B 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 25 Probe Plate part numbers

Reagent Kit p/n	Included Probe design	Probe Plate p/n	Quantity per kit
G9731B (96 Reactions)	Custom 1–499 kb	5190-9690 or 5190-9691*	1 plate (12 strips)
G9731A (32 Reactions)	Custom 1–499 kb	5191-5677 or 5191-5678*	1 plate (4 strips)
G9732B (96 Reactions)	Custom 0.5 –2.9 Mb	5190-9884 or 5190-9955*	1 plate (12 strips)
G9732A (32 Reactions)	Custom 0.5 –2.9 Mb	5191-5679 or 5191-5680*	1 plate (4 strips)
G9733B (96 Reactions)	Custom 3–5.9 Mb	5190-9886 or 5190-9956*	1 plate (12 strips)
G9733A (32 Reactions)	Custom 3–5.9 Mb	5191-5681 or 5191-5682*	1 plate (4 strips)
G9734B (96 Reactions)	Custom 6–11.9 Mb	5190-9888 or 5190-9957*	1 plate (12 strips)
G9734A (32 Reactions)	Custom 6–11.9 Mb	5191-5683 or 5191-5684*	1 plate (4 strips)
G9735B (96 Reactions)	Custom 12–24 Mb	5190-9890 or 5190-9958*	1 plate (12 strips)
G9735A (32 Reactions)	Custom 12–24 Mb	5191-5685 or 5191-5686*	1 plate (4 strips)
G9771B (96 Reactions)	Human All Exon V7	5191-5721	1 plate (12 strips)
G9771A (32 Reactions)	Human All Exon V7	5191-5720	1 plate (4 strips)
G9730B (96 Reactions)	None (provided with empty Magnis Probe Input Strips)	5190-9883 (empty probe input strips)	12 strips

* The Probe Plate p/n appropriate for your custom probe design is determined by Agilent when you place an order for a Magnis SureSelect XT HS Reagent Kit. Both part numbers listed for each reagent kit are fully compatible with the Magnis protocols supported in this publication.

Table 26 Components of Magnis SureSelect XT HS Reagent Plates, ILM kit

Part Number (kit size)	Component provided	Quantity and format
5190-9688 (96 Reactions)	Magnis SureSelect XT HS Reagent Plate, ILM	12 plates (use 1 plate per run)
5191-5672 (32 Reactions)		4 plates (use 1 plate per run)

Table 27 Components of Magnis SureSelect XT HS Index Plate, ILM kit

Part Number (kit size)	Component provided	Quantity and format
5190-9880 (96 Reactions)	Magnis SureSelect XT HS Index Plate, ILM	1 plate of 12 strips (use 1 strip per run)
5191-5673 (32 Reactions)		1 plate of 4 strips (use 1 strip per run)

Table 28 Components of Magnis SureSelect XT HS Beads/Buffers Plates, ILM kit

Part Number (kit size)	Component provided	Quantity and format
5190-9692 (96 Reactions)	Magnis SureSelect XT HS Beads/Buffers Plate, ILM	12 plates (use 1 plate per run)
5191-5674 (32 Reactions)		4 plates (use 1 plate per run)

Table 29 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 30 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)

Table 31 Components of the Magnis Probe Input Strip kit

Part Number (kit size)	Components provided	Quantity and format
5190-9883 (96 Reactions)	Magnis Probe Input Strip	12 empty white, foil-sealed strips
	Magnis Foil Seals	2 sheets (6 single-strip tube foil sealing strips per sheet)

Reference Information for SureSelect XT HS Indexes

Plate Position Information

Primers used to index the sequencing libraries are provided in the Magnis SureSelect XT HS Index Plate, ILM. Plates contain the SureSelect XT HS indexes A01-H04 in single-use aliquots in the individual wells of strip tubes contained in a plate support. The plate provided with 32 Reaction kits (p/n 5191-5673) contains one set of four (4) strips labeled *A1*, *A2*, *A3*, or *A4*, with each of 32 unique indexes A01-H04 provided in a single well. See Table 32 for a plate map.

Table 32 Index map for Magnis SureSelect XT HS Index Plate, ILM provided with **32 Reaction kits**

Plate Column	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Strip Tube Label	**A1**	**A2**	**A3**	**A4**								
	A01	A02	A03	A04	—	—	—	—	—	—	—	—
	B01	B02	B03	B04	—	—	—	—	—	—	—	—
	C01	C02	C03	C04	—	—	—	—	—	—	—	—
	D01	D02	D03	D04	—	—	—	—	—	—	—	—
	E01	E02	E03	E04	—	—	—	—	—	—	—	—
	F01	F02	F03	F04	—	—	—	—	—	—	—	—
	G01	G02	G03	G04	—	—	—	—	—	—	—	—
	H01	H02	H03	H04	—	—	—	—	—	—	—	—

The plate provided with 96 Reaction kits (p/n 5190-9880) contains 12 strips, made up of three sets of the four (4) strips labeled *A1*, *A2*, *A3*, or *A4* with each of 32 unique indexes A01-H04 provided in three wells. See Table 33 for a plate map. Do not use the same index strip number for multiple runs used to prepare libraries that will be multiplexed for NGS.

Table 33 Index map for Magnis SureSelect XT HS Index Plate, ILM provided with **96 Reaction kits**

Plate Column	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Strip Tube Label	**A1**	**A2**	**A3**	**A4**	**A1**	**A2**	**A3**	**A4**	**A1**	**A2**	**A3**	**A4**
	A01	A02	A03	A04	A01	A02	A03	A04	A01	A02	A03	A04
	B01	B02	B03	B04	B01	B02	B03	B04	B01	B02	B03	B04
	C01	C02	C03	C04	C01	C02	C03	C04	C01	C02	C03	C04
	D01	D02	D03	D04	D01	D02	D03	D04	D01	D02	D03	D04
	E01	E02	E03	E04	E01	E02	E03	E04	E01	E02	E03	E04
	F01	F02	F03	F04	F01	F02	F03	F04	F01	F02	F03	F04
	G01	G02	G03	G04	G01	G02	G03	G04	G01	G02	G03	G04
	H01	H02	H03	H04	H01	H02	H03	H04	H01	H02	H03	H04

Index Nucleotide Sequences

The nucleotide sequence of each SureSelect XT HS index is shown in Table 34. Each index is 8 nt in length, and sequencing runs should be completed using 8-bp index reads (see page 60).

Table 34 SureSelect XT HS Indexes A01 through H04

Strip A1		Strip A2		Strip A3		Strip A4	
Index	Sequence	Index	Sequence	Index	Sequence	Index	Sequence
A01	GTCTGTCA	A02	GCGAGTAA	A03	AGCAGGAA	A04	CCGTGAGA
B01	TGAAGAGA	B02	GTCGTAGA	B03	AGCCATGC	B04	GACTAGTA
C01	TTCACGCA	C02	GTGTTCTA	C03	TGGCTTCA	C04	GATAGACA
D01	AACGTGAT	D02	TATCAGCA	D03	CATCAAGT	D04	GCTCGGTA
E01	ACCACTGT	E02	TGGAACAA	E03	CTAAGGTC	E04	GGTGCGAA
F01	ACCTCCAA	F02	TGGTGGTA	F03	AGTGGTCA	F04	AACAACCA
G01	ATTGAGGA	G02	ACTATGCA	G03	AGATCGCA	G04	CGGATTGC
H01	ACACAGAA	H02	CCTAATCC	H03	ATCCTGTA	H04	AGTCACTA

CAUTION

Sequences of the SureSelect XT HS system index A01 through H04 differ from the sequences of Agilent's SureSelect XT system A01 through H04.

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 1–12 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 1–12 can also be found in the run log file.

The specific SureSelect XT HS indexes associated with each Index Strip number 1–12 are shown in Table 35.

Table 35 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)**	**Index by Sample Number in Run**							
		Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**	**Sample 7**	**Sample 8**
1	A1	A01	B01	C01	D01	E01	F01	G01	H01
2	A2	A02	B02	C02	D02	E02	F02	G02	H02
3	A3	A03	B03	C03	D03	E03	F03	G03	H03
4	A4	A04	B04	C04	D04	E04	F04	G04	H04
5	A1	A01	B01	C01	D01	E01	F01	G01	H01
6	A2	A02	B02	C02	D02	E02	F02	G02	H02
7	A3	A03	B03	C03	D03	E03	F03	G03	H03
8	A4	A04	B04	C04	D04	E04	F04	G04	H04
9	A1	A01	B01	C01	D01	E01	F01	G01	H01
10	A2	A02	B02	C02	D02	E02	F02	G02	H02
11	A3	A03	B03	C03	D03	E03	F03	G03	H03
12	A4	A04	B04	C04	D04	E04	F04	G04	H04

Troubleshooting Guide

Troubleshooting guidelines are included below for running the automated SureSelect XT HS 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 use of touchscreen for run setup presents usability issues or if touchscreen appears unresponsive

✔ As an alternative to the touchscreen controls, you can use a USB-connected mouse to make selections and enter data in the Magnis software. Connect the mouse using either of the two USB ports on the front of the instrument. Once connected, use the mouse point-and-click functions to make selections on the interface displayed on the touchscreen.

✔ Reboot the system to reset touchscreen functionality.

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 filled sample strip, index strip and probe strip in left-to-right order.

✔ 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 29 to page 33 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 the scanned Reagent Plate and Probe Input Strip are identified as the *wrong labware***

  When the Reagent Plate and the Probe Input Strip, specifically, are 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 the 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 HS **Rev B** Reagent Kits supplied with **pre-filled probe input strips**	*SSEL XTHS-RevB-ILM* **OR** *LT-SSEL XTHS-RevB-ILM*
Magnis SureSelect XT HS **Rev B** Reagent Kit supplied with **empty probe input strips** (filled at run time)	*SSEL XTHS-EPIS-RevB-ILM* **OR** *LT-SSEL XTHS-EPIS-RevB-ILM*
Magnis SureSelect XT HS Reagent Kits in **original format** supplied with **pre-filled probe input strips**	*SureSelectXT HS-Illumina*
Magnis SureSelect XT HS Reagent Kits in **original format** supplied with **empty probe input strips** (filled at run time)	*SureSelectXT HS-Illumina*

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

  Replace the misplaced labware with the correct labware component 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 library fragment size is larger than expected in electropherograms

✔ Shearing may not be optimal. For intact, high-quality DNA samples, ensure that shearing is completed using the two-round shearing protocol provided, including all spinning and vortexing steps.

✔ Any bubbles present on the microTUBE filament may disrupt complete shearing. Spin the microTUBE for 30 seconds before the first round of shearing to ensure that any bubbles are released.

If yield of post-capture libraries is low

- ✔ 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 37 for more information.
- ✔ Verify that the input DNA sample meets the guidelines for quality and concentration range specified in "Appendix 1: DNA Sample Preparation Guidelines"
- ✔ Verify that the run was set up for the appropriate input DNA concentration and quality. Settings may be checked on the **Run Setup** tab of the **Post Run Data** screen for the run.
- ✔ Verify that DNA shearing was performed in Low TE Buffer, and not in water. Shearing DNA samples in water reduces overall library yield and complexity.
- ✔ 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.

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$^{XT\ HS}$ target-enriched Illumina paired-end multiplexed sequencing libraries using the Magnis NGS Prep System.

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