illumina®

SomaSeq Discovery v2

Product Documentation for use with the Plasma/Serum Kit

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Overview

This resource explains how to prepare and analyze samples to detect and measure protein abundance using the SomaSeq Discovery v2 kit. The SomaSeq Discovery v2 kit measures protein from plasma, serum, and cerebral spinal fluid (CSF) samples, along with certain cell lysate and tissue homogenate samples that are validated for use in the Illumina Multi Matrix sample kit.

The SomaSeq Discovery v2 workflow provides the following features:

- A high throughput proteomics assay that provides over 10,000 SOMAmer reagents corresponding to over 9,000 unique human targets.
- An end-to-end automated workflow on the SomaSeq Automation System (SomaSeqAS) platform that includes the SomaSeq Discovery v2 assay and generation of sequencing-ready libraries.
- Secondary analysis of the sequenced libraries, using the DRAGEN Protein Quantification analysis software. You can perform secondary analysis locally via a v4 DRAGEN server, or in the cloud via BaseSpace Sequence Hub and Illumina BioInsight Platform Core (Platform Core).
  For details, refer to the following resources:
  - BaseSpace Sequence Hub support resources
  - Illumina BioInsight Platform Core support resources
- Tertiary analysis via Illumina Connected Multiomics (ICM).
  For details, refer to the following resource:
  - Illumina Connected Multiomics support resources

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Warnings and Precautions

⚠ **This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.** For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

⚠ Handle all biological samples as if they are potentially infectious agents.

⚠ Certain assay and kit reagents are classified as hazardous and contain warning labels to indicate the hazard. They may cause eye irritation, skin irritation, respiratory irritation, or organ effects with prolonged or repeated exposure. Avoid contact with skin and eyes and avoid inhalation of vapors or aerosols.

- Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and assay reagents. Wash hands thoroughly after handling specimens and assay reagents.
- Do not use any assay components beyond their stated expiration date on the assay box label. Do not interchange assay components from different assay lots. Assay lots are identified on the assay box label. Store the assay components at the specified temperature.
- Failure to follow the procedures as outlined can result in erroneous results or significant reduction in sample result quality.
- For environmental, health, and safety information, refer to the safety data sheets (SDS) at support.illumina.com/sds.html.
- Before beginning the protocol, review the SomaSeqAS safety and compliance information and safety hazards. Refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

Specimen Transport and Storage

⚠ Handle all biological samples as if they are potentially infectious agents.

- Transportation of samples must comply with all applicable governing regulations for the transport of etiologic agents. Expedited shipping and transportation methods are recommended.
- Transport biological specimens or cell or tissue extracts on dry ice. After samples are received, store at -80°C until ready to use.
- If retesting is required, you can cap unused samples and store at -80°C for future use.

Up to three freeze-thaw cycles have been tested on plasma and serum samples only. Specific protein stability for other sample types varies and should be evaluated individually. Avoid extended sample storage at room temperature as this might negatively impact protein stability.

Exposure to elevated temperatures, such as above room temperature, can negatively impact individual sample failure rates and sample performance.

Tips and Techniques

Follow the protocol steps precisely. Changes in volumes, timing, or steps can result in failure of assay quality control metrics.

Unless a safe stopping point is specified in the protocol, proceed immediately to the next step.

Up to three freeze-thaw cycles have been tested on plasma and serum samples only. Specific protein stability for other sample types varies and should be evaluated individually. Avoid extended sample storage at room temperature as this might negatively impact protein stability. This guidance applies to samples and reagents.

Avoiding Cross-Contamination

- Use separate areas for pre-PCR and post-PCR processes and cleanup. PCR products can contaminate reagents, instruments, and samples, delaying normal operations and causing inaccurate results. For lab setup details, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.
- Store supplies used for pre-PCR protocol steps in the pre-PCR area. Transfer supplies to the post-PCR area as needed.
- When adding or transferring samples or reagent master mixes by manual pipette, change tips between ***each sample***.
- Always use new, unused plate seals.
- Always use clean tips and other consumables.
- Follow applicable governing regulations for proper laboratory practice and hygiene when handling potentially infectious samples.
- You might be required to perform some steps in a biosafety cabinet. Follow the applicable governing regulations for your region.

Sealing Plates and Tubes

- Reagents in the SOMAmer-Bead dilution plates are generally stable in indoor ambient lighting but are sensitive to long-wave UV light. For best practice, protect the bead plates from light by covering them with a foil seal until loading onto the deck.
- Before starting the following steps in the protocol, seal the 96-well plate with the adhesive seal. Use a plate paddle seal applicator to cover the plate with the seal.
  - Shaking steps

  - Centrifuge steps
  - Thermal cycling steps
- Use Microseal 'B' adhesive seals for thermal cycling or short-term storage. Microseal 'B' seals are effective at -40°C to 110°C and suitable for skirted or semiskirted PCR plates.
- Microseal 'F' foil seals are effective at -70°C to 105°C and suitable for skirted or semiskirted plates.

Loading Tips

When loading disposable tips onto the instrument deck, stack the tips as straight and centered as possible.

Loading Plates into Centrifuge

To balance the centrifuge during reagent preparation, Illumina recommends creating duplicate plates filled with water and placing them on the opposite side of the centrifuge.

Plasma and Serum Sample Types

This section provides all information necessary to run SomaSeq Discovery v2 and generate sequencing-ready libraries from ~55 µl input of human plasma or serum.

- **Input Recommendations and Requirements**—This includes guidelines for collecting and processing plasma and serum samples.
- **Consumables and Equipment**—This includes all components included in the reagent kit, with storage conditions. This also details the ancillary consumables, equipment, and other prerequisites needed to complete the protocol.
- **Protocol**—This includes all steps necessary to prepare the samples, interact with the Illumina Lab Automation Software Solution (ILASS), prepare for each protocol, clean up and quantify libraries, and finally dilute the libraries to the appropriate concentration for sequencing.

Input Recommendations and Requirements

SomaSeq Discovery v2 is compatible with human plasma, with ethylenediaminetetraacetic acid (EDTA) as anticoagulant, and serum samples. Guidelines for collecting and processing samples for the SomaSeq Discovery v2 protocol are as follows.

⚠ Handle all biological samples as if they are potentially infectious agents.

Avoid processing different sample types together in the same plate. Processing different sample types in the same plate can result in erroneous data.

Samples that have been improperly stored or handled can become unsuitable for use. If unsuitable samples are processed through the workflow, they can clog the pipette tips and cause sample failure or cross-contamination.

Sample Volume

Input Sample Type	Recommended Minimum Volume
Human plasma	55 µl
Human serum	55 µl

Sample Collection

- Whole blood specimens for plasma samples must be collected in EDTA blood collection tubes. Do not freeze whole blood specimens. Illumina cannot guarantee the performance of the SomaSeq Discovery v2 protocol for samples collected using other blood collection tubes (for example, citrate plasma or heparin plasma). Avoid adding anticoagulant to serum samples.
- Check the expiration date on the collection tubes and replace the tubes that have expired.
- This protocol assumes that the venipuncture was performed per institutional guidelines.
- If collecting more than one sample type, follow the collection order specified in the guidelines provided by the tube manufacturer.

General Processing

- Proper processing of the collected samples is critical.
- Do not chill the collection tubes before processing.
- Perform all steps at room temperature.
- For tubes that have minimum and maximum fill lines, follow the fill guidelines indicated.
- Hemolyzed samples (colored pink to red) can prevent accurate biomarker discovery.
- Observe all specified time recommendations.
  - Avoid leaving samples at room temperature longer than the procedure requires.
  - Complete processing and freezing of samples within 2 hours of collection. Freeze processed samples at -80°C.

Plasma Processing

- Begin processing plasma samples within 30 minutes of collection.
- Before placing the collection tubes in the centrifuge, invert a minimum of 5 times to make sure that EDTA and blood are well mixed.
- Do not add additives other than EDTA to the samples.

- Centrifuge collection tubes at room temperature. Centrifuge at 2200 × g (RCF) for 15 minutes. This speed optimizes separation of all cellular contents and platelets from plasma. Standard centrifugation of 1300 × g for 10 minutes provides comparable results.
  - Observe separation of blood cells and plasma, with plasma layer on top.
- Aliquot plasma samples immediately into appropriately labeled 0.5 ml Matrix screw-top tubes. Place into a sample rack.
- Aliquot only the plasma layer. To avoid disturbing the buffy coat, leave a residual of plasma and avoid the cell layer.
- Store aliquoted samples in a -80°C freezer.
- Avoid multiple freeze-thaw cycles of plasma samples.

> Up to three freeze-thaw cycles have been tested on plasma and serum samples only. Specific protein stability for other sample types varies and should be evaluated individually. Avoid extended sample storage at room temperature as this might negatively impact protein stability.

Serum Processing

- Allow serum to clot for 60–90 minutes at room temperature before centrifuging.
- Centrifuge collection tubes at room temperature. Centrifuge at 2200 × g (RCF) for 15 minutes. This speed optimizes separation of clot from serum. Standard centrifugation of 1300 × g for 10 minutes provides comparable results.
  - Observe separation of blood cells and serum, with serum layer on top.
- Aliquot serum samples within 30 minutes of centrifugation into appropriately labeled 0.5 ml Matrix screw-top tubes. Place into a sample rack.
- Aliquot only the serum layer.
- Store aliquoted samples in a -80°C freezer. Storage effects at -20°C have not been evaluated for the SomaSeq Discovery v2 assay.
- Avoid multiple freeze-thaw cycles of serum samples.

> Up to three freeze-thaw cycles have been tested on plasma and serum samples only. Specific protein stability for other sample types varies and should be evaluated individually. Avoid extended sample storage at room temperature as this might negatively impact protein stability.

Product Contents

The SomaSeq Discovery v2 protocol requires library prep reagents and index adapters. Index adapters are sold separately.

Component	Kit Options	Catalog #
Library prep reagents	Illumina SomaSeq Discovery v2, Plasma, 96 Reactions*	20167042
	Illumina SomaSeq Discovery v2, Serum, 96 Reactions*	20167043
Index adapters	Illumina DNA/RNA UD Index v3, Auto (96 Indexes, 96 Samples) One of the following sets:	
	• Set A • Set B • Set C • Set D	• 20141196 • 20141197 • 20141198 • 20141199

* Processes 85 samples and 11 controls.

Reagent Kit Contents

Each SomaSeq Discovery v2 kit includes the components listed in the following tables.

Store all reagent kit boxes in an upright position.

Illumina SomaSeq Discovery v2, Assay Buffer, 4°C Box

Quantity	Reagent	Abbreviation	Storage Temperature
3 (250 ml bottles)	Assay Buffer	AB	2°C to 8°C
3	Loose Assay Buffer Trough Labels	None	2°C to 8°C

Illumina SomaSeq Discovery v2, Ambient Box

Quantity	Reagent	Abbreviation	Storage Temperature
1	MB Prep Plate	MPR	15°C to 30°C
10	Loose Labels	None	15°C to 30°C

Illumina SomaSeq Discovery v2, Proteomics Assay Reagents, 4°C Box

Quantity	Reagent	Abbreviation	Storage Temperature
1	Bead Elution Plate	BEP	2°C to 8°C
1	MB Wash Plate	MBW	2°C to 8°C
1	Photocleavage Plate	PHC	2°C to 8°C
1	Quench Plate	QCH	2°C to 8°C

Illumina SomaSeq Discovery v2, Tag Reagent, -20°C Box

Quantity	Reagent	Abbreviation	Storage Temperature
1	Tag Plate	TAG	-30°C to -10°C

Illumina SomaSeq Discovery v2, SOMAmer 3-Dilution, -20°C Box

Quantity	Reagent	Abbreviation	Storage Temperature
1	SOMAmer-Bead Plate 11k v5.0 Dil 1	SBP1	-30°C to -10°C
1	SOMAmer-Bead Plate 11k v5.0 Dil 2	SBP2	-30°C to -10°C
1	SOMAmer-Bead Plate 11k v5.0 Dil 3	SBP3	-30°C to -10°C

Illumina SomaSeq Discovery v2, Plasma/Serum, -80°C Box

Certain assay and kit reagents are classified as hazardous and contain warning labels to indicate the hazard. They may cause eye irritation, skin irritation, respiratory irritation, or organ effects with prolonged or repeated exposure. Avoid contact with skin and eyes and avoid inhalation of vapors or aerosols.

Quantity	Reagent	Abbreviation	Storage Temperature
1	Plasma/Serum Diluent Plate	PLD/SRD	-30°C to -10°C

Quantity	Reagent	Abbreviation	Storage Temperature
5	Plasma/Serum Calibrator 11K	C-P/C-S	-90°C to -70°C
3	Plasma/Serum QC1 11K	Q-P/Q-S	-90°C to -70°C
3	Blank	None	-90°C to -70°C

Illumina SomaSeq Discovery v2, Library Prep Box 1

Quantity	Reagent	Abbreviation	Storage Temperature
1	Illumina Purification Beads	IPB	15°C to 30°C
1	Resuspension Buffer	RSB	15°C to 30°C

Illumina SomaSeq Discovery v2, Library Prep Box 2

Quantity	Reagent	Abbreviation	Storage Temperature
1	Proteomics Hybridization Plate 2	HP2	2°C to 8°C

Illumina SomaSeq Discovery v2, Plasma and Serum, Library Prep Box 3

Quantity	Reagent	Abbreviation	Storage Temperature
1	Proteomics Probe Plate 2	PP2	-25°C to -15°C
1	Proteomics Amplification Plate 2	AP2	-25°C to -15°C
1	Enhanced Enrichment Wash	EEW	-25°C to -15°C

User-Supplied Consumables and Equipment

The following sections provide information on the required user-supplied consumables and equipment.

Unless otherwise specified, using substitute consumables and equipment can cause system shutdown or erroneous results.

Consumables

Quantity	Consumable	Supplier
6 ml	[Optional] 1N HCl	General lab supplier
2	Abgene 96 Well 0.8ml Polypropylene DeepWell Sample Processing & Storage Plate for Genomics and NGS library preparation, round, conical bottom (V-well)	Thermo Fisher Scientific, catalog # AB0859
2	Axygen Silicone 96 Round Well Compression Flat Mat for Microplates, Nonsterile	Fisher Scientific, catalog # CM-FLAT
Varies[1]	Bleach disinfectant (Sodium hypochlorite, NaOCl)[2]	General lab supplier
Varies[1]	Deionized water	General lab supplier
1	Ethanol 200 proof (absolute) for molecular biology (500 ml)	Sigma-Aldrich, catalog # E7023
1	Ethanol alcohol wipes, 70% (box)	General lab supplier
3	Hard-Shell 96-Well PCR plates, low profile, thin wall, skirted	Bio-Rad, catalog # HSP9601
3	LoBind microcentrifuge tubes, 1.5 ml	Eppendorf, catalog # 022431021
1	Matrix screw-top tube, one of the following: • Matrix 0.5 ml ScrewTop Tubes in Barcoded Latch Racks • Matrix 500 µl ScrewTop Tubes in Latch Racks (you must provide a separate barcode)	Thermo Fisher Scientific: • Catalog # 3744-BR • Catalog # 3744
7	MCA 96 Disposable Tips – Non-filtered, Pure, Nested 8 Stack (Passive Stack), 200 µl	Tecan, catalog # 30038619
4	MCA 96 Disposable Tips – Non-filtered, Pure, Single Stack, 200 µl	Tecan, catalog # 30038616
2[3]	Microseal 'B' PCR Plate Sealing Film, adhesive, optical	Bio-Rad, catalog # MSB1001
3	Microseal 'F' PCR Plate Seal, foil, pierceable	Bio-Rad, catalog # MSF1001B
1	Nuclease-free water (120 µl)	General lab supplier

Quantity	Consumable	Supplier
1	**[Optional]** Pierce 96-Well Polystyrene Plate, Corner Notch	Thermo Fisher Scientific, catalog # 15041
5	Nunc 96-Well Polypropylene Sample Processing & Storage Microplates, round bottom	Thermo Fisher Scientific, catalog # 267334
1	PCR strip, 8-tube, with caps	General lab supplier
2	Pipette tips, 20 µl (box)	General lab supplier
3	Pipette tips, 200 µl (box)	General lab supplier
2	Pipette tips, 1000 µl (box)	General lab supplier
4	Qubit Assay Tubes	Thermo Fisher Scientific, catalog # Q32856
1	Qubit dsDNA HS Quantification Assay Kit	One of the following, depending on the number of reactions: • Thermo Fisher Scientific, catalog # Q32851 • Thermo Fisher Scientific, catalog # Q32854
1	**[Optional]** Pierce Dilution-Free Rapid Gold BCA Protein Assay	Thermo Fisher Scientific, catalog # A55862

Quantity	Consumable	Supplier
3	Reagent reservoirs, 96 well, pyramid base, any of the following: • Reservoir, single cavity, polypropylene, 300 ml, 96 pyramids base geometry • Axygen Single Well Reagent Reservoir with 96-Bottom Troughs, High Profile, Sterile • 96 Well Reservoir, Pyramid Bottom, Natural Polypropylene, 25pk	One of the following: • Agilent, catalog # 201244-100 • Corning Life Sciences, catalog # RES-SW96-HP-SI • Element Lab Solutions, catalog # 390001
2	Solid waste containers, either of the following[4]: • Fisherbrand Burn-up Bin Biohazard Waste Boxes • Clinisafe 60 L Clinical Waste Container	One of the following or equivalent: • Fisher Scientific, catalog # 19-168-430 • Fisher Scientific, catalog # 17641725

[1] Volume required per run depends on cleaning, spillage, and decontamination needs.
[2] Determine the appropriate disinfectant for SomaSeqAS cleaning procedures, in accordance with your applicable regional, national, and local laws and regulations.
[3] The protocol includes several optional steps that require plate sealing film. The quantity specified does not include these steps.
[4] Determine the appropriate waste container in accordance with your applicable regional, national, and local laws and regulations.

Equipment

Equipment	Supplier	Pre-PCR	Post-PCR
8-Channel Screw Cap Decapper	Thermo Fisher Scientific, catalog # 4105MAT, or equivalent	X	

Equipment	Supplier	Pre-PCR	Post-PCR
BioMicroLab Scan barcode reader	SPT LabTech, catalog # BML-Scan, or equivalent	X	
BioMicroLab Thaw rack thawing station	SPT LabTech, catalog # BML-RTS, or equivalent	X	
BioShake XP benchtop shaker	QInstruments, catalog # 1808-0505, or equivalent	X	
Centrifuge 5810 - benchtop centrifuge, or equivalent with the following specifications: • Compatible with deep-well plates • Capable of 1000 × g (RCF)	Eppendorf, catalog # 022625004, or equivalent	X	
Centrifuge, non-refrigerated	General lab supplier		X
[Optional] Biotek Cytation 5 Cell Imaging and Multimode Reader, or equivalent imaging reader with the following specifications: • Excitation: 540/18 • Emission: 579/20 • Gain: 88 • Optics: Top	Agilent, catalog # CYT5MFAV-SN, or equivalent	X	X
DynaMag-2 Magnet (for 1.5–2 ml tubes)	Thermo Fisher Scientific, catalog # 12321D, or equivalent		X
[Optional] DynaMag-96 Magnet Side Skirted Magnet (for 96-well skirted PCR plates)	Invitrogen, catalog # 12-027, or equivalent		X
Flammables cabinet	General lab supplier		X
Freezer, -20°C	General lab supplier	X	X
Freezer, -80°C	General lab supplier	X	
Minifuge	General lab supplier		X
Pipettes, multichannel, 20 µl	General lab supplier		X

Equipment	Supplier	Pre-PCR	Post-PCR
Pipettes, single channel, 20 µl	General lab supplier		X
Pipettes, single channel, 200 µl	General lab supplier		X
Pipettes, single channel, 1000 µl	General lab supplier		X
Plate sealing paddle (plastic)	General lab supplier	X	X
Quad Microplate Shaker-Incubator Thermoshaker, Grant Instruments	VWR, catalog # 97025-554	X	
Qubit 4 Fluorometer	Thermo Fisher Scientific, catalog # Q33238, or equivalent		X
Reagent reservoirs for manual pipetting	General lab supplier	X	X
Refrigerator, 4°C	General lab supplier	X	X
Vortexer, tube	General lab supplier		X

* Pre-PCR and post-PCR both require one thermal cycler block. The pre-PCR thermal cycler must have a deep well block. The pre- and post-PCR thermal cyclers must accommodate Bio-Rad skirted PCR plates. Refer to the *Consumables* on page 10 table for details.

Thermal Cyclers

The following table lists recommended thermal cyclers or specifications. Thermal cyclers must be capable of supporting the sample volumes and temperature profiles used in this workflow, with appropriate thermal accuracy and block uniformity to ensure consistent incubation and amplification performance. Validate the thermal cycler before performing the protocol.

Performance may vary depending on the specific thermal cycler and consumables used. Minor workflow optimization may be required to account for instrument and consumable specific differences.

Thermal Cycler*	Supplier/Description

Thermal cycler with the following specifications:
• Heated lid
• Ramp rate range:
  • Min 0.1°C/sec
  • Max ≥ 2.5°C/sec
• Temperature control range:
  • Min ≤ 4°C
  • Max ≥ 99°C
• Temperature accuracy: ±0.25°C
• Temperature uniformity: ±0.5°C
• Capable of supporting reaction volumes of 100 µl
• Compatible with 96-well PCR plates (full or semi-skirted), or suitable for the applicable workflow.
	General lab supplier

PTC Tempo Deepwell Thermal Cycler (one block)	Bio-Rad, catalog # 12015392

* Thermal cyclers are required in both pre-PCR and post-PCR workflows. Pre-PCR and post-PCR both require one thermal cycler block. The pre-PCR thermal cycler must have a deep well block. The pre- and post-PCR thermal cyclers must accommodate Bio-Rad skirted PCR plates. Refer to the *Consumables* on page 10 table for details.

Consumables for Diluting and Denaturing Libraries

Additional user-supplied consumables are required for diluting and denaturing libraries.

- For NovaSeq 6000, refer to the Denature and Dilute Protocol Generator and follow the NovaSeq 6000 Standard Loading instructions.
- For NovaSeq X Series, refer to the Denature and Dilute Protocol Generator and follow the NovaSeq X Series instructions for use with the applicable control software for your lab.
  - SomaSeq Discovery v2, control software v1.4 and later
  - Illumina Protein Prep, control software v1.3

Illumina Equipment

The SomaSeq Discovery v2 protocol requires the following Illumina equipment.

Equipment	Supplier
SomaSeq Automation System (SomaSeqAS)	Illumina, catalog # 20148741

Protocol

This section describes the SomaSeq Discovery v2 protocol.

- Review the planned complete sequencing workflow, from sample through analysis, to ensure compatibility of products and experiment parameters.
- Before proceeding, confirm the kit contents and make sure that you have the required components, equipment, and consumables. This protocol requires library prep reagents and index adapters. Index adapter plates are ordered separately. Refer to *Product Contents* on page 6 for details.
- If you are combining plates for sequencing, each SampleID must be unique per sample sheet.
- **[NovaSeq 6000]** Index sequences must be unique within a sequencing run. Avoid combining plates that contain the same index sequences.
- **[NovaSeq X Series]** Index sequences can be repeated on separate lanes. Make sure that samples are designated appropriately to lanes in the sample sheet.
- The SomaSeq protocol is time sensitive. Unless a safe stopping point is specified, proceed immediately to the next step. The protocol has safe stopping points at the end of the following steps:
  - *SomaSeq Assay (Automated)* on page 31
  - *SomaSeq Assay QC (Optional)* on page 36
  - *Index PCR* on page 44
  - *Pooled Library Cleanup* on page 47
  - *Dilute Libraries to the Starting Concentration* on page 49
- Follow the protocol steps in the order shown, using the specified volumes and incubation parameters. Changes in volumes, timing, or steps can result in failure of assay quality control metrics.
- Perform all steps, including centrifuge steps, at room temperature.

Illumina Lab Automation Software Solution

Run all automated assay workflows using the Illumina Lab Automation Software Solution (ILASS) touch screen or system computer peripherals, as follows.

- Follow the prompts to load consumables, confirm deck layout, and complete steps. To proceed, select **Next**.
- When prompted to check the waste containers, empty the containers.
- Access the options to Abort or Pause the run from the lower-right corner of the screen.
  - **Abort Setup** terminates the run setup process.
  - **Abort Batch** terminates a run that is in progress.

  The ILASS requires confirmation of the request to abort the run before initiating termination.
- Only select **Continue** when prompted to do so by the main ILASS window.
- You must select **Execute Run** to initiate the automation scripts.

- The ILASS automatically closes login sessions that have been idle for 15 minutes. You must log in again to resume the workflow or to pause or abort a workflow step.

For more information, refer to the *ILASS Product Documentation (document # 200015136)*.

SomaSeq Discovery v2 Workflow

The following diagram illustrates the SomaSeq Discovery v2 workflow. Time estimates are based on processing 96 samples. Hands-on time represents the manual steps that are completed before or after an automation script. This time does not include the manual interaction that occurs during script execution.

Prepare Sample Plate and Generate Manifest File

The day before Day 1 activities, prepare the sample rack, scan the sample barcodes, and generate a sample manifest file for the SomaSeq Discovery v2 run.

When preparing the sample plate, work quickly to avoid thaw. Best practice is to keep the sample rack and the control tubes on dry ice during the plate preparation and manifest generation steps.

Avoid processing different sample types together in the same plate. Processing different sample types in the same plate can result in erroneous data.

The manifest file is a comma-separated values (CSV) file that provides the (SomaSeqAS) with information about the input samples. Only CSV files are accepted. There are no file naming constraints.

During the automated Sample Prep procedure, you must upload the manifest file to the (ILASS). Make sure to save your completed file to a location that is accessible from the SomaSeqAS computer.

Excel regional settings determine whether a comma (,) or semicolon (;) is used as the default delimiter for separating items in a file. Before saving your manifest file, you might need to adjust the settings to make sure that a comma is used as the delimiter.

Consumables

- Sample rack (barcoded).
- Matrix tubes containing the samples.
- Sample manifest template file for your sample type (plasma or serum). Download the appropriate template from the Illumina support site or from the ILASS Batch Creation screen in the Sample Prep workflow.
- The following controls:

Table 1 -90°C to -70°C Storage

Reagent	Box Name	Instructions
Blank	Illumina SomaSeq Discovery v2, Plasma/Serum -80°C Box	While the controls are frozen, add them to the sample rack. For details, refer to *Prepare the Sample Rack* on page 21.
Plasma/Serum Calibrator (C-P/C-S)		
Plasma/Serum QC Sample (Q-P/Q-S)		

Manifest File Contents

The manifest file contains the data columns listed in the following table. The columns appear beneath the header row in the file and are presented in the order specified in the table. Do not delete columns or add new columns to the manifest template file.

The manifest file columns are included in the SomaSeqAS output file that is generated after automated library preparation is completed. ILASS adds additional information to the output file during automated library preparation.

Manifest File Column	Description
[Optional] Project	The project associated with the sample. It is permitted to have different project names for each sample. The project name must be 3–36 characters in length. Only letters, numbers, dashes, and underscores are permitted.
PlateBarcode	The unique barcode of the sample matrix rack. The barcode must be 8–20 characters in length. Only letters, numbers, dashes, and underscores are permitted. Every row of the manifest file must contain the same plate barcode value.
[Optional] MatrixTubeBarcode	The unique tube barcode. The barcode must be 8–20 characters in length. Only letters, numbers, dashes, and underscores are permitted.
WellPosition	The well position for the sample. All 96 positions must be present and unique. Permitted values: A01–H12.
SampleID	The sample identifier for samples and controls (Blank, Calibrator, and QC). Only letters, numbers, dashes, and underscores are permitted. The SampleID must be 3–100 characters in length. If you are combining plates for sequencing, each SampleID must be unique per sample sheet. • [Optional] You can append a globally unique identifier to control SampleIDs to make sure that they are always unique. The **Suffix Control Samples ID with GUID** checkbox in the ILASS System Configuration screen controls this feature. When the checkbox is selected, ILASS appends identifiers automatically for all runs.
InputType	The input sample type. Permitted values: Blank, Plasma, Serum • The entire plate must use the same sample type for the entire plate. Mixing plasma and serum samples on the same sample plate is not permitted. • The sample plate must contain at least three Blank, five Calibrator, and three QC input types. • The ILASS validates that the InputType from the manifest file matches the Kit Type drop-down menu selected during the automated Sample Prep workflow.

Manifest File Column	Description
Control	The input control type. Permitted values: Blank, Calibrator, QC, Empty • If the control used is Blank, the InputType selected must also be Blank. • If the control used is a QC or Calibrator, the InputType selected must be Plasma or Serum.

Preparation

1. Create a copy of the sample manifest template file.
2. Fill out the manifest file columns for your sample plate. Follow the guidance provided in the Manifest File Contents table.
3. Save the file (CSV format).
4. Proceed as follows:
   - If you intend to scan the matrix tube barcodes, save the file to the barcode scanner computer in preparation for scanning.
   - If you do not intend to scan the matrix tube barcodes, save the file to a location that is accessible from the SomaSeqAS computer.

Prepare the Sample Rack

1. Remove the controls from the box.
2. While still frozen, distribute the Blank, Calibrator, and QC controls in the sample plate as follows.
   - Blank controls: B02, D06, G11.
   - Calibrator controls: A01, C03, G05, H08, D12.
   - QC controls: E04, F07, B10.
   - Place samples into the remaining well locations.
   - For well locations that you cannot fill with a sample, place a matrix tube containing 55 µl Assay Buffer into that position. In the manifest file, refer to this as Blank in the InputType and Control columns.

	01	02	03	04	05	06	07	08	09	10	11	12
A	Calibrator											
B		Blank								QC		
C			Calibrator									
D						Blank						Calibrator

E	QC		
F		QC	
G	Calibrator		Blank
H		Calibrator	

3. **[Optional]** Proceed immediately to scan the sample barcodes. Refer to *[Optional] Scan the Matrix Tube Barcodes* on page 22.
4. Store the sample matrix rack at -80°C until ready to begin *Prepare for Protocol* on page 23 steps.

[Optional] Scan the Matrix Tube Barcodes

If scanning the matrix tube barcodes, proceed as follows.

1. Place the sample rack on the scanner.
   Make sure that the A1 tube positions for the sample rack and the scanner are aligned.
2. Use the barcode scanner to scan the barcode on each sample tube.
3. Open the sample manifest template file. Copy and paste the scanned barcodes into the Matrix Tube Barcode column.
   Make sure that the order of the barcodes aligns with the well positions listed in the manifest file.
4. Enter the input sample type into the Input Type column of the sample manifest file. Refer to the table in Manifest File Contents for information about permitted values.
5. Save the completed manifest file (CSV format) to a location that is accessible from the SomaSeqAS computer.
6. Store the sample rack at -80°C until ready to begin *Prepare for Protocol* on page 23 steps.

Start the SomaSeq Automation System

⚠ Before beginning this procedure, review the SomaSeqAS safety and compliance information and safety hazards. Refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

This procedure starts up the SomaSeqAS and initializes the (ILASS for operation. The software prompts guide you through the equipment and deck setup.

1. Power on the SomaSeqAS.
   The power button is on the lower right corner of the instrument. Press the power button and briefly hold it to power on the instrument.
2. To avoid component collision during instrument initialization and operation, do as follows.
   a. Make sure that the Robotic Gripper Arm (RGA) fingers are leveled.
   b. Make sure that the RGA and Multi-Channel Arm (MCA) are positioned to the left side of the automation deck.
   c. Make sure that the waste chutes are clicked into position.

For more information, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

3. Open the ILASS from the desktop.
   Wait for the software to load before proceeding.
4. Make sure that the biohazard waste collection bottle is connected to the waste trough.
   For details, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.
5. Make sure that the solid waste containers and the liquid waste bottle are in place below the waste chutes.

   The solid waste containers must be centered below the chutes. This alignment prevents plates from piling up and interfering with RGA movement or falling out of the container.

6. In the ILASS, open the SomaSeq Maintenance drop-down menu and select and perform any necessary maintenance tasks.
   For details, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

Prepare for Protocol

This preparation is required to perform the protocol steps leading up to the overnight hybridization step. Illumina recommends that you gather and thaw reagents at the beginning of the day.

1. Remove the reagents from the box and thaw as follows.

Table 2 Room Temperature Storage

Reagent	Box Name	Instructions
MB Prep Plate (MPR)	Illumina SomaSeq Discovery v2, Ambient Box	Use at room temperature.

Table 3 2°C to 8°C Storage

Reagent	Box Name	Instructions
Assay Buffer (AB) (3)	Illumina SomaSeq Discovery v2, Assay Buffer, 4°C Box	Bring to room temperature. Can be used cold.
MB Wash Plate (MBW)	Illumina SomaSeq Discovery v2, Proteomics Assay Reagents, 4°C Box	Bring to room temperature.
Bead Elution Plate (BEP)	Illumina SomaSeq Discovery v2, Proteomics Assay Reagents, 4°C Box	Bring to room temperature.

Reagent	Box Name	Instructions
Photocleavage Plate (PHC)	Illumina SomaSeq Discovery v2, 4°C Box	Bring to room temperature.
Quench Plate (QCH)	Illumina SomaSeq Discovery v2, Proteomics Assay Reagents, 4°C Box	Bring to room temperature.

Table 4 -30°C to -10°C Storage

Reagent	Box Name	Instructions
Tag Plate (TAG)	Illumina SomaSeq Discovery v2, Tag Reagent, -20°C Box	Bring to room temperature.
Proteomics Probe Plate 2 (PP2)	SomaSeq Hyb Capture, Plasma and Serum, Library Prep Box 3	Bring to room temperature.
SOMAmer-Bead Plate 11k v5.0 Dil 1 (SBP1)	Illumina SomaSeq Discovery v2, SOMAmer 3-Dilution, -20°C Box	Bring to room temperature.
SOMAmer-Bead Plate 11k v5.0 Dil 2 (SBP2)	Illumina SomaSeq Discovery v2, SOMAmer 3-Dilution, -20°C Box	Bring to room temperature.
SOMAmer-Bead Plate 11k v5.0 Dil 3 (SBP3)	Illumina SomaSeq Discovery v2, SOMAmer 3-Dilution, -20°C Box	Bring to room temperature.

Table 5 -30°C to -10°C Storage

Reagent	Box Name	Instructions
Plasma/Serum Diluent Plate (PLD/SRD)	Illumina SomaSeq Discovery v2, Plasma/Serum, -80°C Box	Bring to room temperature using a thawing station for ~15 minutes.

Retain the -80°C box until the reagent scanning portion of the ILASS automation procedure is complete.

2. Save the following Hyb program on the pre-PCR thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - Set the reaction volume to 100 µl
   - 95°C for 10 minutes
   - Decrease by 0.1°C per second until reaching 50°C
   - 50°C for 1 hour
   - Decrease by 0.1°C per second until reaching 45°C
   - 45°C for 2 hours

- Decrease by 0.1°C per second until reaching 40°C
- 40°C for 4 hours
- Decrease by 0.1°C per second until reaching 37°C
- 37°C for 8 hours
- Hold at 37°C

Sample Prep (Automated)

⚠ Before beginning this procedure, review the SomaSeqAS safety and compliance information and safety hazards. Refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

This step prepares a dilution series and prepares SOMAmer-Bead dilution plates for binding. The binding step takes 3.5 hours to complete.

Consumables

- Assay Buffer (AB) (1 x 250 ml)
- Plasma/Serum Diluent Plate (PLD/SRD)
- SOMAmer-Bead Plate 11k v5.0 Dil 1 (SBP1)
- SOMAmer-Bead Plate 11k v5.0 Dil 2 (SBP2)
- SOMAmer-Bead Plate 11k v5.0 Dil 2 (SBP3)
- 96-well Nunc round bottom plates (5)
- 96-well pyramid base reagent reservoir (1)
- Prepared Sample Rack, 96 tubes (1)
- Microseal 'F' PCR plate seals (3)
- **[Optional]** Microseal 'F' PCR plate seal (1)
- Nested tips, 200 µl (2 stacks)
- Single Stack Pipette tips, 200 µl (1 stack)
- The barcode labels with the following prefix:
  - ABT (1)
  - DIL (5)
- The following SomaSeqAS cleanup consumables:
  - Bleach disinfectant
  - Deionized water

Determine the appropriate disinfectant for SomaSeqAS cleaning procedures, in accordance with your applicable regional, national, and local laws and regulations.

About Reagents

- SOMAmer-Bead dilution plates
  - Do not centrifuge or vortex.
  - Store in an upright position.

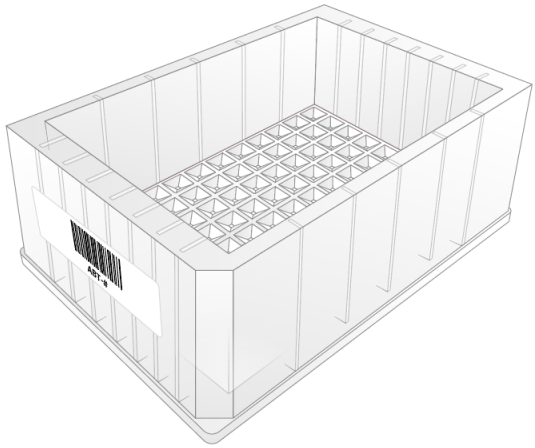
SOMAmer-Bead dilution plates thaw quickly at room temperature. If the plates thaw and are jostled or tilted, beads can stick to the foil seal. Remove the foil seal while the plates are still frozen.

If the beads are not at the bottom of the plate or are adhered to the foil seal, contact Illumina Technical Support. Return the samples to the freezer until ready to proceed. If there is only buffer adhered to the seal, you can proceed with the protocol.

Preparation

Labware Preparation

Apply barcode labels to labware as follows.

Barcode Label Prefix	Barcode Label Numbers	Labware Type	Label Position
ABT	Refers to lot number	96-well pyramid base reagent reservoir (1)	Place the barcode in the center of the left side of the reservoir. Make sure that the barcode is as straight as possible. 
DIL	Refers to serial number	96-well Nunc round bottom plates (5)	Align the lower edge of the label with the upper edge of the plate skirt or trough skirt. 

Reagent Preparation

1. Prepare the Plasma/Serum diluent plate.
    a. Remove the Plasma/Serum diluent plate from the rack thawing station.

b. Confirm that the plate contents are thawed. Only proceed when fully thawed.
c. Shake the plate at 1350 rpm for 1 minute.
d. Centrifuge the plate at 280 × g for 15 seconds.

Sample Preparation

1. Retrieve the sample rack containing samples and controls.
2. Remove the lid from the rack and thaw contents on a thawing station for at least 15 minutes.

   The maximum thaw time is 1 hour. Exceeding 1 hour can affect the assay results.
3. Confirm that the rack contents are thawed. Only proceed when fully thawed.
4. Centrifuge the rack at 1000 × g for 1 minute.
5. Using a decapper, remove the caps from all tubes in the sample rack.
   You might be required to perform this step in a biosafety cabinet. Follow the applicable governing regulations for your region.

Procedure

Sample Prep

1. In the ILASS SomaSeq Discover Module, open the SomaSeq drop-down menu and select **Sample Prep**.
2. Make sure that the doors to the deck and lower cabinet are closed, and then select **Next** to run the UVA light check.
   - If the UVA light check fails, select **Retry**.
   - If the UVA light check fails after a retry, select **Abort Setup** and power cycle the SomaSeqAS.
   - Repeat step 1. If the UVA light check fails again, contact Illumina Technical Support.
3. When prompted by the software, make sure that the solid waste containers are set up correctly and are empty. Then close the doors to the deck and lower cabinet to prepare for the liquid waste check.
4. When prompted, make sure that the liquid waste wafer is in the correct position on the deck.
5. When prompted, make sure that the magnets are in the correct positions on the deck.
6. When prompted, enter the batch name and select a sample manifest file.
7. From the Kit Type drop-down menu, select **Plasma** or **Serum**.
8. When prompted, place a pyramid base reagent reservoir with the barcode prefix ABT onto the instrument deck. Select **Next**.
9. Carefully pour the entire contents of one Assay Buffer bottle into the pyramid base reagent reservoir. Select **Next**.
10. When prompted, place the sample rack, reagent plates and consumables onto the instrument deck. Select **Next**.

Make sure that plates placed on the ABI magnets are seated correctly.

Make sure that there are no lids or seals on the consumables on deck.

The following consumables are loaded empty:

- 96-well Nunc round bottom plates with the barcode prefix DIL (5)

11. When prompted, close the instrument door, and then select **Next** to initiate barcode scanning.
12. If prompted with a plate detection or barcode error, make sure that the reagents and labware are placed in the correct positions on the instrument deck.
    - If there are misplaced items, reposition them and select **Queue for Autoscan**.
    - If all items are placed correctly, hand-scan or type the barcode and select **Submit**.
    - Select **Continue** to proceed.
13. Start the run.
14. When prompted, make sure that the samples have aspirated evenly.
    - If samples are aspirated evenly, select **Continue**.
    - If abnormalities are observed, select **Retry**.
        a. Pull out the sample rack and recap the samples.
        b. Centrifuge the sample rack at 1000 × g for one minute.
        c. Uncap the samples and replace on the deck.
    - If abnormalities are observed after retrying, select **Recover + Abort**. Contact your Illumina representative.
15. During pipetting, watch for abnormalities such as short volume transfers, bubbles, and bead aspiration. Document your observations.
    The Sample Prep script runs for ~45 minutes.
    Observe the SomaSeqAS during the automated steps. Monitor the ILASS interface for prompts and operator instructions.

Complete the following steps within 15 minutes.

1. When prompted, remove the SOMAmer-Bead dilution plates from the instrument deck.
2. Seal each plate with a Microseal 'F' plate seal.
3. Place the sealed plates into the binding shaker and run the following program:
    - 3 h 30 min
    - 28°C
    - 850 rpm

    Record the start time.

SomaSeqAS Cleanup

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

1. Remove the sample rack from the instrument deck.
   Discard the racks and tubes in accordance with applicable standards for your region. Alternatively, you can cap and refreeze the sample rack if > 55 µl volume remains.
2. **[Optional]** If performing a sample QC, remove the leftover DIL plate. Seal the plate with a Microseal 'F' plate seal and store -20°C for up to 4 days. Refer *Protein Quantification by Absorbance QC (Optional)* on page 51 for instructions.
3. Remove the remaining tips from the instrument deck.
4. Save the remaining tips and restack them in columns of eight wafers per stack.
   Avoid touching the tips with your gloves. Handle the tips by the black wafer to prevent contamination.
5. **[Optional]** Clean the assay buffer pyramid base reagent reservoir as follows.
   a. Remove the barcode.
   b. Rinse with deionized water.
   c. Leave to air dry.
6. Remove the biohazard liquid waste wafer from the liquid waste trough.
7. Decontaminate the wafer as follows.
   a. Spray with or immerse in bleach disinfectant. Refer to the manufacturer instructions for contact time.
   b. Rinse with deionized water.
   c. Leave to air dry.
8. Rinse the liquid waste trough with deionized water.
9. Place the dry wafer back on the liquid waste trough.
10. Discard the biohazard waste and used deionized water according to applicable standards for chemical waste for your region.
11. If spillage has occurred, wipe down the deck and ring magnet as follows.
    - Wipe down the deck with a bleach disinfectant. Wipe down the ring magnet with 70% EtOH.
      For detailed deck and magnet cleaning procedures, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

- Discard the waste according to applicable standards for chemical waste for your region.

SomaSeq Assay (Automated)

Before beginning this procedure, review the SomaSeqAS safety and compliance information and safety hazards. Refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

This section describes the steps for processing SOMAmer-Bead plates after the binding step is completed. At the end of the main assay steps, SOMAmer reagents are eluted and prepared for overnight hybridization with the probe master mix.

Begin the ILASS SomaSeq Assay automated procedure with 30–45 remaining in the 3.5 hr binding step.

Transfer to the SOMAmer-Bead dilution plates from the binding shaker to the SomaSeqAS and begin the ILASS SomaSeq Assay automated procedure within 15 minutes of completing the binding step.

Consumables

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

- Assay Buffer (AB) (2 x 250 ml)
- Bead Elution Plate (BEP)
- MB Prep Plate (MPR)
- MB Wash Plate (MBW)
- Photocleavage Plate (PHC)
- Proteomics Probe Plate 2 (PP2)
- Quench Plate (QCH)
- Tag Plate (TAG)
- 0.8 ml deep-well Abgene plate (1)
- 96-well Hard-Shell PCR plates (2)
- Pyramid base reagent reservoirs (2)

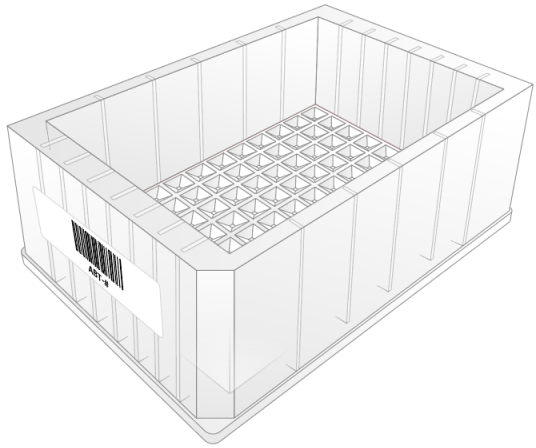
- Microseal 'B' adhesive seal (1)
- Nested tips, 200 µl (3 stacks)
- Single Stack Pipette tips, 200 µl (2 stacks)
- 96-Well Microplate Lid (1)
- The barcode labels with the following prefix:
  - ABT (2)
  - ELU (1)
  - HYB (1)
  - MGB (1)

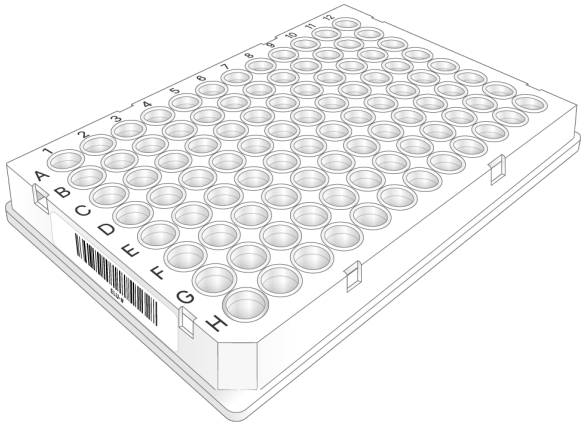
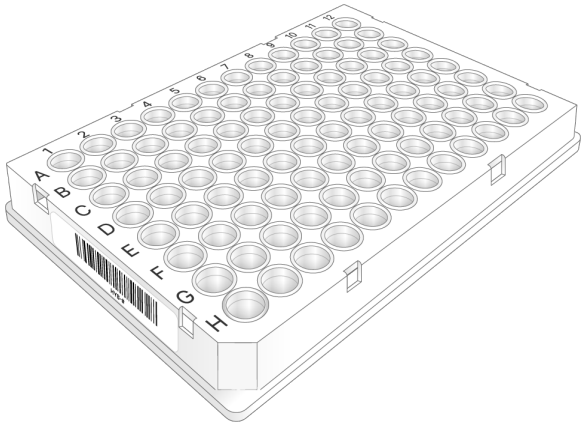
Preparation

Labware Preparation

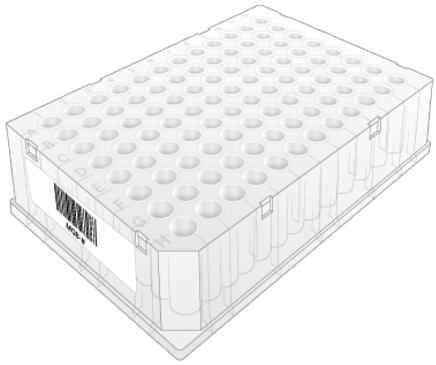
Apply barcode labels to labware as follows.

Barcode Label Prefix	Barcode Label Numbers	Labware Type	Label Position
ABT	Refers to lot number	96-well pyramid base reagent reservoir (2)	Place the barcode in the center of the left side of the reservoir. Make sure that the barcode is as straight as possible.



Barcode Label Prefix	Barcode Label Numbers	Labware Type	Label Position
ELU	Refers to serial number	96-well Hard-Shell PCR plate (1)	Align the lower edge of the label with the upper edge of the plate skirt or trough skirt. 
HYB	Refers to serial number	96-well Hard-Shell PCR plate (1)	Align the lower edge of the label with the upper edge of the plate skirt or trough skirt. 

Barcode Label Prefix	Barcode Label Numbers	Labware Type	Label Position
MGB	Refers to serial number	0.8 ml deep-well Abgene plate (1)	Place the barcode in the center of the left side of the plate. Make sure that the barcode is as straight as possible.



Reagent Preparation

Prepare reagents while the SOMAmer-Bead dilution plates are in the binding step.

1. Prepare the Proteomics Probe Plate 2 and Tag Plate as follows.
   a. Confirm that the plate contents are thawed. Only proceed when fully thawed.
   b. Shake at 1350 rpm for 1 minute.
   c. Centrifuge at 280 × g for 15 seconds.
   d. Remove the seal.
2. Prepare the Quench Plate, Photocleavage Plate, MB Prep Plate, and MB Wash Plate as follows.
   a. Shake at 1350 rpm for 1 minute.
   b. Centrifuge at 280 × g for 15 seconds.
   c. Remove the seal.
3. Prepare the Bead Elution Plate as follows.
   a. Centrifuge at 280 × g for 15 seconds.
   b. Shake at 1350 rpm for 1 minute.
   c. Remove the seal.

Procedure

1. Make sure that the Robotic Gripper Arm (RGA) fingers are leveled. For details, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.
2. In the ILASS, open the SomaSeq drop-down menu and select **SomaSeq Assay**.
3. Make sure that the doors to the deck and lower cabinet are closed, and then select **Next** to run the UVA light check.
4. When prompted by the software, make sure that the solid waste containers are set up correctly and are empty. Then close the doors to the deck and lower cabinet to prepare for the liquid waste check.
5. When prompted, make sure that the liquid waste wafer is in the correct position on the deck.
6. When prompted, make sure that the magnets are in the correct positions on the deck.
7. When prompted, browse to and select the output file generated from the Sample Prep step.
   - The file name is in the following format:
     `Sample_Prep_BatchID_Date_Time`
   - By default, ILASS output files are saved to the following location:
     `C:\OutputFiles`
8. Select **Next**.
9. When prompted, place two pyramid base reagent reservoirs with the barcode prefix ABT onto the instrument deck. Select **Next**.
10. Carefully pour the entire contents of one Assay Buffer bottle into one pyramid base reagent reservoir.
11. Then, carefully pour the entire contents of the other Assay Buffer bottle into the other pyramid base reagent reservoir. Select **Next**.
12. When prompted, place the reagent plates and consumables onto the instrument deck. Place items from left to right.

    Transfer the SOMAmer-Bead dilution plates within 15 minutes of being prompted to load the deck. Otherwise, assay results can be affected.

    Make sure that plates placed on the ABI magnets are seated correctly.

    Make sure that the seals are removed from all reagent plates.

    Make sure that the lid is placed flat side up on the deck.

    The following consumables are loaded empty:

    - 96-well Hard-Shell PCR plate with the barcode prefix ELU (1)

- 96-well Hard-Shell PCR plate with the barcode prefix HYB (1)
- 0.8 ml deep-well Abgene plate with the barcode prefix MGB (1)

13. When prompted, close the instrument door, and then select **Next** to initiate barcode scanning.
14. If prompted with a plate detection or barcode error, make sure that the reagents and labware are placed in the correct positions on the instrument deck.
    - If there are misplaced items, reposition them and select **Queue for Autoscan**.
    - If all items are placed correctly, hand-scan or type the barcode and select **Submit**.
    - Select **Continue** to proceed.
15. Start the run.
    The SomaSeq Assay script runs for ~3 hours.
    Observe the SomaSeqAS during the automated steps. Monitor the ILASS interface for prompts and operator instructions.
16. After the automation completes, and when prompted, remove the HYB plate from the instrument deck.
17. Use a plate paddle applicator to seal each row and column securely with a Microseal 'B' plate seal.
18. Transfer to a plate shaker and shake at 1350 rpm for 1 minute to mix.
19. Centrifuge at 280 × g for 15 seconds.

SAFE STOPPING POINT

- If you are not performing the optional QC step before hybridization, do as follows.
  - If you are stopping, store the sealed HYB plate at -20°C for up to 4 days, and then proceed to the *Hybridization* on page 37 step.
  - If you are not stopping, proceed directly to the *Hybridization* on page 37 step.
- If you are performing the optional QC step, proceed directly to the *SomaSeq Assay QC (Optional)* on page 36 step.

SomaSeq Assay QC (Optional)

In this step, fluorescence signal from the Cyanine 3-labeled SOMAmers is measured in solution. The ratio between the fluorescence reads for calibrator samples and blanks indicates the success of the SomaSeq Assay v2.

Perform the following steps to make sure that the SomaSeq Assay v2 completed successfully.

1. Place the sealed HYB plate on the imaging reader.
2. Configure the imaging reader settings as follows. The settings may require adjustment based on your chosen imaging reader. For details, refer to the manufacturer documentation.
    - Excitation: 540/18
    - Emission: 579/20

- Gain: 88
- Optics: Top

3. Run the imaging reader program.
4. From the results, calculate the median Calibrator and Blank relative fluorescence units (RFU) values.
5. Use the following formula to determine the SomaSeq Assay pass/fail result:

$$\frac{CalibratorRFUMedian}{BlankRFUMedian} >= 2$$

If the result is less than 2, contact your Illumina representative. Store the plate at -20°C for up to 4 days.

SAFE STOPPING POINT

If you are stopping, seal the HYB plate and store at -20°C for up to 4 days. If you are not stopping, proceed directly to the next step.

Hybridization

In this step, eluted SOMAmers are hybridized with the probes for library preparation. The HYB plate is placed in the thermal cycler and overnight hybridization is performed.

Consumables

- Silicone mat for PCR microplate (1)
- Microseal 'B' adhesive seal (1) (Only required if the HYB plate was stored frozen.)
- The following SomaSeqAS cleanup consumables:
  - Bleach disinfectant
  - Deionized water

  Determine the appropriate disinfectant for SomaSeqAS cleaning procedures, in accordance with your applicable regional, national, and local laws and regulations.

Procedure

1. If the HYB plate was stored frozen, do as follows.
   a. Thaw the plate at room temperature for at least 20 minutes.
   b. Centrifuge at 280 × g for 15 seconds.
   c. Remove and discard the seal.
   d. Apply a new Microseal 'B' plate seal. Use a plate paddle applicator to seal each row and column securely.
   e. Transfer to a plate shaker and shake at 1350 rpm for 1 minute to mix.
   f. Centrifuge at 280 × g for 15 seconds.
2. Place the sealed HYB plate on the preprogrammed thermal cycler and cover with a silicone mat.

3. To prevent evaporation, do as follows.
   a. Seal the plate with a plate sealing paddle. Make sure that all raised well rings are sealed firmly with no gaps. Improper sealing can lead to evaporation and sample failure.
   b. Make sure that the silicon mat is aligned correctly.
   c. Make sure that the thermal cycler lid is shut firmly.
4. Run the Hyb program. Record the start time.
   - Choose the preheat lid option and set to 100°C
   - Set the reaction volume to 100 µl
   - 95°C for 10 minutes
   - Decrease by 0.1°C per second until reaching 50°C
   - 50°C for 1 hour
   - Decrease by 0.1°C per second until reaching 45°C
   - 45°C for 2 hours
   - Decrease by 0.1°C per second until reaching 40°C
   - 40°C for 4 hours
   - Decrease by 0.1°C per second until reaching 37°C
   - 37°C for 8 hours
   - Hold at 37°C

   The Hyb program duration is 19.5 hours. However, the plate can be removed any time between 18.5 hours and 21.5 hours without affecting the assay results.

SomaSeqAS Cleanup

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

Before beginning this procedure, review the SomaSeqAS safety and compliance information and safety hazards. Refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

1. Remove the remaining tips from the instrument deck.
2. Save the remaining tips and restack them in columns of eight wafers per stack.

Avoid touching the tips with your gloves. Handle the tips by the black wafer to prevent contamination.

3. Remove the remaining labware from the instrument deck and discard in accordance with applicable standards for your region.
4. **[Optional]** Clean the assay buffer pyramid base reagent reservoir as follows.
   a. Remove the barcode.
   b. Rinse with deionized water.
   c. Leave to air dry.
5. Remove the biohazard liquid waste wafer from the liquid waste trough.
6. Decontaminate the wafer as follows.
   a. Spray with or immerse in bleach disinfectant. Refer to the manufacturer instructions for contact time.
   b. Rinse with deionized water.
   c. Leave to air dry.
7. Rinse the liquid waste trough with deionized water.
8. Place the dry wafer back on the liquid waste trough.
9. Discard the biohazard waste and used deionized water according to applicable standards for chemical waste for your region.
10. If spillage has occurred, wipe down the deck and ring magnet as follows.
    - Wipe down the deck with a bleach disinfectant. Wipe down the ring magnet with 70% EtOH. For detailed deck and magnet cleaning procedures, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.
    - Discard the waste according to applicable standards for chemical waste for your region.
11. Select the **X** in the upper-right corner of the screen to close ILASS.
12. Use the Windows Start menu shutdown procedure to shut down the SomaSeqAS computer. The SomaSeqAS instrument also shuts down and the lights turn off. Illumina recommends shutting down the SomaSeqAS computer at the end of each day.

Illumina recommends beginning day 2 preparation activities ~90 minutes before the Hyb thermal cycler program completes.

Prepare for Protocol

This preparation is required to perform the protocol steps leading up to the next safe stopping point.

1. Power on the SomaSeq Automation System (SomaSeqAS) if it is not already on. Refer to *Start the SomaSeq Automation System* on page 22.
2. Remove the reagents and index plates from the box and thaw as follows.

Table 6 2°C to 8°C Storage

Reagent	Box Name	Instructions
Proteomics Hybridization Plate 2 (HP2)	Illumina SomaSeq Discovery v2, Library Prep Box 2	Bring to room temperature for a minimum of 30 minutes, not exceeding 3 hours.

Table 7 -25°C to -15°C Storage

Reagent	Box Name	Instructions
Enhanced Enrichment Wash (EEW)	Illumina SomaSeq Discovery v2, Plasma and Serum, Library Prep Box 3	Thaw at room temperature for a minimum of 30 minutes, not exceeding 3 hours.
Proteomics Amplification Plate 2 (AP2)	Illumina SomaSeq Discovery v2, Plasma and Serum, Library Prep Box 3	Use one of the following methods: • Thaw at room temperature for a minimum of 90 minutes before the HYB thermal cycler program ends. • Thaw on the thawing station for 20 minutes before the HYB thermal cycler program ends.
Index adapter plate	Illumina DNA/RNA UD Index v3, Auto (96 Indexes, 96 Samples)	Thaw at room temperature for a minimum of 30 minutes, not exceeding 3 hours.

3. Save the following Index PCR program on the post-PCR thermal cycler:
   - Choose the preheat lid option and set to 100°C
   - Set the reaction volume to 50 µl
   - 98°C for 3 minutes
   - 16 cycles of:
     - 98°C for 10 seconds
     - 60°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 4°C

Hyb Capture (Automated)

Before beginning this procedure, review the SomaSeqAS safety and compliance information and safety hazards. Refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

This section describes the steps to perform hyb capture, wash, and elute reporters ready for index PCR reaction.

Consumables

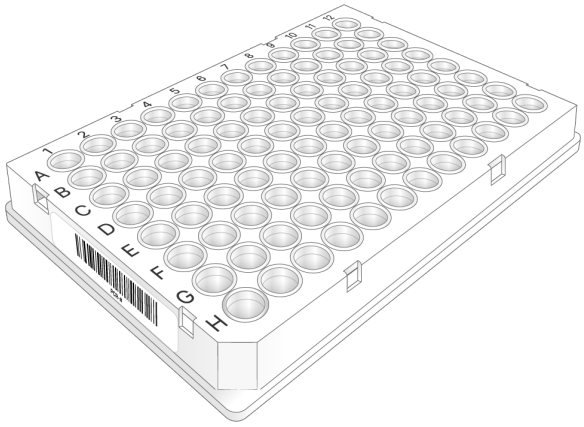
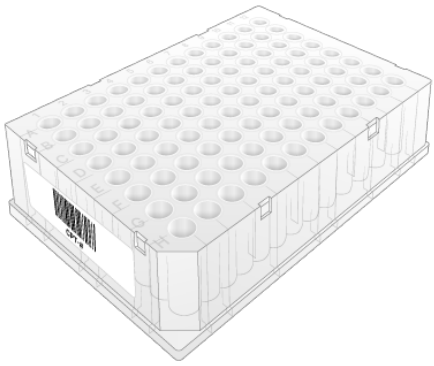
This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

- Enhanced Enrichment Wash (EEW)
- Proteomics Amplification Plate 2 (AP2)
- Proteomics Hybridization Plate 2 (HP2)
- Index adapter plate
- 0.8 ml deep-well Abgene plate (1)
- 96-well Hard-Shell plate (1)
- Microseal 'B' adhesive seal (1)
- Nested tips, 200 µl (2 stacks)
- Single Stack Pipette tips, 200 µl (1 stack)
- 96-Well Microplate Lid (1)
- The barcode labels with the following prefix:
  - PCR (1)
  - CPT (1)

Preparation

Labware Preparation

Apply barcode labels to labware as follows.

Barcode Label Prefix	Barcode Label Numbers	Labware Type	Label Position
PCR	Refers to serial number	96-well Hard-Shell PCR plate (1)	Align the lower edge of the label with the upper edge of the plate skirt or trough skirt. 
CPT	Refers to serial number	0.8 ml deep-well Abgene plate (1)	Place the barcode in the center of the left side of the plate. Make sure that the barcode is as straight as possible. 

Reagent Preparation

1. Prepare the EEW and AP2 as follows.
   a. Confirm that the plate contents are thawed. Only proceed when fully thawed.
   b. Shake at 1350 rpm for 1 minute.
   c. Centrifuge at 280 × g for 15 seconds.

d. Remove the seal.

2. Prepare the HP2 as follows.
   a. Centrifuge at 280 × g for 15 seconds.
   b. Shake at 1800 rpm for 3 minutes.
3. Prepare the index adapter plate as follows.
   a. Confirm that the plate contents are thawed. Only proceed when fully thawed.
   b. Shake at 1350 rpm for 1 minute to mix.
   c. Centrifuge at 280 × g for 15 seconds.
   d. Keep the foil seal intact.

Procedure

Begin the following procedure when ~30 minutes remain before completion of the Hyb thermal cycler program.

1. In the ILASS SomaSeq Discovery Module, open the SomaSeq drop-down menu and select **Hyb Capture**.
2. When prompted by the software, make sure that the solid waste containers are set up correctly and are empty. Then close the doors to the deck and lower cabinet to prepare for the liquid waste check.
3. When prompted, make sure that the liquid waste wafer is in the correct position on the deck.
4. When prompted, make sure that the magnets are in the correct positions on the deck.
5. When prompted, browse to and select the output file generated from the SomaSeq Assay step.
   - The file name is in the following format:
     `SomaSeq_Assay_BatchID_Date_Time`
   - By default, ILASS output files are saved to the following location:
     `C:\OutputFiles`
6. Select **Next**.
7. When prompted, enter the index plate barcode information. Select **Next**.
8. When prompted, confirm correct preparation of the HP2.
9. When prompted, place the reagent plates, index adapter plate, and other consumables onto the instrument deck. Keep the foil seal on the index adapter plate intact.

   Make sure that the lid is placed flat side up on the deck.

   The following consumables are loaded empty:

   - 96-well Hard-Shell PCR plate with the barcode prefix PCR (1)
   - 0.8 ml deep-well Abgene plate with the barcode prefix CPT (1)

10. After the Hyb program is complete, remove the HYB plate from the thermal cycler.
    - Do not vortex or centrifuge.
    - Remove the seal carefully, making sure that the plate contents do not splash.
    - Place the HYB plate on the instrument deck.
11. When prompted, close the instrument door, and then select **Next** to initiate barcode scanning.
12. If prompted with a plate detection or barcode error, make sure that the reagents and labware are placed in the correct positions on the instrument deck.
    - If there are misplaced items, reposition them and select **Queue for Autoscan**.
    - If all items are placed correctly, hand-scan or type the barcode and select **Submit**.
    - Select **Continue** to proceed.
13. Start the run.
    The Hyb Capture for NGS script runs for ~1 hour and 45 minutes.
    Observe the SomaSeqAS during the automated steps. Monitor the ILASS interface for prompts and operator instructions.
14. When prompted, remove the PCR output plate from the instrument deck.
15. Seal firmly with Microseal 'B' plate seal.
16. On the SomaSeqAS computer, browse and select the SomaSeqAS output file.
    - The file name is in the following format:
      `BatchID_PlateBarcode_output_Date_Time.csv`
    - By default, SomaSeqAS output files are saved to the following location:
      `C:\OutputFiles`

    An additional file with all barcodes listed is also generated and can be used for troubleshooting purposes.
17. The output file is required for BaseSpace Sequence Hub run planning. Save a copy of the file to a location accessible from the computer used for run planning.

Index PCR

This step amplifies library fragments and adds index sequences for multiplexing and sequencing.

Consumables

- Silicone mat for PCR microplate
- The following SomaSeqAS cleanup consumables:
  - Bleach disinfectant
  - Deionized water

Determine the appropriate disinfectant for SomaSeqAS cleaning procedures, in accordance with your applicable regional, national, and local laws and regulations.

Procedure

1. Transfer the PCR plate to a plate shaker and shake at 1500 rpm for 1 minute to resuspend.
2. If splashing occurs, centrifuge at 280 × g for 15 seconds to make sure that no liquid is adhered to the plate seal.
3. Visually inspect the plate wells. If bead pellets have reformed, repeat steps 1 and 2.
4. Place the PCR plate on the preprogrammed thermal cycler and cover with a silicone mat.
5. Run the Index PCR program.
   - Choose the preheat lid option and set to 100°C
   - Set the reaction volume to 50 µl
   - 98°C for 3 minutes
   - 16 cycles of:
     - 98°C for 10 seconds
     - 60°C for 30 seconds
     - 72°C for 30 seconds
   - 72°C for 5 minutes
   - Hold at 4°C
6. When the program has completed, remove the PCR output plate from the thermal cycler.

SAFE STOPPING POINT

If you are stopping, store the sealed PCR plate at -25°C to -15°C for up to 30 days.

After Index PCR, complete all subsequent steps in the post-PCR area.

SomaSeqAS Cleanup

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

⚠ Before beginning this procedure, review the SomaSeqAS safety and compliance information and safety hazards. Refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.

1. Remove the index plate from the instrument deck.
2. Remove the remaining consumables and tips from the instrument deck.
3. Save the remaining tips and restack them in columns of eight wafers per stack.
   Avoid touching the tips with your gloves. Handle the tips by the black wafer to prevent contamination.
4. Remove the biohazard liquid waste wafer from the liquid waste trough.
5. Decontaminate the wafer as follows.
   a. Spray with or immerse in bleach disinfectant. Refer to the manufacturer instructions for contact time.
   b. Rinse with deionized water.
   c. Leave to air dry.
6. Rinse the liquid waste trough with deionized water.
7. Place the dry wafer back on the liquid waste trough.
8. Discard the biohazard waste and used deionized water according to applicable standards for chemical waste for your region.
9. If spillage has occurred, wipe down the deck and ring magnet as follows.
   - Wipe down the deck with a bleach disinfectant. Wipe down the ring magnet with 70% EtOH.
     For detailed deck and magnet cleaning procedures, refer to the *SomaSeq Automation System Product Documentation (document # 200082762)*.
   - Discard the waste according to applicable standards for chemical waste for your region.
10. Select the **X** in the upper-right corner of the screen to close ILASS.
11. Use the Windows Start menu shutdown procedure to shut down the SomaSeqAS computer.
    The SomaSeqAS instrument also shuts down and the lights turn off.
    Illumina recommends shutting down the SomaSeqAS computer at the end of each day.

Prepare for Protocol

This preparation is required to perform the protocol steps leading up to the next safe stopping point.

1. If stored at -25°C to -15°C, remove the sealed PCR output plate from storage and thaw at room temperature. Only proceed with the *Pooled Library Cleanup* on page 47 procedure when the plate is fully thawed.
2. Remove the following reagents from the box:

Table 8 Room Temperature Storage

Reagent	Box Name	Instructions
Illumina Purification Beads (IPB)	Illumina SomaSeq Discovery v2, Library Prep Box 1	Use at room temperature.
Resuspension Buffer (RSB)	Illumina SomaSeq Discovery v2, Library Prep Box 1	Use at room temperature.

Pooled Library Cleanup

This step uses a single-sided Solid Phase Reversible Immobilization (SPRI) bead purification procedure to purify the libraries. Complete the following steps in the post-PCR area.

Consumables

- Illumina Purification Beads (IPB)
- Resuspension Buffer (RSB)
- 1.5 ml LoBind microcentrifuge tube (4)
- 8-tube PCR strip with caps (1)
- Pipette tips, 20 µl (1 box)
- Pipette tips, 200 µl (1 box)
- Pipette tips, 1000 µl (1 box)
- EtOH (absolute ethanol)
- Nuclease-free water

About Reagents

- Illumina Purification Beads
  - Use Illumina Purification Beads at room temperature.
  - Vortex the stock reagent tubes thoroughly until the beads are resuspended.
  - Pipette slowly to minimize foaming. To avoid resettling the beads, centrifugation before pipetting is not recommended.
  - Do not agitate the labware while it is on the magnetic stand.
  - Avoid disturbing the bead pellet when washing.
  - If beads are aspirated into pipette tips, dispense back into the tube on the magnetic stand and wait until the liquid is clear (~2 minutes).
  - If liquid becomes adhered to the side or top of the tube, seal and centrifuge at 280 × g for 3 seconds to collect contents.

Preparation

1. Prepare reagents as follows.
   - Illumina Purification Beads—Vortex to mix.
   - Resuspension Buffer—Vortex to mix.
2. For each pool, combine the following volumes to prepare 200 µl fresh 80% EtOH:
   - EtOH (160 µl)
   - Nuclease-free water (40 µl)

   These volumes produce 200 µl 80% EtOH per pool, including overage.
3. Vortex prepared 80% EtOH to mix.

Procedure

1. **[Optional]** Place the PCR output plate on the magnetic stand and wait until the liquid is clear (~5 minutes).
2. Avoiding the bead pellet, transfer 15 µl supernatant from each well to an 8-tube PCR strip. Use new pipette tips for each column of the plate.
   The total volume per tube is 180 µl.
   Illumina recommends saving the PCR plate in case repooling is required. Store at -25°C to -15°C for up to 30 days.
3. Cap the PCR strip.
4. Vortex to mix, and then microcentrifuge briefly.
5. Label a 1.5 ml LoBind tube LP1.
6. To make the 96 plex library pool, transfer 50 µl from each tube of the 8-tube PCR strip to the LP1 tube.
   The total pool volume is 400 µl.
7. Vortex to mix, and then microcentrifuge briefly.
8. Label a new 1.5 ml LoBind tube LP2.
9. Transfer 50 µl from the LP1 tube into the LP2 tube.
   [Optional] Save the LP1 tube with the remaining 96 plex library pool. Cap the LP1 tube and store at -25°C to -15°C for up to 30 days.
10. Vortex Illumina Purification Beads to resuspend.
11. Add 90 µl Illumina Purification Beads to the LP2 tube.
12. Pipette to mix.
13. Incubate at room temperature for 2 minutes.
14. Place on the magnetic stand and proceed as follows.
    a. Wait until the liquid is clear (~5 minutes).

   b. Remove and discard all supernatant.
15. Keep on the magnetic stand and wash the beads as follows.
   a. Add 180 µl fresh 80% EtOH.
   b. Wait 30 seconds.
   c. Remove and discard all supernatant.
16. Cap the LP2 tube, centrifuge briefly, and then return to the magnetic stand.
17. Using a pipette set to 20 µl, remove all residual EtOH.
18. Uncap and air-dry the LP2 tube on the magnetic stand for 2 minutes.
19. Remove from the magnetic stand and add 200 µl Resuspension Buffer.
20. Pipette to mix.
21. Incubate at room temperature for 2 minutes.
22. Place on the LP2 tube magnetic stand and proceed as follows.
   a. Wait until the liquid is clear (~2 minutes).
   b. Label a new 1.5 ml LoBind tube LP3.
   c. Transfer 190 µl supernatant from the LP2 tube to the LP3 tube.

SAFE STOPPING POINT

If you are stopping, cap the LP3 tube and store at -25°C to -15°C for up to 30 days.

Library Quantification

Perform the following to check the quantity and quality of the purified library prep product.

1. Quantify 5 µl library using a Qubit dsDNA HS Quantification Assay Kit.
   Expect a ≥ 2 ng/µl library yield. Contact Illumina Technical Support if the library yield is < 2 ng/µl.

Dilute Libraries to the Starting Concentration

This step dilutes the pooled libraries to the starting concentration for the sequencing system and is the first step in a serial dilution. After diluting to the starting concentration, libraries are ready to be denatured and diluted to the final loading concentration.

The final loading concentration is a starting point and general guideline. Optimize concentrations for your workflow and quantification method over subsequent sequencing runs or by flow cell titration.

Illumina does not recommend including a PhiX control in the sequencing run. As there are < 26 cycles per read, no PhiX metrics can be generated.

Procedure

1. Calculate the molarity value of the libraries. Use the following formula:
   Use 164 bp as the average library size.

$$\frac{ng/\mu l \times 10^6}{660\frac{g}{mol} \times average\ library\ size\ (bp)} = Molarity\ (nM)$$

2. Using the molarity value, calculate the volumes of RSB and library needed to dilute libraries to the starting concentration for your system.

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
NovaSeq 6000, S4	1	200
NovaSeq X Series, 5B	2	35
NovaSeq X Series, 10B	2	35
NovaSeq X Series, 25B	2	43

3. Dilute each library pool to the starting concentration for your system. Refer to the table below for pooling options.
   - If you are combining plates for sequencing, each SampleID must be unique per sample sheet.
   - **[NovaSeq 6000]** Index sequences must be unique within a sequencing run. Avoid combining plates that contain the same index sequences.
   - **[NovaSeq X Series]** Index sequences can be repeated on separate lanes. Make sure that samples are designated appropriately to lanes in the sample sheet.

Sequencing System	Total Library Pool Required (µl)	Number of Pools	Minimum Volume per Pool*
NovaSeq 6000, S4	310	2	155 µl at 1 nM (each plate)
NovaSeq X Series, 5B	55	1	55 µl at 2 nM
NovaSeq X Series, 10B	55	2	27.5 µl at 2 nM (each plate)
NovaSeq X Series, 25B	110	2 4	55 µl at 2 nM (each plate) 27.5 µl at 2 nM (each plate)

* If combining pools. Do not combine pools when using a 5B flow cell.

4. Follow the denature and dilute instructions for your system.
   - For NovaSeq 6000, refer to the Denature and Dilute Protocol Generator and follow the NovaSeq 6000 Standard Loading instructions.
   - For NovaSeq X Series, refer to the Denature and Dilute Protocol Generator and follow the NovaSeq X Series instructions for use with the applicable control software for your lab.
     - SomaSeq Discovery v2, control software v1.4 and later

- Illumina Protein Prep, control software v1.3

SAFE STOPPING POINT

If you are stopping, cap the tube and store at -25°C to -15°C for up to 30 days.

Protein Quantification by Absorbance QC (Optional)

This QC assessment is intended to aid in troubleshooting if a `SampleNorm` flag in non-control samples is associated with extreme total protein concentrations. This protocol describes how to measure total protein concentration from leftover material in the DIL plate retained from step 2 of *SomaSeqAS Cleanup* on page 30 from the *Sample Prep (Automated)* on page 25 protocol.

Consumables

- Microseal 'B' PCR plate seal (1)
- Pierce Dilution-Free Rapid Gold BCA Protein Assay (1)
- Pierce 96-well Corner Notch Plate (1)
- 1N HCl (6 ml/50 µl per well)

Preparation

Imaging Reader Prep

1. Save the following SomaSeq settings on the imaging reader.
   - Read Type: Absorbance
   - Wavelength: 480 nm

Sample Prep

1. Thaw and remove the seal from the DIL plate retained from step 2 of *SomaSeqAS Cleanup* on page 30 from the *Sample Prep (Automated)* on page 25 protocol to room temperature.
2. Add 10 µL of each standard curve titration point provided in the Pierce Dilution-Free Rapid Gold BCA Protein Assay Kit to a new Pierce 96-well Corner Notch Plate.

   A standard curve should be included on every plate processed. For improved accuracy, it is recommended to run at least two replicates of each standard curve titration point.

3. Add 10 µL from the each Calibrator and Blank plate controls in the DIL plate to the Pierce 96-well Corner Notch Plate. Refer to the sample manifest for control well locations if they differ from the recommended wells.

Sample Plate Well	Sample ID
A01	CAL
C03	CAL
G05	CAL
H08	CAL
D12	CAL
G11	BLANK
D06	BLANK
B02	BLANK

4. Add 10 µL from each sample well under evaluation to the Pierce 96-well Corner Notch Plate.

Procedure

- To ensure accurate quantification, minimize variability in development time across all samples, controls, and standards.
- It is recommended to use a multichannel pipette and to pipette mix immediately with the same tips before discarding and moving to the next column.
- Plate reading must be completed within 1 hour of Working Reagent addition.

1. Follow the Pierce Dilution-Free Rapid Gold BCA Protein Assay User Guide microplate procedure to prepare the Working Reagent (WR) at a 20:1 ratio (Reagent A:Reagent B). Prepare sufficient WR for all samples, controls, and standards to be processed.
2. Add 200 µL of WR to each well of the Pierce 96-well Corner Notch Plate.
3. Pipette to mix.
4. Incubate for 5 minutes at room temperature.
5. Add 50 µL 1N HCl to each well to stop the reaction.
   There will be some 1N HCl left from the initial 6 ml.
6. Pipette to mix.
7. Seal the Pierce 96-well Corner Notch Plate with a Microseal 'B' PCR plate seal.
8. Centrifuge at 1000 × g for 1 minute to minimize bubbles.
9. Load the sealed plate into the imaging reader tray and run the SomaSeq settings.
10. Export the absorbance data once imaging is complete.
11. Use the exported data to further investigate potential causes of the `SampleNorm` flags. Contact Illumina Technical Support for assistance in interpreting results.

Resources & References

The SomaSeq Discovery v2 support pages on the Illumina support site provide additional resources. Always check the support pages for the latest versions.

For secondary analysis documentation, refer to BaseSpace Sequence Hub support resources and Illumina BioInsight Platform Core support resources.

For tertiary analysis documentation, refer to Illumina Connected Multiomics support resources.

Revision History

Document	Date	Description of Change
Document # 200076585 v00	August 2026	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website:	www.illumina.com
Email:	techsupport@illumina.com

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.

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