SomaScan® 11K Assay: Automated Kit

User Manual for the SomaScan 11K Assay: Plasma and Serum Kits (RUO)

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This user manual describes the steps for the processing of plasma and serum sample using the SomaScan 11K Assay kit and is applicable to kit part numbers 900-00050 and 900-00051.

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1. Introduction

This kit contains the components required to analyze 85 human plasma (kit part number 900-00050) or 85 human serum samples (kit part number 900-00051) in the SomaScan® 11K Assay format.

2. Principle of the Assay

The SomaScan 11K Assay Kits are designed for use with the Tecan Fluent 780 equipped to run the SomaScan 11K Assay.

The SOMAmer® reagents are supplied pre-bound onto a magnetic resin in 96-well format and for each of the three sample dilutions a unique SOMAmer Bead (Catch 0) Plate is supplied. The neat biological samples and controls are diluted 1 to 5 in a matrix specific diluent. Two additional dilutions are prepared (1 to 200 and 1 to 20,000) using a serial dilution. The diluted samples are pipetted into the appropriate 96-well plate. After a binding step the unbound substances are washed away. A biotinylation reagent is added to each well for the labeling of the proteins bound to the SOMAmer reagents on the beads. Following washes to remove excess biotinylation reagent the samples are exposed to UV light which releases the SOMAmer reagents and their binding partners from the magnetic resin. Magnets separate the magnetic resin from the solution, which is transferred onto a new set of magnetic beads. After binding the biotinylated proteins with corresponding SOMAmer reagents to the new magnetic beads a series of wash steps removes the SOMAmer reagents that did not bind to a protein. The addition of a buffer elutes the bound SOMAmer reagents from the proteins. Magnets separate the magnetic beads from the solution which is recovered. The eluted SOMAmer reagents are added to microarrays. The SOMAmer reagents bind to complementary probes on the array during the hybridization reaction. Following the microarray washing the microarray slides are processed in an Agilent Microarray scanner system where a laser excites the fluorophore intrinsic to the SOMAmer reagent. The fluorescence intensity is proportional to the available epitopes of the corresponding proteins in the original sample.

3. Limitations of the Procedure

3.1. For Research Use Only

The SomaScan® Assay 11k, SomaScan Assay 11k Kits, and SOMAmer® reagents may only be used for their intended purpose as described in and in strict accordance with the protocols and other materials accompanying them. The SomaScan 11K Assay, SomaScan 11K Assay Kits, and SOMAmer reagents are developed, designed, intended, and sold to the Institution for research purposes only and are not for use in diagnostic procedures. The SomaScan 11K Assay, SomaScan 11K Assay Kits and SOMAmer reagents are not to be used by the Institution or any Third Party for human consumption, diagnostic, clinical or therapeutic applications, or be included or used in any drug intended for human, agricultural or cosmetic use. All care and attention should be exercised in the handling of the SomaScan 11K Assay, SomaScan 11K Assay Kits, and SOMAmer reagents by following appropriate research lab practices.

3.2. Sample Matrices

The SomaScan 11K Assay method was validated using human EDTA plasma and serum samples. Samples collected using other blood collection tubes (for example citrate plasma or heparin plasma) should work as well but SomaLogic cannot guarantee the performance of the kits.

3.3. Procedure

1. The kit should not be used beyond the expiration date on the kit label.
2. To ensure accurate results, the SomaScan 11K Assay protocol must be precisely followed. Changes in volumes, timing, or steps can lead to failure of assay quality control metrics resulting in difficult, time-consuming, and resource-intensive troubleshooting. Once started, the SomaScan 11K Assay has no natural stopping points and is time sensitive. Be prepared - make sure all required reagents, consumables and equipment are ready and labeled appropriately as outlined below.
3. All samples must be aliquoted into 0.5 mL 2D barcoded tubes from Matrix (ThermoFisher Scientific PN: 3744) and must be placed into a Matrix rack along with the 11 SomaScan assay controls provided with the kit. It Is recommended to distribute the controls across all 12 column and all 8 rows.
4. Use new reagents and containers and be mindful of aerosol-based contamination as minor contamination can result in assay artifacts.
5. Reagent reservoirs and plates must be clearly labeled, to facilitate identification, confirm orientation, and allow for efficient performance of procedural steps.
6. The only difference between a serum and plasma kit for the SomaScan 11K Assay is the sample diluent and controls that are part of the -80C components kit.
7. SOMAmer reagents in the SOMAmer Bead (Catch 0) Plates are light sensitive. They are generally stable in indoor ambient lighting but are sensitive to long-wave UV light. The best practice is to keep all SOMAmer Bead Plates protected from light, such as by covering them with foil.
8. A user checklist (SomaScan 11K Assay - Experienced User Checklist Serum and Plasma, D0006494) is provided for trained, experienced users to follow during the performance of the assay and to complete as a record of each study conducted.

9. Certain steps of the SomaScan 11k Assay protocol require specific volumes that are critical to the success of the assay run. These volumes are called out in this manual and the Experienced User Checklist.

10. When running a Fluent method, the analyst will follow prompts and select **Next Page** or **Continue** as instructed in **TouchTools** after completing advised steps.

11. **Note:** The option to **Stop** or **Continue** Is always visible within **TouchTools** located in the bottom right-hand corner of the screen while the method is running. Only select **Stop** when needing to conduct an emergency stop of the method. Only select **Continue** when instructed to do so by a prompt in the method.

3.4. Restrictions

The transfer or sale of any SomaScan Assay or SOMAmer reagent (alone or in any Institution panel or kit), outside of the scope of the agreement is strictly prohibited. For purposes of clarity, these restrictions do not prohibit the Institution from providing SomaScan services to a third party or conducting research in collaboration with another entity. Further, any and all reverse engineering or alteration of any SOMAmer reagent to determine its sequence and/or structure is strictly prohibited.

3.5. Patents and Trademarks

Please visit the following link to view the patent and trademark notice:

https://somalogic.com/list-of-patents/

https://somalogic.com/somascan-patents-trademarks/

https://somalogic.com/somamer-reagent-patent-and-trademark-notice/

3.6. Notice

All reagents, equipment and other supplies (hereinafter "Materials") recommended, by way of example, for use within this Application Note and purchased by the user are to be used by the user in compliance with the terms and conditions, and any restrictions and limitations, as set forth by the third party supplier or manufacturer from which the user purchased or obtained the Materials.

3.7. Copyright

3.8. For Technical Support

For help and technical information, please submit your questions via email to TechSupport@somalogic.com or call 1-844-SOMAmer.

4. Warnings

All biological samples should be considered as containing infectious disease pathogens. Follow your institutional guidelines for biosafety.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

The SomaScan Elution Buffer is classified with a warning label and can cause eye irritation and skin irritation. For further details please refer to the SDS document (available upon request).

The 10x Slide Block is classified with a warning label and can cause eye irritation and skin irritation. For further details please refer to the SDS document (available upon request).

The 2x Hybridization Buffer is classified with a warning label and causes serious eye damage and can cause skin irritation. The 2x Hybridization Buffer may cause damage to organs through prolonged or repeated exposure. The 2x Hybridization Buffer is harmful to aquatic life with long lasting effects. For further details please refer to the SDS document (available upon request).

Wear personal protective equipment (lab coat, protective gloves, safety glasses). Wash hands thoroughly after handling. Refer to the SDS, available upon request, to use the kits.

5. Initial Setup Requirements

5.1. Required Equipment

Please refer to the SomaScan Assay - Equipment List (D0004619) for a complete equipment list.

5.2. Equipment Monitoring

To ensure consistent performance of the SomaScan 11K Assay, it is recommended that routine maintenance, calibration and monitoring of key pieces of equipment be performed. In particular, the supplemental maintenance and calibration protocols supplied by SomaLogic for the Tecan instrument and by Agilent for the microarray hybridization oven and the microarray scanner system should be followed.

6. Material Provided and Storage Conditions

For a detailed list of the components provided in a SomaScan 11K Assay Kit and the types of kits available, please see Appendix 2.

Upon receipt of the SomaScan 11K Assay Kits the components should be stored at the indicated temperature. The SomaScan 11K Assay Kit is shipped in three parts, one on dry ice one on blue ice and one at ambient temperature.

Kit Component Name	Part Number	Storage Temperature
SomaScan 11K Assay, ambient components	899-00054	Ambient (+10 to +30 °C)
SomaScan 11K Assay, ambient components, no slides	899-00059	Ambient (+10 to +30 °C)
SomaScan 11K Assay, 4C components	899-00055	+4 °C (+2 to +8 °C)
899-00056 SomaScan 11K Assay, -20C components	899-00056	-20 °C (-10 to -30 °C)
SomaScan 11K Assay, -80C Plasma components	899-00057	-80 °C (-70 to -90 °C)
SomaScan 11K Assay, -80C Serum components	899-00058	-80 °C (-70 to -90 °C)
Filled Bottle, Assay Buffer	651-00125	+4 °C (+2 to +8 °C)

7. Other Supplies Required but Not Supplied in the SomaScan 11K Assay Kit

Please refer to the SomaScan 11K Assay – Consumable List (D0006492) for a complete list of required disposables. This list is also available in Appendix 1 at the end of this document.

8. Sample Collection and Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

8.1. General Blood Sample Processing Requirements

Proper processing of the collected samples is critical. Many tubes have a minimum and maximum fill line and these requirements should be followed and no additional additives should be added to the samples. It is particularly important that time constraints are observed and that samples are not left at room temperature longer than necessary. Samples should be processed and frozen at **-80 °C** within **2 hr** of collection.

8.2. EDTA Plasma Processing

1. Process samples within **30 min** of collection.
2. Perform all steps at **room temperature**. Do not chill the vacutainer prior to processing.
3. Centrifuge plasma tubes at **room temperature**. If within the tube manufacturer's specification, spin at **2200 x g** (2200 rcf – not rpm) for **15 min**. Set the centrifuge to maximum acceleration with full brake.

4. Draw off only the plasma layer. Take care not to disturb buffy coat when aliquoting by leaving some plasma behind and avoiding the cell layer.

5. Aliquot the sample **immediately** into appropriately labeled tubes (130 µL total sample or two 55 µL aliquots in preferred tubes).

6. Place aliquoted samples in a **-80 °C** freezer. If samples must be frozen at -20 °C rather than -80 °C, an aliquot volume of 0.5 mL to 1 mL is required. Maximum recommended storage time at -20 °C is 3 weeks.

8.3. Serum Processing

1. Perform all steps at **room temperature**. Do not chill the vacutainer prior to processing.

2. Allow the serum to clot for **60-90 min** at **room temperature** prior to centrifugation.

3. Centrifuge serum tubes at **room temperature**. If within the tube manufacturer's specification, spin at **2200 x g** (2200 rcf – not rpm) for **15 min**. Set the centrifuge to maximum acceleration with full brake.

4. Draw off only the serum layer.

5. Aliquot sample within **30 min** of centrifugation into appropriately labeled tubes (130 µL total sample or two 55 µL aliquots in preferred tubes).

6. Place aliquoted samples in a **-80 °C** freezer. If samples must be frozen at -20 °C rather than -80 °C, an aliquot volume of 0.5-1 mL is required. Maximum recommended storage time at -20 °C is 3 weeks.

9. Setup of the SomaScan 11K Assay: Sample Preparation (Duration: ~1.25 hr)

9.1. Consumables Preparation

1. Obtain and label five Nunc Round Bottom Plates on the front and left-hand side, where the notch is towards the analyst and in the lower left-hand corner.
    - Dil 1 (**D1**)
    - Extra Dil 1 (**ED1**)
    - Dil 2 (**D2**)
    - Extra Dil 2 (**ED2**)
    - Dil 3 (**D3**)

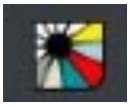
2. Obtain three stacks of 200 µL nested tips, 8 wafers per stack, white cover removed.

3. Obtain one stack of 50 µL nested tips, 8 wafers per stack, white cover removed.

9.2. Tecan Fluent Startup and Platform Initialization

NOTE: Complete the following actions prior to thawing the biological samples as a conservative measure if unforeseen technical issues arise with the instrument. If multiple assays are performed within a day the following steps only need to be completed once.

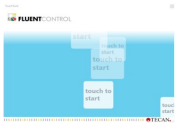
1. Startup of robot.
   - Turn on the computer connected to the Tecan Fluent robot.
     - Wait to initiate the Fluent software until all hardware is turned on.
   - Turn on the Fluent robot (Myrius box).
   - Turn on the power strip controlling the Thermal Magnetic Shaker (TMS), control box, and BioShake shakers.
     Open the **FluentControl** software.

     NOTE: Do not interrupt the software while it is loading. The Driver Framework window will pop up and then disappear when the software is ready.

     When instructed, touch anywhere on the **TouchTools** screen.

     
   - Log into the **FluentControl** software.
     - Select username
     - Enter password

2. If the MCA head adapter is attached to the MCA head, proceed to Step 3.

   a. If the MCA head adapter is not attached to the MCA head, follow the steps below:

   - Ensure the Deck Segment System MCA is mounted to **grid position 23** on the robotic deck.
     - Ensure the deck segment is securely in position with the yellow switch on the bottom of the carrier flipped to the right, locking it in place.
   - Ensure Rack Head Adapter MCA is mounted on the Deck Segment System MCA with label on rack facing analyst at **position 23-2**.
   - Ensure both robotic arms are away from the Photocleavage light source to prevent collision errors during instrument initialization.
   - From the **TouchTools** home screen select.
     - **System Care**
     - **Get Head Adapter**
     - Green **Play** Button to start the **Get Head Adapter** method

        - ◊ The MCA will perform the action of initializing then attaching the Head Adaptor.
    - After method completion, remove deck segment with MCA head adapter cradle from Fluent **deck grid position 23**.
        - Flip the yellow switch on the bottom front of carrier to the left.
            - ◊ This will unlock the carrier from the robotic deck allow for front to back movement.
        - Remove carrier by moving it forward and up.
            - ◊ Careful of the plastic pin-attachments in the rear, they are prone to breakage if the carrier is forcefully removed.
    - Install Nest Segment, 6-Position, 7mm on **grid position 23** of Fluent deck.
        - Ensure the rear pins of the deck segment are secure to the robot deck.
        - Flip the yellow switch on the bottom front of carrier from left to right to secure the carrier in place.

3. Inspection of gripper fingers.
    - Inspect gripper fingers for deformities, damage, and debris.
        - If debris is present, clean gripper fingers with an alcohol wipe.
        - If there are deformities or damage, do not proceed and notify your robotics specialist, or contact SomaLogic Technical Support.
    - Check leveling of the RGA fingers.
        - Gently move the RGA to an unobstructed carrier position with a flat surface.
        - Gently pull the RGA head down until the fingers are just above the flat surface.
            - ◊ If both fingers are parallel with surface, manually raise the RGA head.
            - ◊ If the fingers are not evenly parallel with the surface, see **Appendix 3** for instructions on how to level the RGA gripper fingers.

4. Shaker Communication Test.

⚠ If any portion of the Shaker Communication Test fails, do not proceed, and notify SomaLogic Technical Support.

    - From the **TouchTools** home screen, select:
        - **Method Starter**
        - **Shaker Communication Test**
        - Green **Play** button to initiate the **Shaker Communication Test** method
    - The method will execute a series of tests.
        - Watch each test carefully.
    - The method requires the user to acknowledge the successful completion of each test.
        - Follow the robotic prompts.

5. Waste collection bottle:
    - Connect the waste collection bottle to the waste trough.
        - Screw the waste bottle cap to the bottle.
        - Attach the tubing from the waste trough to the bottle cap using the quick connector.

      NOTE: To allow proper venting, make sure the barbs are securely connected in the quick-release fitting.

Reagent Preparation

1. Electronic documentation:
   - Obtain a copy of the SomaScan 11K Assay Workbook (D0006495).
   - Save the workbook with the study ID.
     - The study ID should consist of a letter code for your institution followed by a digit year, a consecutive number, and Set#.
       - ◊ Example: SL-23-002_Set001

2. Retrieve samples:
   - Retrieve the sample rack containing the randomized samples (85 research samples).
     - If not done previously, add the SomaScan 11K Assay controls from the matrix-specific kit, SomaScan 11K Assay, -80 ºC components (899-00057 or 899-00058).
     - 11 SomaScan control samples:
       - ◊ 11K Calibrator in quintuplet
       - ◊ 11K QC1 Sample in triplicate
       - ◊ Blank in triplicate
     - The control samples should be distributed across the number of columns of samples analyzed.
       - ◊ As a general guidance one control per column and at least one control per row.
       - ◊ Do not place the replicates next to each other.

3. Obtain the following reagents:

NOTE: Make sure to use the kit type (serum or plasma) that matches the samples and controls in the assay. The only difference between serum and plasma kits is the sample diluent and the controls.

For details of the components within each kit, refer to Appendix 2.

Reagent/Kit	Amount	Item/Part Number	Storage Location
SomaScan 11K Assay Kit, Ambient Components	1 each	899-00042	Ambient room temperature
Assay Buffer (**AB**)	1 each	651-00125	4 °C storage
SomaScan 11K Assay Kit, 4 °C Components	1 each	899-00043	4 °C storage
SomaScan 11K Assay Kit, -20 °C Components	1 each	899-00044	-20 °C storage
Sample Diluent (**D-P** or **D-S**) from SomaScan 11K Assay, -80 °C Components	1 each	651-00072 in 899-00057 or 651-00073 in 899-00058	-80 °C storage

   - Record the lot numbers of the kit components and the **AB** in **Tab 2 - Reagents** of the workbook.
   - Store the 11K Catch 0 Plates (part of SomaScan 11K Assay, -20 °C components) on the bench top until needed.
     NOTE: SOMAmer Bead Plates are referred to as "Catch 0" plates in the -20C components list.

4. Prepare reagents:
    - Sample rack
        - Remove the Matrix™ rack lid.
        - Place the sample rack on a rack thawing station and turn on.
        - Set a timer and thaw the samples for at least 15 min.
    - Sample diluent (**D-P** or **D-S**)
        - Confirm 25 °C water bath temperature is 25 °C (± 2.0 °C).
        - Place the sample diluent in the water bath.
        - Set a timer and thaw the sample diluent for at least 20 min.
    - 10x Slide Block (**3-B**)
        - Thaw Slide Block in a 25 °C water bath.
        - Retrieve at the end of the sample preparation protocol for auxiliary reagent preparation.
    - 100x Tag Reagent (**1-T**) and Tag Diluent (**1-D**)
        - Place the 100x Tag Reagent aliquot in a 25 °C water bath.
        - Place the Tag Diluent aliquot in 25 °C water bath.
        - Retrieve at the start of the assay protocol.

9.3. SomaScan 11K Assay – Sample Preparation Script

1. Fluent preparation:
    - Set up the gravity feed trough.
        - Fill refillable trough bottle with 1 L of Assay Buffer (**AB**).
        - Place bottle base on deck position left rail pin #7 and the refillable trough on deck position 16-4.
            - ◊ Route the tubing so it's positioned in front and under deck carriers 2 and 9, just above the TouchTools screen.
        - Insert a refillable trough bottle into the bottle base.
            - ◊ The bottle will click into place when properly secured.
        - Ensure trough fills.
            - ◊ Ensure no large bubbles are present in tubing, as this will prevent trough filling.
    - From TouchTools home screen, select:
        - Method Starter
        - SomaScan 11K Assay - Sample Prep
        - Green Play button
        - Acknowledge the RUO prompt

2. Fluent deck setup:
    - Obtain the consumables prepared in **Step 9.1**.
        - Dil 1 (**D1**)
        - Extra Dil 1 (**ED1**)
        - Dil 2 (**D2**)
        - Extra Dil 2 (**ED2**)
        - Dil 3 (**D3**)
        - Three stacks of 200 µL Nested Tips, 8 wafers per stack, no cover
        - One stack of 50 µL nested tips, 8 wafers per stack, no white cover
        - Three foil seals
    - Obtain a clean empty tip wafer.
    - Obtain the 11K Catch 0 Plates and place in Thermal adapter. Carefully remove the foils seal.
        - Make sure to securely hold the 11K Catch 0 Plates while removing the seals.
          NOTE: Failing to place 11K Catch 0 Plates in a thermal adapter can lead to spilling and Assay Failure.
    - When prompted by the robotic script, add the following items to the Fluent deck and wait to add samples and Dil 1 plate as instructed.

Deck Position	Item
2-3, 2-4	Stack of 200 µL Nested Tips (8 wafers per stack, no lid)
2-6	Stack of 50 µL Nested Tips (8 wafers per stack, no lid)
23-1	Dil 3 (**D3**)
23-2	Extra Dil 2 (**ED2**)
23-3	Dil 2 (**D2**)
23-4	Extra Dil 1 (**ED1**)
30-4 (waste trough)	Clean empty tip wafer
TMS 1	11K Catch 0 Plate - **Dil 1 (in Thermal Adapter)**
TMS 2	11K Catch 0 Plate - **Dil 2 (in Thermal Adapter)**
TMS 3	11K Catch 0 Plate - **Dil 3 (in Thermal Adapter)**

    - With 7 min left in diluent thaw press **Continue** within Fluent Control to initiate pipetting Assay Buffer to the dilution plates.
        - Carefully watch all buffer transfers to the dilution plates and take note of any short transfers, bubbles or other abnormalities.
        - The robot will pause again and prompt to add **samples** and **Dil 1** (**D1**) plate to deck.

3. Preparing Samples:
    - After the 15 min sample thaw, remove the sample rack from the rack thawing station.
        - Ensure samples are completely thawed by observing tubes in the bottom of the rack.
        - If not thawed return rack to the thawing station and thaw for additional 5 min.
    - Turn the rack thawing station off.
    - Centrifuge sample rack at 1000× g for 1 min.
        - Remove sample tube caps using a decapper.
            - ◊ Confirm that no sample is present in caps.

- If sample is present in the caps.
  - ◊ Replace caps on tubes
  - ◊ Centrifuge sample rack at 1000× g for 1 min.

- Cover sample Matrix rack with the plastic rack lid until further Instruction.

4. Preparing Dilution plates
   - After 20 min sample diluent thaw, prepare **Dil 1** sample plate.
     - Remove sample diluent from the 25 °C water bath
     - Ensure diluent is completely thawed
     - Invert diluent several times to thoroughly mix, avoid creating bubbles
   - Pour sample diluent into a clean reagent reservoir.
   - Pipette **140 µL sample diluent** (**critical volume**) into wells of **Dil 1** sample plate.
   - Centrifuge plate at 1000× g for 1 min.
   - When prompted, add the following items to the robot deck.

Deck Position	Item
23-5	Dil 1 (**D1**)
23-6	Sample Rack (no lid, lock tabs facing analyst)

   - Select **Continue** within the TouchTools prompt to initiate the sample dilution and 11K Catch 0 Plate preparation steps.
     - Carefully watch for short volume transfers, bubbles, bead aspiration or any other abnormality during pipetting.
     - Document any observation in the Plate Map tab of the SomaScan 11K Assay Workbook.
   - When prompted carefully remove 11K Catch 0 Plates.

9.4. Binding Reaction

1. Binding setup
   - Place 11K Catch 0 plates on the Thermal Adapter Removal Tool "Load" pins and carefully release the wire clips from the top of the plate (see D0005558).
   - Seal each 11K Catch 0 Plate with a foil seal.
     - Ensure each well is tightly sealed.
   - Carefully remove 11K Catch 0 Plates from Thermal Adapter using the Thermal Adapter Removal Tool by placing the 11K Catch 0 Plate and thermal adapter on the "Eject" pins and gently pushing down on the thumb tabs on the left and right hand sides of the thermal adapter (see D0005558).
   - Place the sealed 11K Catch 0 Plates in the binding shaker.
     - Confirm that the shaker is set to:

Category	Setting
Temperature	28 °C
Time	3 hr 30 min
Mix speed	850 rpm

- Turn the shaker on and confirm the shaker reaches **850 rpm**.
- Record the following In the Plate Map tab of the SomaScan 11K Assay workbook.
  - Binding start time.
  - Calculated binding end time (**start time + 3.5 hours**).

9.5. Robot Cleanup

1. Fluent Cleanup
   - Remove sample rack from deck and retain to populate **Tab 3- Assayed Sample List**.
   - Clear deck.
     - Remove remaining tip boxes.
     - Remove and discard sample dilution plates and wafer the from the waste trough into a biohazardous waste bin.
     - Leave the Tecan powered on and the Fluent Control running.
     - Recommendation: Wipe down deck with a disinfectant cloth.

9.6. Sample Plate Map

1. A Sample Import Sheet is required for SomaLogic to normalize and calibrate the data set. The **Tab 7 – Sample Import Sheet** is part of the SomaScan 11K Assay Workbook and is being populated through information entered in **Tab 2 - Reagents** and the Plate Map tab of the workbook.

2. Complete the **Tab 3 - Assayed Sample List** in the following way (all fields are case sensitive).
   - The following information is required for SomaLogic control samples (part of the kit):
     - SampleType
       - ◊ Calibrator
       - ◊ QC
       - ◊ Buffer
     - StudyId
       - ◊ Control
     - Barcode
       - ◊ Lot number of the control (from label)
         - · For example: 200169B
     - SampleID
       - ◊ Lot number digits only (i.e. the first 6 numbers from the barcode)
         - · For example: 200169
   - The following information is mandatory for Customer samples:
     - StudyId
     - SampleId
   - The following column must be blank for Customer samples:
     - Sample Type
     - Barcode

- Optional Information for Customer samples ONLY:
  - TimePoint
  - SampleGroup
  - SampleDescription
  - Matrix Type
  - Volume submitted
  - Optional1
  - Optional2

An example of a filled-out T**ab 3 - Assayed Sample List** is shown below:

Sample Number	SampleType	StudyId	Barcode	SampleId	TimePoint	SampleGroup	SampleDescription	Sample Matrix	Volume Submitted	Optional1	Optional2
1	Calibrator	Control	200169D								
2		SL-22-002		X1234569	1	treatment	Ind 1	Serum	130	low dose	site A
3		SL-22-002		X1234566	2	native	Ind 2	Serum	130		Site B
4	QC	Control	200170C								
5		SL-22-002		X1234567	2	treatment	Ind 3	Serum	130	high dose	Site A
6		SL-22-002		X1231234	1	native	Ind 4	Serum	130		Site B
7		SL-22-002		X1234576	1	native	Ind 5	Serum	130		Site A
8	Blank	Control	210331								

3. Complete the "Plate Map" tab of the SomaScan 11K Assay Workbook **Tab 4 - Plate Map** in the workbook is intended to be used for tracking information during the hybridization and readout part of the SomaScan 11K Assay. Some information in the table is populated through the information entered in **Tab 3 - Assayed Sample List**.
   - Enter or scan the SlideID #.
   - If samples clogged or had low volume, select the checkbox in the column "Clogged" or "Low Volume".
     - The selection will show up in the Sample Comments column of **Tab 4 - Plate Map**.
   - Assay Notes
     - If aspiration of beads during the assay process after photocleavage are observed select the check box in the column "C2 Aspiration"
     - If the subarray leaked during the hybridization or slide loading process, select the check box in the column "Leak"
     - Enter any other well specific assay noted into the "Assay Notes" column. This is a free text field.
     - All these notes will show up in the Assay Notes column of **Tab 4 - Plate Map**.
   - The bottom of **Tab 4 - Plate Map** allows for the tracking of additional information.
     - Verification of the slide ID (important if multiple assay versions are performed within the lab).
     - Initial and date for tracking the execution of procedure steps.
     - Tracking of hybridization oven, timer used for the hybridization, hybridization start and end time and dates.
4. Notes that impact the entire run or all samples on a single slide (i.e. temperature or reagent wash issues) should be submitted with the Agilent data in the body of the data upload email for the respective SlideId. The issue(s) will be entered into the Experiment notes in the database and will be returned in the SQS header of the data set.

10. SomaScan 11K Assay - Auxiliary Reagent Prep (Duration: ~10 min)

10.1. Auxiliary Preparation

NOTE: The following plates may be prepared in any order.

1. Magnetic Bead (**M-B**) plate preparation
    - Obtain an Adapter Plate and label Mag Beads (**M-B**)
    - Retrieve the Magnetic Beads from the 4 °C kit
    - Vortex Magnetic Beads for at least 1 min to ensure proper bead resuspension
    - Immediately pour vortexed beads into a clean reagent reservoir
    - Pipette **75 µL** Magnetic Beads (**critical volume**) into wells of Mag Bead Plate
    - Seal plate with a foil seal
    - Store plate at 4 °C
2. MB Prep Buffer (**M-P**) plate preparation
    - Obtain a Nunc Round Bottom Plate and label MB Prep Buffer (**M-P**)
    - Pour the MB Prep Buffer into a clean reagent reservoir
    - Pipette **100 µL** MB Prep Buffer into wells of MB Prep Buffer plate
    - Seal plate with a foil seal
    - Store plate at 4 °C
3. 10X Slide Block (**3-B**) plate preparation
    - Obtain a V-Bottom Plate and label Slide Block **(3-B**)
    - Retrieve 10X Slide Block aliquot from 25 °C water bath
    - Vortex for approximately 15 sec
    - Quick spin in a microfuge
    - Pour into a clean reagent reservoir
    - Pipette **15 µL** 10X Slide Block into wells of Slide Block plate
    - Seal plate with a foil seal
    - Store plate at 4 °C
4. MB Block (**2-B**) Plate Preparation
    - Obtain a V-Bottom Plate and label MB Block (**2-B**)
    - Retrieve the MB Block aliquot from 4 °C kit
    - Vortex MB Block and pour into a clean reagent reservoir
    - Pipette **30 µL** MB Block into wells of MB Block plate
    - Seal plate with a foil seal
    - Store plate at 4 °C

10.2. Sample Documentation

1. Sample preparation and well-specific notes.
    - Ensure any general sample preparation notes, well specific notes, or observations have been recorded in the SomaScan 11k Workbook.
    - Update **Tab 1 - Assay Overview** of the workbook.
    - Transfer sample preparation notes to Section 2 of **Tab 1 - Assay Overview** ; initial and date.
    - If there were no sample preparation notes, enter “None”.
        - Update **Tab 4 - Plate Map** of the workbook.
    - Add all well-specific notes to **Tab 4 - Plate Map** of the workbook.
    - Save the workbook file.

11. Setup of the SomaScan 11K Assay (Duration: ~2.5 h)

NOTE: Consumable and reagent preparation (11.1 - 11.2) should be Initiated ~ 30 min prior to Binding End Time.

11.1. Consumables preparation

1. Obtain four Pyramid Base Troughs and label on the front and left-hand side, where the notch is towards the analyst on the bottom left-hand corner.
    - Quench Buffer (**1-Q**)
    - Photo-Cleavage (**1-PC**)
    - Elution Buffer (**2-E**)
    - Tag
2. Obtain one V-Bottom Trough and label on the front and left-hand side, where the notch is towards the analyst on the bottom left-hand corner.
    - MB Wash Buffer (**2-W**)
3. Obtain three Adapter Plates and label on the front and left-hand side, where the notch is towards the analyst on the lower left-hand corner.
    - Catch-1 Elution (**C1E**)
    - Elution
    - Archive
        - Include Plate Name, assay date, and initial.
4. Obtain one V-Bottom Plate and label on the front and right-hand side, where the notches are towards the analyst.
    - Hyb
5. Obtain one Black Spec Plate.

11.2. Buffer Preparation

1. Retrieve the plates prepared during the auxiliary reagent preparation from 4˚C storage.
    - Mag Beads (**M-B**)
    - MB Block (**2-B**)
    - 10X Slide Block (**3-B**)
    - MB Prep Buffer (**M-P**)
2. Centrifuge the following plates at 1000× g for 1 min.
    - MB Block (**2-B**)
    - 10X Slide Block (**3-B**)
    - MB Prep Buffer (**M-P**)
3. Firmly place Mag Bead, C1E, Elution, and Archive plates into Thermal Adapters.
    - Make sure that the plate is securely placed in the Thermal Adapter.
    - Remove foil seal from the Mag Bead Plate.
    - Cover each plate with a lid.
4. Add the following reagents to the prepared Pyramid Base Troughs, each aliquot Is premeasured with the correct volume.
    - Quench Buffer (**1-Q**) (85 mL)
    - Photo Cleavage (**1-PC**) (40 mL)
    - Elution Buffer (**2-E**) (20 mL)
    - MB Wash Buffer 20% (**2-W**) (85 mL)
    - Cover each trough with a lid.

11.3. SomaScan 11K Assay Script

1. Fluent preparation
    - Log in to FluentControl (if not completed previously).
        - Select username
        - Enter password
2. From TouchTools home screen select
    - Method Starter
    - **SomaScan 11K Assay**
    - Green Play Button
3. Fluent deck setup
    - When prompted, verify both lightbulbs in the Photo-Cleavage station are on.
    - When prompted, add the following to deck.

Deck Position	Labware	Lid
2-2, 2-3, 2-4	200 µL (8 wafers per stack, no lid)	⊗
2-6	50 µL (8 wafers per stack, no lid)	⊗
9-2, 9-3	200 µL (8 wafers per stack, no lid)	⊗
9-5	Black Spec Plate (no lid)	⊗
9-6	Hyb plate (no lid)	⊗
16-1	Quench Buffer Trough(**1-Q**) (lidded)	+
16-2	Photo Cleavage Trough (**1-PC**) (lidded)	+
16-3	Elution Buffer Trough (**2-E**) (lidded)	+
16-6	MB Wash Buffer 20% Trough (**2-W**) (lidded)	+
23-2	Elution Plate (**E**) in thermal adapter (lidded)	+
23-3	MB Block Plate (**2-B**) (lidded)	+
23-4	10X Slide Block (**3-B**) (lidded)	+
23-5	MB Prep Buffer (**M-P**) (lidded)	+
23-6	Tag Trough (empty, no lid)	⊗
30-1	**C1E** plate in thermal adapter (lidded)	+
30-2	Mag Bead plate (**M-B**) in thermal adapter (lidded)	+
30-3	Archive plate In thermal adapter (lidded)	+
30-4	Waste trough with clean wafer on top	∅

- When prompted, verify the MB Wash Buffer 20% trough Is locked into place (**16-6**).
- Advance the robotic prompts until instructed to add 11K Catch 0 Plates to the robot deck.

4. Post Binding
   - At the end of the 3.5 hour binding step:
     - Remove 11K Catch 0 Plates from shaker.
     - Carefully insert 11K Catch 0 Plates into thermal adapters.
     - Carefully remove foil seals.

5. Continue with Fluent deck setup.
   - When prompted, add the following to deck:

Deck Position	Labware
TMS 1	**Dil 1** – 11K Catch 0 Plate **in thermal adapter**
TMS 2	**Dil 2**– 11K Catch 0 Plate **in thermal adapter**
TMS 3	**Dil 3**– 11K Catch 0 Plate **in thermal adapter**

- Advance the robotic prompts until instructed to add Tag Reagent to the robot deck.

6. Tag
    - Complete the Tag Dilution step, when prompted:
        - Retrieve Tag Diluent from 25 °C water bath.
        - Retrieve 100x Tag Reagent from 25 °C water bath.
            - ◊ Vortex 100x Tag Reagent for approximately 5 sec on highest setting.
            - ◊ Spin down Tag Reagent in microfuge for approximately 5 sec.
    - Add **400 µL** Tag Reagent (**1-T**) (**Critical Volume**) to Tag Diluent (**1-D**) bottle.
    - Gently mix by inversion, avoid creating bubbles.
    - Pour into the Tag trough at deck position **23-6**.
7. Robot pipetting
    - Advance the robotic prompts to continue the assay method.
        - The method will initiate pipetting aspiration and dispensing.
        - Observe MCA tip loading and RGA plate movements.
        - Carefully watch for short volume transfers, bead aspiration, or any other sample or assay abnormality.
        - Record all observed abnormalities in the SomaScan 11K Assay Workbook.
8. Photo-Cleavage
    - At the start of Photo-Cleavage, ensure both UV bulbs on Photo-Cleavage station turn on.
        - If either bulb does not turn on, promptly contact Tech Support.
9. Run Completion Tasks
    - Near the end of the robotic script remove Black Spec Plate from deck position 9-5 when prompted.
        - Centrifuge Black Spec Plate 1000× g for 1 min.
    - At the completion of the script remove, save and foil seal the following from Fluent deck when prompted.

Deck Position	Labware
TMS 1	Archive plate
9-6	Hyb plate

11.4. End of Assay Tasks

1. Fluent deck cleanup
    - Remove, save, and consolidate remaining compatible robotic tips.
    - Remove, save, and foil seal Slide Block Plate.
      NOTE: The Slide Block plate is saved as a precautionary measure if for any reason the Hyb plate needs to be remade during the slide dropping procedure **Step 12**. Otherwise, this plate can be discarded into the biohazard waste stream.
    - Clear remaining troughs, plates and waste trough tip wafer from deck.
        - Discard into biohazard waste stream.
    - Remove Assay Buffer refillable trough bottle and trough.
        - Dispose of Assay Buffer and rinse with tap water followed by DI water.
          NOTE: Ensure water is completely drained from the tubing before leaving to dry overnight.
    - Wipe down deck and waste chute with a disinfecting wipe.
    - Remove waste bottle from waste trough and add bleach.
        - Allow the waste bottle to bleach for at least 30 min, or overnight.
    - Exit FluentControl software.
        - Exit out of the FluentControl software by selecting File > Exit.
    - Turn off the power strip connected to the TMS control box and BioShake shakers.
    - Turn off the Fluent robot (Myrius box).
    - Shut down computer connected to the Fluent.
2. Assay and well-specific notes
    - Ensure general assay notes, well specific noted and any observations have been recorded in the SomaScan 11K Assay workbook.
    - Update **Tab 4 - Plate Map** of the workbook.

11.5. Cyanine 3 Reading of Eluate

NOTE: If a Molecular Devices SpectraMax Plate reader is used, SomaLogic can provide a template file with all the required settings.

NOTE: The instructions below are a guideline and will vary depending on the make and model of the fluorescence plate reader used

1. Considerations for the fluorescence of Cyanine 3 are:
    - Excitation wavelength maximum: 554 nm.
    - Emission wavelength maximum: 568 nm.
    - Laser lines used for excitation: 532 nm.
    - Common filter set used: TRITC (excitation 540/25 (550/25) nm, emission 575 (605/70) long pass, beam splitter 564 (570) nm).
2. General instructions
    - Within the fluorescent plate reader software create a new plate.
    - Load the plate into the fluorescent plate reader.

- Read the plate using the settings (example given for SpectraMax plate reader series).
  - Fluorescence
  - Bottom read
  - Excitation wavelength: 535 nm
  - Emission wavelength: 575 nm
  - Cutoff wavelength: 570 nm

3. After the plate has been read:
   - Remove and retain until analysis is complete.
   - Save file as Plate Name.
   - Export a .txt file.
   - Open the .txt file in Excel and copy the data to **Tab 5 - Cyanine 3 Analysis** of the workbook.
     - The layout must be a matrix of eight rows and 12 columns corresponding to the format of the 96-well plate
   - **Tab 5 - Cyanine 3 Analysis** will automatically calculate the following:
     - Calibrators
       - ◊ Average RFU
       - ◊ %CV
     - Blank Control
       - ◊ Median RFU
       - ◊ %CV
       - ◊ Median Blank Control RFU + 3 Standard Deviations
     - QC Sample
       - ◊ Average RFU
       - ◊ %CV
     - Sample
       - ◊ Median RFU
   - Evaluate Cyanine 3 results.
     - Acceptance criteria:
       - ◊ Average RFU Calibrator > Median Blank Control RFU + 3 Standard Deviations.
     - The workbook will assess this acceptance criteria and will provide a pass/fail result.
   - Discard black spec plate after evaluating Cyanine 3 results.

12. Microarray Hybridization (Duration: ~30 min)

NOTE: Detailed instructions on using the SureHyb chambers can be found on Agilent's web site at https://www.agilent.com/cs/library/usermanuals/public/G2534-90004_HybridizationChamber_A1.pdf

NOTE: The hybridization plate can be remade using sample eluate from the archive plate if the sample(s) become compromised. Follow procedure in **Appendix 4**.

1. Retrieve the following reagents:
    - 2× Hybridization Buffer (**3-H**)
    - Microarray slides
    - Slide ID: 2587401
    - Gasket slides
2. Prepare Hybridization (Hyb) plate.
    - Transfer 2× Hybridization Buffer (**3-H**) into a clean reagent reservoir.
    - Add 25 µL 2× Hybridization Buffer (**3-H**) (**Critical Volume**) to all tested wells of the "Hyb" plate.
        - 2× Hybridization Buffer (**3-H**) is extremely viscous. Pipette slowly and drag the tips on the side of the trough to remove any residual volume.
    - Carefully mix by pipetting up and down slowly 5 times, then faster 10 times.
        - Avoid creating bubbles.
    - Centrifuge at 1000x g for 1 minute.
    - Foil seal plate.
3. Document slide barcode numbers.
    - Enter the microarray slide barcodes into **Tab 4 - Plate Map** of the workbook.
        - The barcodes can be entered into the workbook using a hand-held barcode reader.
    - Print a copy of **Tab 4 - Plate Map**.
4. Verify the following settings on the 6-channel spanning P300 pipette:
    - Volume is set at 40 µL.
    - Spanning knob is set to 16.
5. Load Microarray Slides.
    - Slide the lever on slide dropper to the "drop" position.
    - Load a SureHyb chamber base onto slide dropper with the label opening on the left.
    - Load a Gasket Slide with the barcode label to the left and gasket o-rings facing upwards.
    - Slide lever on slide dropper to the "load" position.
    - From the Hyb plate, load **40 µL** (**Critical Volume**) of each sample onto a gasket slide in the order shown in **Figure 1**.
        - Do not dispense past first stop while dispensing sample to gasket slide.
        - Ensure that sample does not touch the gasket edge
        - Avoid creating bubbles

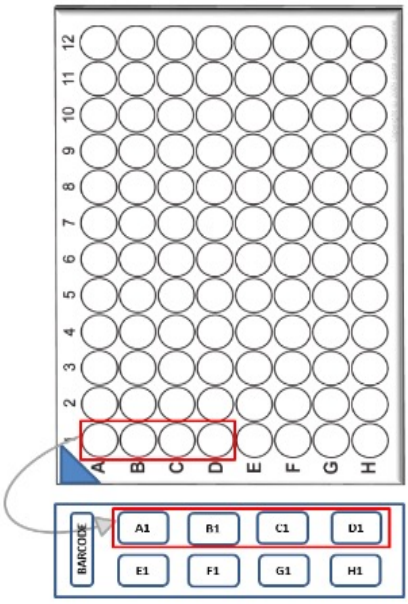


Figure 1

- Verify the microarray slide number against the **Tab 4 - Plate Map** printout as slides are being loaded.
- Place microarray slide onto the slide dropper with **Agilent** label facing down and to the lef.
  - Handle microarray by edges only; do not touch the interior portion of the microarray.
- Slide lever on slide dropper to the “drop” position.
- Assemble the Hybridization chamber.
  - Place chamber top on gasket-slide sandwich.
  - Place clamp over assembled hybridization chamber and tighten.
  - Ensure the bubble moves freely in each subarray when the assembly is rotated.
  - Ensure there is equal volume in each subarray.
  - Note any abnormalities (for examples low volume or leaks) in **Tab 7 – Sample Import Sheet**.
    - ◊ Repeat for remaining samples.

6. Load hybridization oven
   - Ensure hybridization oven temperature is 55.0 °C ± 2.0 °C.
   - Set rotator speed to 20 rpm.
   - Place hybridization chambers in oven.
     - Ensure hybridization chambers are balanced in oven.
   - Start a timer for 19 hours.
   - Record Hybridization Start Date and Time on **Tab 4 - Plate Map** printout.
   - Record Hybridization Calculated End Date and Time on **Tab 4 - Plate Map** printout.
     - Calculated End Time = Start Date and Time +19 hours.

13. Clean-up (Duration: ~10 min)

13.1. Slides and Archive Plate

1. Store unused microarray slides in nitrogen-purged desiccator cabinet.
2. Seal Archive Plate.
    - Seal the Archive plate with a foil seal.
        - Make sure that the seal is tightly adhering to all wells of the plate.
          NOTE: For longer term storage it is recommended to heat-seal the Archive plate. Simple adhesive sealing will not hold up at -20 °C over a long period of time and sample loss might occur. If heat-sealing is not available, store in an airtight plastic bag.
3. Store Archive plate in -20 °C storage.
   NOTE: The "Archive" plate should be stored for at least two weeks after hybridization to enable re-hybridization if a hybridization error is detected.

13.2. Documentation

- Ensure well-specific notes have been recorded on **Tab 4 - Plate Map** printout.
- Update **Tab 1 - Assay Overview** of the workbook.
- Transfer SomaScan Assay notes to Section 2 of **Tab 1 - Assay Overview**; initial and date.
- If there were no SomaScan Assay notes, enter "None".
- Update **Tab 4 - Plate Map** of the workbook.
- Transfer well-specific notes from **Tab 4 - Plate Map** printout to **Tab 4 - Plate Map** of the workbook.
- Save the workbook file.

13.3. Day 2 Preparation

1. Verify the temperature of the 37 °C incubator and that it contains at least 300 mL Agilent Wash 2.

14. Microarray Processing (Duration: ~0.75 h + 3.5 h scan time)

14.1. Slide Washing

This section describes the protocol for washing microarray slides. It is broken up into two sections for either the automated protocol (14.1.1) or manual (14.1.2) depending on equipment in the laboratory. If you follow the automated protocol (14.1.1) skip section 14.1.2. If you follow the manual protocol (14.1.2) skip section 14.1.1.

Note: Automated slide washing reduces the risk of wash artifacts on the microarray slides, if you choose to follow manual wash, it is important to follow the steps closely for the best results.

14.1.1. Slide Washing – Automated

Note: Agilent Wash Buffer 2 is pre-warmed in the 37°C incubator. If it stored at Room temperature, please allow for >30 minutes of setup time to allow Wash Buffer 2 to stabilize at 37°C.

14.1.1.1. Automated Slide Washing – Preparation

1. Retrieve Agilent Wash 1, Agilent Wash 2, and Acetonitrile (ACN).
2. Record lot numbers and expiration dates in **Tab 2 - Reagents** of the workbook.
3. Turn **ON** the main power switch on the right side of the SciGene Little Dipper.
4. Set up baths:
    - Ensure baths are free of dust and debris.
    - Place the 3 baths into the slide washer base slots 1, 2 and 3.
    - Add a stir bar to all three bath.
    - Fill baths to fill line (~0.5 cm from the top).
        - Bath 1 with Agilent Wash 1
        - Bath 2 with pre-warmed 37 °C Agilent Wash 2
        - Bath 3 with ACN
    - Set stir dial to ~5 for each bath.
      NOTE: The stir setting is dependent on the slide washer unit and size of stir bar being used. Increase stir speed until dimpling on the surface of the liquid is observed.
    - Lower temperature probe for Bath 2 all the way down into the bath.
    - Turn ON the power switch for Bath 2.
        - Press & hold the Set button to view temperature setting:
        - Bath 2 should be set at 37 °C
            - ◊ To set the correct temperature press & hold Set and use the ▲ or ▼ buttons.
    - Cover Baths 1 and 2 with plastic lids if more than 10 min remain until the slide washing.
    - Cover Bath 3 with a metal lid.
5. Set up disassembling dish.
    - Place slide rack into the disassembly dish.
    - Add Wash Buffer 1 to the disassembling dish with slide rack.
        - Ensure volume of Agilent Wash 1 will thoroughly cover slides in slide rack.
    - Cover disassembling dish until use.

14.1.1.2. Automated Slide Washing – Processing

1. When 19 hour hybridization is complete:
    - Visually confirm hybridization oven temperature is 55.0 °C ± 2.0 °C.
    - Visually confirm hybridization oven speed is set to 20.
    - Document hybridization deviations on the plate map tab in the Assay Workbook.
    - Hybridization End Time may be up to 5 min after Calculated End Time on Plate Map.
2. Separate slide-gasket slide sandwich (sandwich).
    - Remove 2 Hybridization chambers at a time from the hybridization oven.
    - Open the first hybridization chamber.

- Grasp the slide gasket sandwich by the edges and remove it from the chamber.
- Inspect the slide gasket sandwich for any abnormalities (volume changes, leaks.
  - Note any abnormalities in the plate map.
- Submerge the slide-gasket sandwich into the disassembling dish.
- Gently insert forceps between the slide and the gasket at the barcode.
- Holding the slide upright and separating the gasket from the slide, allow the gasket slide to fall away.
- Place the slide in the slide rack.

3. Repeat for the remaining hybridization chambers.
4. Gently slide fingers across the tops of the slides to ensure proper placement in the rack.
5. Run Wash 1+2 protocol on the SciGene Slide washer.
   - Remove the covers from the baths if present.
   - On the touch screen of the SciGene slide washer:
     - Press the Run Protocol button
     - Select program "Wash 1+2"
     - Press Select
     - Press Start Now
   - Quickly and steadily transfer the slide rack from the disassembling dish to Bath 1.
     - Submerge the slide rack in Bath 1.
     - Align the slide rack with the black lines on the instrument.
     - Align the holes in the handle with the two bumps in the gripper paddle as it lowers.

     NOTE: If the gripper closes before the holes align, manually adjust slide rack using the thumb tab.
   - When slides are in Bath 1 (This step can be completed after the slide washing procedure is finished.):
     - Discard gasket slides.
     - Empty buffer from disassembling dish into sink.
     - Rinse disassembling dish with tap water.
     - Rinse disassembling dish with DI water.
     - Dry disassembling dish with a lint-free wipe.
   - When slides have transferred to Bath 2:
     - Turn off stir dial for Bath 1.
     - Remove Bath 1 from base and empty buffer into sink.
     - Rinse Bath 1 with tap water.
     - Rinse Bath 1 with DI water.
     - Dry Bath 1 with a lint-free wipe.
   - When "Wash 1+2" protocol is complete:
     - Remove slide rack from gripper paddle using thumb tab.
     - Place slide rack on a lint-free wipe to absorb excess liquid.
     - Press □ button on touch screen.

6. Run ACN protocol on the SciGene Slide washer.
    - On the touch screen of the SciGene slide washer:
        - Select program “ACN”
        - Press Select
        - Press Start Now
    - Move slide rack into empty Bath 1.
    - Align the slide rack with the black lines on the instrument.
    - Align holes in slide rack handle with nodes on gripper paddle.
    - While the “ACN” program is running:
        - Turn off the temperature power switch and stir bar for Bath 2
        - Raise the temperature sensor out of Bath 2
        - Remove Bath 2 from the base and empty into the sink
        - Rinse Bath 2 with running tap water
        - Rinse Bath 2 with DI water
        - Dry Bath 2 with lint-free wipes
7. Once the “ACN” program is complete:
    - Remove the slide rack from gripper paddle using the thumb screw.
    - Place the slide rack on a lint-free wipe to absorb any remaining liquid.
    - Turn off the stir bar for Bath 3.
    - Turn OFF the main power switch on the right side of the SciGene Little Dipper.
    - Record any abnormalities during slide washing in the Sample Import Sheet.
    - Dispose of the ACN according to institutional requirements.

14.1.2. Slide Washing – Manual

Note: Automated slide washing reduces the risk of wash artifacts on the microarray slides, if you choose to follow manual wash, it is important to follow the steps closely for the best results.

Note: Agilent Wash Buffer 2 and Staining Dish 3 are pre-warmed in the 37°C incubator. If they are stored at Room temperature, please allow for >30 minutes of setup time to allow Wash Buffer 2 to stabilize at 37°C.

14.1.2.1. Manual Slide Washing – Preparation

1. Retrieve Agilent Wash 1, Agilent Wash 2, and Acetonitrile (ACN)
2. Ensure that both stir plates are plugged in to an active power outlet
    a. Stir plate 1 – Room temperature magnetic Stir plate.
    b. Stir plate 2 – Magnetic Hotplate stirrer with active temperature control.
3. Record lot numbers and expiration dates in **Tab 2 - Reagents** of the workbook
4. Turn **ON** the power switch on the Room Temperature (RT) Stir Plate and the Magnetic Hotplate Stirrer (37 °C stir plate)

5. Set up baths.
    - Ensure baths are free of dust and debris.
    - Add a stir bar to all three baths.
    - Fill baths with ~350 mL of buffer (enough to completely submerge the slides in the slide rack).
        - Bath 1 with Agilent Wash 1
            - ◊ Place Bath 1 on Stir plate 1 (room temperature)
        - Bath 2 with pre-warmed 37 °C Agilent Wash 2
            - ◊ Place Bath 2 on Stir plate 2 (37 °C)
        - Bath 3 with ACN
    - Increase the stir dial until a small dimple forms on the surface of the liquid.

      NOTE: The stir setting is dependent on the slide washer unit and size of stir bar being used. Slowly increase stir speed until dimpling on the surface of the liquid is observed.

    - Lower temperature probe for Bath 2 until the sensor is fully submerged in the buffer.
    - Turn the temperature dial on Bath 2 (37 °C Stir plate) until the target temperature of 37 °C is reached.
        - To set the correct temperature turn the dial clockwise until the display reads 37 °C.

      NOTE: The Magnetic Hotplate Stirrer (37 °C stir plate) works via active temperature control. It is important to ensure that the temperature probe is fully submerged in the heated buffer and the temperature is set to 37 °C.

    - Cover Baths 1 and 2 with lids if more than 10 min remain until the slide washing.
    - Cover Bath 3 with a lid.
6. Set up disassembling dish.
    - Place slide rack into the disassembly dish.
    - Add Wash Buffer 1 to the disassembling dish with slide rack.
        - Ensure volume of Agilent Wash 1 will thoroughly cover slides in slide rack.
    - Cover disassembling dish until use.

14.1.2.2. Manual Slide Washing – Processing

1. When 19 hour hybridization is complete:
    - Visually confirm hybridization oven temperature is 55.0 °C ± 2.0 °C.
    - Visually confirm hybridization oven speed is set to 20.
    - Document hybridization deviations on the plate map tab in the Assay Workbook.
    - Hybridization End Time may be up to 5 min after Calculated End Time on Plate Map.

2. Separate slide-gasket slide sandwich (sandwich).
    - Remove 2 Hybridization chambers at a time from the hybridization oven.
    - Open the first hybridization chamber.
    - Grasp the slide gasket sandwich by the edges and remove it from the chamber.
    - Inspect the slide gasket sandwich for any abnormalities (volume changes, leaks).
        - Note any abnormalities in the plate map.
    - Submerge the slide-gasket sandwich into the disassembling dish.
    - Gently insert forceps between the slide and the gasket at the barcode.
    - Holding the slide upright and separating the gasket from the slide, allow the gasket slide to fall away.
    - Place the slide in the slide rack.
3. Repeat for the remaining hybridization chambers.
4. Gently slide fingers across the tops of the slides to ensure proper placement in the rack.
5. Perform Wash 1, Wash 2, and ACN.
    - Remove the covers from the baths if present.
    - Quickly and steadily transfer the slide rack from the disassembling dish to Bath 1 on the Room Temperature stir plate.
        - Submerge the slide rack in Bath 1.
        - Ensure that the stir bar is not-contacting any of the slides.
        - Start a 5 minute timer for Wash Buffer 1 wash.
    - When slides are in Bath 1 (This step can be completed after the slide washing procedure is finished).
        - Discard gasket slides.
        - Empty buffer from disassembling dish into sink.
        - Rinse disassembling dish with tap water.
        - Rinse disassembling dish with DI water.
        - Dry disassembling dish with a lint-free wipe.
    - After 5 minute Wash Buffer 1 timer has completed, manually transfer the slide rack to the Wash Buffer 2 bath on the 37C temperature controlled stir plate.
        - Remove the slide rack from Wash Buffer 1 using a steady speed.
    - Start a 5 minute timer.
    - When slides have transferred to Bath 2.
        - Turn off stir dial for Bath 1.
        - Remove Bath 1 from Room Temperature Stir plate and empty buffer into sink.
        - Rinse Bath 1 with tap water.
        - Rinse Bath 1 with DI water.
        - Dry Bath 1 with a lint-free wipe.
        - Add the ACN bath to the Room temperature stir plate.
        - Turn on the stir dial for the Room temperature Stir plate 1 (ACN) and ensure that a small dimple on the surface of the ACN is observed.

- After 5 minute timer is complete, **SLOWLY** remove the slide rack from the 37C Wash Buffer 2 bath and transfer to the ACN bath.
  - It should take approximately 15 seconds to completely remove the slide rack from Wash 2.

  Note: Slowly removing the slides from this buffer allows the aqueous wash solution to "peel" away from the hydrophobic slide surface, minimizing water transfer to the Acetonitrile solution and reducing artifacts on the slide.
- Start a 5 minute Timer for the ACN wash.
- During the 5 minute timer of the "ACN" protocol:
  - Turn off the temperature power switch and stir bar for Bath 2.
  - Raise the temperature sensor out of Bath 2.
  - Remove Bath 2 from the stir plate and empty into the sink.
  - Rinse Bath 2 with running tap water.
  - Rinse Bath 2 with DI water.
  - Dry Bath 2 with lint-free wipes.
- After the 5-minute ACN timer is complete, **SLOWLY** remove the slide rack from the ACN bath.
  - It should take approximately 15 seconds to completely remove the slide rack from ACN.

  Note: When removing the rack slowly, the meniscus of the ACN moves across the slides without tearing and leaves no droplets behind. Removing slides too fast can leave droplets of ACN that dry on the slide surface and lead to scanning artifacts.
- Place the slide rack on a lint-free wipe to absorb any remaining liquid.
- Turn off the stir plate for ACN bath.
- Record any abnormalities during slide washing in the Sample Import Sheet.
- Dispose of the ACN according to institutional requirements.

14.2. Microarray Scanning and Feature Extraction (FE)

1. If the Agilent scanner computer is not on, turn on the Agilent scanner computer.
2. If the Agilent microarray scanner is not on, turn on the Agilent Microarray scanner using the power switch on the front.
   - a. Wait until the scanner is done initializing
3. Open the Agilent Microarray Scan Control software on the Agilent scanner computer.
4. Insert slides into slide holders.
   - Place a slide holder on a flat surface.
   - Gently push in and pull up on tabbed end of clear plastic cover to open slide holder.
   - Retrieve a slide from the slide rack and hold slide by the edges.
     - Avoid contact with the face of microarray side.
   - Ensure that the Agilent label of the slide is facing up and gently lower slide into slide holder with label oriented near hinges.
   - Close plastic slide cover.
     - Push on the tab until it clicks.
   - Ensure clear tab is flush with holder and that the slide is level.
     - If clear tab is not flush with slide holder, use a different holder.
   - Complete for remaining slides.

5. Scan slides
    - Select Open Door in Agilent Microarray Scan Control software.
    - Insert each slide holder into an open slot with the arrow pointing to the left and finger grip facing up.
    - Select Close Door in Agilent Microarray Scan Control software.
    - Under Output Folder use the Shift or Ctrl key highlight slides to be scanned.
    - Select the "..." icon on the right side of the last slide.
    - Navigate to the designated folder for the study.

    Note: Make a new folder and enter the folder name as Plate ID or Plate Name if not already present.

    - Select OK.
    - Confirm Scan Protocol defaults to "AgilentG3_GX_1Color".
    - Add slides to queue.
    - Select Start Scan if software is not already actively scanning.
6. Initiate Agilent Feature Extraction (FE).
    - Open the Feature Extraction software.
    - Select File > New > On-Time Project.
    - Change the settings of the following fields:

Field	Setting
Operator	Analyst initials
Input/Incoming Image Folder	Folder where the .tif images are saved
Grid	Local file only
Time Out Setting	Ensure settings are configured to allow all slide images for a plate to extract

    - Select the Run project (start) icon 
    - In popup window select "yes" to save the changes.
        - Navigate to the Incoming Image Folder.
        - Name the project Plate ID_FE or Plate Name_FE.
    - Select Save.
    - If an additional pop-up window appears:
        - Select All to Extract
        - Select OK
7. Clean the slide washer and hybridization chambers.
    - Clean temperature sensor of Bath 2 with DI water and a lint-free wipe.
    - If there are no succeeding washes for the day, dispose of the acetonitrile according to institutional requirements.
    - Return empty Bath 3 to the slide washer, dry with a lint-free wipe, and cover.
    - Turn OFF the main power switch on the right side of the SciGene Little Dipper.
    - Clean and store hybridization chambers.
        - Rinse with DI water
        - Dry with a clean paper towel

15. QC and Data Upload (Duration: ~30 min)

15.1. QC of Feature Extraction output

1. Navigate to the folder where the output file from the Agilent Feature Extraction Software are located.
2. Open each .pdf file within this folder and review.
    - Grid Alignment
        - For the four corners of each array, ensure that the crosses that indicate the feature location are in the center of the green circles.
    - Histogram of signal
        - The histogram of the blank sample should have a narrow histogram with the peak at ~2 log.
        - The histogram for a serum or plasma sample should have a peak at ~3 log.
3. Record any observation on **Tab 4 - Plate Map** of the workbook.

15.2. Data Upload

1. Create a folder named with the study ID.
    - The study ID should consist of a letter code for your institution followed by a digit year, a consecutive number and Set#.
        - Example: SL-23-002_Set001
2. Copy the completed study workbook into the folder.
3. Copy the *.txt from the Feature Extraction output into the folder.
4. Compress the folder to a zip file.
5. Log in to Box (www.box.com) using the username and password assigned to your institution.
6. Navigate to the folder:
    - SomaScan Data and click on the folder with the year.
        - Example: SomaScan Data > 2023
7. Upload the .zip file to this folder.
8. Email SomaLogic BI Services bi-services@somalogic.com with the study name in the subject line, and include any study notes, plate notes, or specific instructions.
9. SomaLogic BI Services will:
    - Enter the experimental data into the SomaLogic database and perform the primary data analysis.
    - Deposit the SQS and adat files to the study folder on Box.
    - Send an email with a notification that the data is available.

16. References

Document ID	Document Title
D0004619	SomaScan Assay - Equipment List
D0006492	SomaScan 11K Assay - Consumables List
D0006494	SomaScan 11K Assay - Experienced User Checklist Serum and Plasma
D0006495	SomaScan 11K Assay - Workbook
D0005558	Thermal Adapter Tool - IFU

17. Appendix 1: Available Kits and Kit Components

Common Name	Amount consumed per run	Amount needed per run*
Matrix Tubes with Rack	85 each	85
50 µL Nested Tips	8 wafers	16 wafers
200 µL Nested Tips	50 wafers	56 wafers
Pyramid Base Trough	4 each	4 each
V-Bottom Trough	1 each	1 each
Lid	11 each	11 each
Adapter Plate	4 each	4 each
Nunc Round-Bottom Plate	6 each	6 each
Nunc V-Bottom Plate	3 each	3 each
Black Spec Plate	1 each	1 each
Foil Seals	12 each	12 each
300 µL Tips	1 box	1 box
Gasket Slides	12 each	12 each
Agilent Wash 1	700 mL	700 mL
Agilent Wash 2	350 mL	350 mL
Acetonitrile	350 mL	350 mL
Reagent Reservoir	6 each	6 each
20 µL Tips for multi-channel pipette	2 boxes	2 boxes
200 µL Tips for multi-channel pipette	6 boxes	6 boxes
1000 µL Tips for single channel pipette	1 tip	1 tip

18. Appendix 2: Available Kits and their Components

Kit Part Number	Name	Subcomponent Part Number	Subcomponent Name	Storage Temperature
900-00050	SomaScan 11K Assay – Plasma Kit	899-00054	SomaScan 11K Assay, ambient components	Ambient (+10 to +30 °C)
		899-00055	SomaScan 11K Assay, 4C components	+4 °C (+2 to +8 °C)
		899-00056	SomaScan 11K Assay, -20C components	-20 °C (-10 to -30 °C)
		899-00057	SomaScan 11K Assay, -80C Plasma components	-80 °C (-70 to -90 °C)
		651-00125	Assay Buffer, 1000 mL	+4 °C (+2 to +8 °C)
900-00051	SomaScan 11K Assay – Serum Kit	899-00054	SomaScan 11K Assay, ambient components	Ambient (+10 to +30 °C)
		899-00055	SomaScan 11K Assay, 4C components	+4 °C (+2 to +8 °C)
		899-00056	SomaScan 11K Assay, -20C components	-20 °C (-10 to -30 °C)
		899-00058	SomaScan 11K Assay, -80C Serum components	-80 °C (-70 to -90 °C)
		651-000125	Assay Buffer, 1000 mL	+4 °C (+2 to +8 °C)
900-00052	SomaScan 11K Assay – Plasma Kit, No Slides	899-00059	SomaScan 11K Assay, ambient components, no slides	Ambient (+10 to +30 °C)
		899-00055	SomaScan 11K Assay, 4C components	+4 °C (+2 to +8 °C)
		899-00056	SomaScan 11K Assay, -20C components	-20 °C (-10 to -30 °C)
		899-00057	SomaScan 11K Assay, -80C Plasma components	-80 °C (-70 to -90 °C)
		651-00125	Assay Buffer, 1000 mL	+4 °C (+2 to +8 °C)
900-00053	SomaScan 11K Assay – Serum Kit, No Slides	899-00059	SomaScan 11K Assay, ambient components, no slides	Ambient (+10 to +30 °C)
		899-00055	SomaScan 11K Assay, 4C components	+4 °C (+2 to +8 °C)
		899-00056	SomaScan 11K Assay, -20C components	-20 °C (-10 to -30 °C)
		899-00058	SomaScan 11K Assay, -80C Serum components	-80 °C (-70 to -90 °C)
		651-00125	Assay Buffer, 1000 mL	+4 °C (+2 to +8 °C)

Component Part Number	Name	Subcomponent Part Number	Subcomponent Name	Quick Code
899-00054	SomaScan 11K Assay, ambient components	651-00126	MB Prep Buffer, 11mL	M-P
		651-00033	2x Hybridization Buffer, 3.5mL	3-H
		300-00010-12	Microarray, 8x60k, 8 per slide, SomaScan 11K Assay, 12 pk	--
		520-00010	Adapter 96-Well Plate	--
899-00055	SomaScan 11K Assay, 4C components	651-00003	Quench Buffer, 85 mL	1-Q
		651-00005	Elution Buffer, 20 mL	2-E
		651-00007	Photo-Cleavage Buffer, 40 mL	1-PC
		651-00030	MB Block, 3.5 mL	2-B
		651-00031	Magnetic Beads, 8.5 mL	M-B
		651-00035	Tag Diluent, 40mL	1-D
		651-00074	MB Wash Buffer 20%, 85 mL	2-W
899-00056	SomaScan 11K Assay, -20C components	651-00131	11K Catch 0 Plate - Dilution 1	--
		651-00132	11K Catch 0 Plate - Dilution 2	--
		651-00133	11K Catch 0 Plate - Dilution 3	--
		651-00008	100x Tag Reagent, 460 uL	1-T
		651-00009	10x Slide Block, 1.8 mL	3-B
899-00057	SomaScan 11K Assay, -80C Plasma components	650-00075	11K Plasma Calibrator	--
		650-00076	11K Plasma QC1 Sample	--
		650-00033	Blank, 140uL	--
		651-00072	Plasma Diluent, 17mL	D-P
899-00058	SomaScan 11K Assay, -80C Serum components	650-00077	11K Serum Calibrator	--
		650-00081	11K Serum QC1 Sample	--
		650-00033	Blank, 140uL	--
		651-00073	Serum Diluent, 17mL	D-S
899-00059	SomaScan 11K Assay, ambient components, no slides	651-00126	MB Prep Buffer, 11mL	M-P
		520-00010	Adapter 96-Well Plate	--
899-00060	SomaScan 11K Assay, Ambient Components, 2 Slides	651-00126	MB Prep Buffer, 11mL	M-P
		651-00033	2x Hybridization Buffer, 3.5mL	3-H
		300-00010-002	Microarray, 8x60k, 8 per, SomaScan 11K Assay, 2 pk	--
		520-00010	Adapter 96-Well Plate	--

19. Appendix 3: Instructions for Adjustment of RGA Gripper Fingers

NOTE: Additional details can be found in the Tecan Fluent robot user manual D0004621.

1. Remove one of the nest carrier deck segments and replace it with the flat deck segment.
2. Manually move RGA head over flat deck segment and lower until fingers are just above surface.
3. Take instrument out of Zero-Gravity mode.
4. Loosen screws on gripper fingers.
5. Using the Move tool in FluentControl, step RGA down until Z = 0.0.
6. Adjust the gripper fingers so they align flush with the carrie.
7. Tighten the screws on both gripper fingers (do not overtighten).
8. Using the Move tool, put RGA back in to Zero-Gravity mode.
9. Manually raise RGA head.

20. Appendix 4: Re-hybridization From Frozen Archive Plate Procedure

NOTE: This procedure is for the hybridization, or re-hybridization, from a frozen SomaScan Archive plate. For Re-hybridization follow Step 20.1.1.1. If Hybridizing (for the first time) from a frozen archive plate skip directly to step 20.1.1.2.

20.1.1.1. New Workbook for Re-Hybridization reagent tracking

1. Open the completed plate **Workbook** and read notes in the **Assay Overview** tab.
2. Save **Workbook** using **Save As** with current name and "rehyb" appended at the end of Plate Name.
    - e.g. SL-23-002_Set001_rehyb
3. Delete the following information from the newly saved **Workbook**:
    - Readout and Data Upload notes on **Tab 1_Assay Overview**.
    - The following reagent lots and expiration dates from the **Tab 2_Reagents** tab:
        - -20C Components
            1. If using the same lot of -20C components then do not delete this lot number
            2. Enter new Slide Block lot number information if using a different lot from the original -20C components in the Assay Overview Tab under "Readout and Data Upload" notes section
        - Agilent Gasket Slides
        - Ambient Components
            1. If using the same lot of Ambient components then do not delete this lot number
            2. Enter new Hyb Buffer lot number information if using a different lot from the original ambient components in the Assay Overview Tab under "Readout and Data Upload" notes section.
        - Agilent Wash Buffer 1
        - Agilent Wash Buffer 2
        - Acetonitrile
4. Add Agilent slide numbers in **Plate Map**.
5. Add Slide hybridization and slide washing notes in **Plate Map**.

20.1.1.2. Reagent Preparation

1. Obtain aliquot of 10X Slide Block (3-B) from -20 °C storage and document -20C Components lot number and expiration date in Reagents tab of the workbook.
2. Confirm the 25 °C water bath temperature is 25.0°C ± 2.0°C.
3. Place 10X Slide Block in water bath and thaw for at least 15 minutes.
4. Retrieve the following reagents:
    - 2× Hybridization Buffer (2× Hyb Buffer)
    - Microarray Slides
    - Gasket Slides
5. Document lot numbers and expirations dates of reagents in the **Reagents** tab of the **Workbook**.

20.1.1.3. Archive Plate Preparation

1. Obtain Archive plate from -20°C storage.
2. Confirm 37 °C incubator temperature is 37.0°C ± 2.0°C.
3. Thaw Archive plate in incubator for at least 15 minutes.
4. Confirm plate shaker is set at:

Category	Setting
Time	10 minutes
Temperature	25 °C
Shaking Speed	850 rpm

5. Shake Archive plate for 10 minutes at 850 rpm.
6. Centrifuge Archive plate at 1000 x g for 1 min.

20.1.1.4. Hybridization Plate Preparation

1. Obtain a v-bottom plate.
    - Label plate “Hyb”
2. Carefully remove foil seal from Archive plate.
3. Place Archive plate on a ring magnet.
4. Let Archive plate magnetize for at least 3 minutes.
5. Pipette **20 µL sample** from Archive plate into Hyb plate. Aspirate from the center of each well.
    - Do not remove the Archive plate from the magnet.
6. Vortex thawed 10X Slide Block and pour into a clean reagent reservoir.
7. Pipette **5 µL** 10X **Slide Block** into each sample well of the Hyb plate.
8. Continue by following **section 12** of this Document for Hybridization procedure.

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