somalogic

SomaScan® 7K Assay v4.1: Single Dilution Assay User Manual

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This user manual describes the SomaScan Assay procedure after the binding step through the data upload for the Single Dilution SomaScan Assay 7K kits and is applicable to kit part numbers: 900-00025, 900-00026, and 900-00027.

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1. Introduction

This document describes the SomaScan Assay protocol in the Single Dilution SomaScan Assay 7K format. This procedure is intended to be initiated after the completion of the appropriate sample preparation process and the protein-SOMAmer® reagents binding step. The following single dilution kits can be used with this protocol:

- CSF (900-00025)
- Urine (900-00026)
- Cell and Tissue (900-00027)

2. Ensure Setup of the Fluent Robotic 780 System

Completion of Fluent startup and platform initialization should be completed prior to sample preparation and only needs to be completed once per day. See D0004920 or D0004922 for instructions.

3. The Single Dilution SomaScan 7K Assay Procedure (Duration: ~2.25 hours)

NOTE: Consumable and reagent preparation (**Steps 3.1 - 3.2**) should be initiated at least 30 minutes prior to the estimated binding end time.

3.1. Consumable Preparation

1. Obtain four pyramid base troughs and label on the front and left-hand side, where the notch in the trough 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 in the trough is towards the analyst on the bottom left-hand corner
    - MB Wash Buffer 20% (**2-W**)
3. Obtain two Abgene plates and label on the front and left-hand side, where the notch in the plate is towards the analyst on the lower left-hand corner
    - Catch-1 Elution (**C1E**)

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- Archive
  - Include study ID, 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
   - Hybridization (**Hyb**)
5. Obtain one black spec plate
6. Obtain ten lids
7. Obtain one clean tip wafer

   NOTE: The tip wafer is a clean wafer with no tips from a 200 µL or 50 µL nested tip stack

3.2. Buffer Preparation

1. Retrieve the plates prepared during the auxiliary reagent preparation from 4 ˚C storage
   - Magnetic Bead (**M-B**) Plate
   - MB Block (**2-B**) Plate
   - 10x Slide Block (**3-B**) Plate
   - MB Prep Buffer (**M-P**) Plate
2. Centrifuge the following plates at 1000 × g for 1 minute
   - MB Block (**2-B**) Plate
   - 10x Slide Block (**3-B**) Plate
   - MB Prep Buffer (**M-P**) Plate
3. Carefully place Magnetic Bead (**M-B**), Catch-1 Elution (**C1E**), and Archive plates into thermal adapters

   CAUTION: Be careful and gentle when placing the Magnetic Bead (**M-B**) Plate into the thermal adapter and when removing the foil seal
   - Make sure that the plate is securely placed in the thermal adapter
   - Remove foil seal from Magnetic Bead (**M-B**) Plate
   - Cover each plate with a lid
4. Add the following reagents to the labeled pyramid base troughs by pouring the entire aliquot directly into the trough and cover each trough with a lid
   - Quench Buffer (**1-Q**) (85 mL aliquot)
   - Photo-Cleavage (**1-PC**) (40 mL aliquot)
   - Elution Buffer (**2-E**) (20 mL aliquot)

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- MB Wash Buffer 20% (**2-W**) (85 mL aliquot)

3.3. Single Dilution SomaScan Assay 7K Method

1. Fluent preparation
   - Log into FluentControl (if necessary)
     - Select username
     - Enter password
2. From the TouchTools home screen, select the following options in the order listed to initiate the liquid handling protocol:
   - Method Starter
     - **SomaScan Assay Single Dilution**
     - Green "play" button
   - When prompted, verify the method
   - When prompted, enter the study ID
   - When prompted, verify both lightbulbs in the photo-cleavage station are **On**
3. Fluent deck setup
   - When prompted, add the following labware/reagents to the Fluent deck

Deck Position	Labware
Left Rail, Pin 7*	Refillable Reagent Base and Bottle with Assay Buffer (**AB**)
16-4*	Refillable Reagent Trough with Assay Buffer (**AB**)
In front of deck* segments 2 & 9	Refillable Reagent Base/Trough Tubing
2-2, 2-3, 2-4	200 µL nested tips (8 wafers per stack, no lid)
2-6	50 µL nested tips (8 wafers per stack, no lid)
9-2, 9-3	200 µL nested tips (8 wafers per stack, no lid)
9-5	Black Spec Plate (no lid)
9-6	Hybridization (**Hyb**) Plate (no lid)
16-1	Quench Buffer (**1-Q**) Trough (lidded)
16-2	Photo-Cleavage (**1-PC**) Trough (lidded)
16-3	Elution Buffer (**2-E**) Trough (lidded)
16-6	MB Wash Buffer 20% (**2-W**) Trough (lidded)
23-2	MB Block Plate (**2-B**) Plate (lidded)
23-3	10x Slide Block (**3-B**) Plate (lidded)

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Deck Position	Labware
23-5	MB Prep Buffer (**M-P**) Plate (lidded)
23-6	Tag Trough (empty, no lid)
30-1	Catch-1 Elution (**C1E**) Plate in Thermal Adapter (lidded)
30-2	Magnetic Bead (**M-B**) Plate in Thermal Adapter (lidded)
30-3	Archive Plate in Thermal Adapter (lidded)
30-4	Waste Trough with clean Tip Wafer on top

*Refillable reagent base, bottle and trough may already be setup on the robot deck, depending on the sample preparation protocol used

- When prompted, verify the MB Wash Buffer 20% (**W-2**) Trough Is locked into place (deck position **16-6**)
- Advance the robotic prompt and wait to be instructed to add the SOMAmer-Bead Plate onto the robot deck

4. Post binding
   - At the end of the 3.5-hour binding step:
     - Remove the SOMAmer-Bead Plate from the shaker, place into a thermal adapter, and remove foil seal

       CAUTION: Splashing the SOMAmer-Bead Plate can compromise the assay. Be careful and gentle when placing the plate into the thermal adapter and when removing the foil seal
   - When prompted, add the following to robot deck:

Deck Position	Labware
37-3 (TMS 3)	Single Dilution SOMAmer-Bead Plate **in thermal adapter** (no lid, no foil seal)

   - Verify the deck has been properly setup with all labware and reagents
   - Advance the robotic prompt to be instructed to add tag reagent to the robot deck
   - Complete the tag reagent dilution step, when prompted
     - Retrieve Tag Diluent (**1-D**) Aliquot from the 25 ºC water bath
     - Retrieve 100x Tag Reagent (**1-T**) Aliquot from the 25 ºC water bath
       - Vortex 100x Tag Reagent (**1-T**) Aliquot for approximately 5 seconds on highest setting
       - Spin down 100x Tag Reagent (**1-T**) Aliquot in microfuge for approximately 5 seconds

- Add **400 µL** (**CRITICAL VOLUME**) 100x Tag Reagent (**1-T**) to the Tag Diluent (**1-D**) bottle
- Gently mix by inversion, avoid creating bubbles
- Pour into the tag trough (deck position **23-6)**

5. 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 Assay 7K Workbook
       - Well-specific notes should be added to the Plate Map tab
       - Abnormalities impacting all wells should be recorded on the v4-1 tab.
6. 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, pause the method, and promptly contact Technical Support.
7. Run completion tasks
   - Near the end of the robotic method, remove the black spec plate from deck position **9-5,** when prompted
     - Centrifuge black spec plate at 1000 × g for 1 min
     - Use clean pipette tips to pop any remaining bubbles
   - At the completion of the method, remove, save, and foil seal the following plates from fluent deck when prompted:

Deck Position	Labware
TMS 1	Archive Plate
9-6	Hybridization (**Hyb**) Plate

3.4. End of Assay Tasks

1. Fluent deck cleanup
   - Remove and save remaining robotic tips
   - Optional: Remove, save, and foil seal the 10x Slide Block (**3-B**) Plate
     NOTE: The 10x Slide Block (**3-B**) Plate is saved as a precautionary measure if, for any reason, the Hybridization (**Hyb**) Plate needs to be remade during

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the Microarray Hybridization Procedure (**Step 4**). Otherwise, this plate can be discarded into the biohazard waste stream.

- Remove remaining troughs, plates, and tip wafer from deck
  - Discard into biohazard waste stream

    NOTE: Remove plates from thermal adapters by using the thermal adapter removal tool prior to discarding plates into waste steam. See D0004625 for instructions.
- Remove Assay Buffer (**AB**) from the refillable reagent bottle and trough
  - Dispose of Assay Buffer (**AB**) 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 minutes or overnight
- Exit FluentControl software
  - In the FluentControl software on the main computer, select **File > Exit**
  - Wait for the driver windows to close
- 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 Assay 7K Workbook

3.5. 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. Otherwise, the instructions below are a guideline and will vary, depending on the make and model of the fluorescent plate reader used.

1. Considerations for the fluorescence of Cyanine 3 are:
   - Excitation wavelength maximum: 554 nm
   - Emission wavelength maximum: 568 nm

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- Excitation: 532 nm
- Common filter set: TRITC (excitation 540/25 (550/25) nm, emission 575 (605/70) nm long pass, beam splitter 564 (570) nm)

2. General instructions
   - Within the fluorescent plate reader software, create a new plate
   - Load the black spec 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 plate until analysis is complete
   - Save file using the assigned plate name
   - Export a .txt file
   - Open the .txt file in Excel and copy the data to the Cyanine 3 Analysis tab of the workbook
     - Paste the data as values into the Cyanine 3 Analysis tab
     - The layout must be a matrix of eight rows and 12 columns, corresponding to the format of the 96-well plate
   - The Cyanine 3 Analysis worksheet will automatically calculate the following:
     - Calibrators
       - Average RFU
       - %CV
     - Blank Control
       - Median RFU
       - %CV
       - Median Blank Control RFU + 3 Standard Deviations
     - QC Sample – <u>if applicable</u>
       - Average RFU
       - %CV

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- Sample
  - ➢ Median RFU
- Evaluate Cyanine 3 results
  - Acceptance criteria:
    - ➢ Average Calibrator RFU > Median Blank Control RFU + 3 Standard Deviations
  - The workbook will assess this acceptance criteria and will provide a pass or fail result

  NOTE: The acceptance criteria may not be applicable for all dilute samples and can be disregarded.
- The black spec plate may be discarded into the biohazard waste stream after evaluating the Cyanine 3 results

4. Microarray Hybridization Procedure (Duration: ~30 min)

NOTE: Detailed instructions on using the SureHyb chambers can be found on the Agilent website.

NOTE: If necessary, the Hybridization (**Hyb**) Plate can be remade using sample eluate from the Archive Plate if the sample(s) become compromised by following the procedure in **Appendix 2.**

1. Retrieve the following reagents:
   - 2x Hybridization Buffer (**3-H**)
   - Microarray slides
     - Slide ID: **2586338**
   - Gasket slides
2. Prepare Hybridization (**Hyb**) Plate
   - Add **25 µL** (**CRITICAL VOLUME**) of 2x Hybridization Buffer (**3-H**) to all tested wells of the Hybridization (**Hyb**) Plate

     NOTE: 2x Hybridization Buffer (**3-H**) is extremely viscous. Pipette slowly and drag the tips on the side of the trough to remove any residual volume on the outside of the tips.
   - Carefully mix by pipetting up and down slowly 5 times, then faster 10 times
     - Avoid creating bubbles
   - Centrifuge at 1000 x g for 1 minute
   - Foil seal plate

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3. Document slide barcode numbers
    - Enter the microarray slide barcodes into Plate Map tab of the workbook
        - The barcodes can be entered into the workbook manually or by using a hand-held barcode reader
    - Print a copy of the Plate Map tab
4. Set the 6-channel spanning P300 pipette
    - Volume is set to **40 µL**
    - Spanning knob is set to **16**
5. Load microarray slides

    NOTE: See D0002969 SomaLogic Slide Dropper IFU for more information on using the slide dropper
    - 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 the first stop (i.e., blow out the tips) while dispensing sample to gasket slide
        - Ensure that sample does not touch the gasket edge
        - Avoid creating bubbles

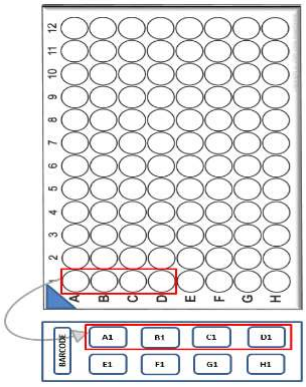

Figure 1

    - Verify the microarray slide number against the plate map printout as slides are being loaded
    - Place microarray slide onto the slide dropper with **Agilent** label facing down and to the left

      CAUTION: 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

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- Slide clamp, from right to left, over the assembled hybridization chamber and tighten
- Remove the assembled hybridization chamber from the slide dropper and inspect
  - ➢ Ensure the bubble moves freely in each subarray when the assembly is rotated
  - ➢ Ensure there is equal volume in each subarray
- Note any abnormalities (e.g., low volume or leaks) in the Sample Import Sheet tab
- 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
     - Balance hybridization chambers in oven
       - ➢ Use an empty assembled hybridization chamber to create balance if an odd number of slides is loaded
   - Start a timer for **19 hours**
   - Record hybridization start date and time on plate map printout
   - Record hybridization calculated end date and time on plate map printout
     - **Calculated End Time = Start Date and Time + 19 hours**

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

5.1. 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. Adhesive sealing may 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

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NOTE: The archive plate should be retained for at least two weeks after hybridization to enable re-hybridization if a hybridization error is detected.

5.2. Documentation

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

5.3. Day 2 Preparation

1. Place at least 300 mL Agilent wash 2 at 37 °C

6. Microarray Processing (Duration: ~45 min + up to 3.5 hours scan time)

6.1. Preparation

1. Retrieve Agilent Wash Buffer 1, Agilent Wash Buffer 2, and Acetonitrile (**ACN**)
2. Record lot numbers and expiration dates in Reagents' tab 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 at their designated locations
   - Add a stir bar to all 3 baths
   - Fill baths to fill line (~0.5 cm from the top):
     - Bath 1 with **Agilent Wash Buffer 1**
     - Bath 2 with pre-warmed 37 °C **Agilent Wash Buffer 2**
     - Bath 3 with Acetonitrile (**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 a slight dimpling on the surface of the liquid is observed.

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- Lower temperature probe for Agilent Wash Buffer 2 all the way down into the bath
- Turn **ON** the temperature power switch for the Agilent Wash Buffer 2 bath
  - Press and hold the Set button to view temperature setting:
    - Setting should be set to 37 °C
    - To set the correct temperature press and hold Set and use the ▲ or ▼ buttons
- Cover Agilent Wash Buffer 1 and 2 baths with plastic lids if more than 10 minutes remain until the slide washing
- Cover Acetonitrile (**ACN**) bath with a metal lid

5. Set up disassembling dish
   - Place slide rack into the disassembling dish
   - Add Agilent Wash Buffer 1 to the disassembling dish with slide rack
     - Ensure volume of Agilent Wash Buffer 1 will thoroughly cover slides in slide rack
   - Cover disassembling dish until use

6.2. Slide Washing

1. When 19-hour hybridization is complete:
   - Confirm that the hybridization oven temperature was set to 55.0 °C ± 2.0 °C
   - Confirm that the hybridization oven speed was set to 20
   - Document hybridization deviations on the Plate Map tab in the assay workbook
2. Separate slide-gasket sandwich
   - Remove 2 to 3 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 (e.g., volume changes, leaks)
     - Note any abnormalities in the Plate Map tab
   - Submerge the slide-gasket sandwich into the disassembling dish
   - Gently insert forceps between the slide and the gasket at the barcode

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- 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
- Repeat for the remaining hybridization chambers

3. Gently slide fingers across the tops of the slides to ensure proper placement in the rack
4. 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 Agilent Wash Buffer 1 bath
     - Align the slide rack handle with the black lines on the instrument
     - Align the holes in the handle with the two bumps in the gripper paddle as it lowers
       - ➢ If the gripper closes before the holes align, manually adjust slide rack using the thumb tab
     - The SciGene will continue with the Wash 1+2 protocol for 10 minutes
   - When Wash 1+2 protocol is complete
     - Remove Agilent Wash Buffer 1 bath from the SciGene
     - Remove slide rack from gripper paddle using thumb tab
     - Place slide rack on a lint-free wipe to absorb excess liquid
     - Press the back button on touch screen
5. 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 handle with the black lines on the instrument

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- Align holes in slide rack handle with nodes on gripper paddle
- The SciGene will continue with the ACN protocol for 5 minutes

6. Once the ACN program is complete:
   - Remove the slide rack from gripper paddle using the thumb tab
   - Place the slide rack on a lint-free wipe to absorb any remaining liquid
   - Record any abnormalities during slide washing in the Sample Import sheet

6.3. Microarray Scanning and Feature Extraction (FE)

1. If the Agilent scanner computer is not already on, turn it on now
2. If the Agilent microarray scanner is not already on, turn it on now using the power switch on the front
   - 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 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 to highlight slides to be scanned
   - Select the "**...**" icon on the right side of the last slide

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- 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**

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 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. General SciGene Little Dipper cleanup
   - Turn off the stir bars for Baths 1, 2, and 3
   - For Bath 2, turn off the temperature power switch and raise the temperature sensor, wipe sensor with DI water and a lint-free wipe
   - Clean Agilent wash baths 1 and 2 and the disassembling dish
     - Dispose of Agilent wash 1 and 2 buffers into the sink

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- Rinse baths with running tap water
- Dry with lint-free wipes

- 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

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

7.1. QC of Feature Extraction Output

1. Navigate to the folder where the output file from the Agilent feature extraction software is 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 the Plate Map tab of the workbook

7.2. 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 number
     - Example: SL-21-002_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 into Box (www.box.com)using the username and password assigned to your institution
6. Navigate to the following folder:
    - "SomaScan Data" and click on the folder with the correct year
        - Example: SomaScan Data > 2021
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. 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

8. Reference Documents

Document ID	Document Title
D0004619	SomaScan Assay 7K Kit - Equipment List
D0004924	SomaScan Assay 7K kit - Single Dilution Consumable List
D0004920	SomaScan Assay 7K Sample Preparation CSF Kit – User Manual
D0004921	SomaScan Assay 7K Sample Preparation Urine Kit – User Manual
D0004922	SomaScan Assay 7K Sample Preparation Cell and Tissue Kit – User Manual
D0004923	SomaScan Assay 7K - Workbook
D0004966	Single Dilution SomaScan Assay 7K Cell & Tissue and CSF kit - Experienced User Checklist
D0002969	SomaLogic Slide Dropper IFU
D0004625	SomaLogic Thermal Adapter Removal Tool IFU

Appendix 1: Consumables and Amounts

Common Name	Amount Consumed Per Run	Amount Needed Per Run*
Matrix Tubes with Rack	85 each	85
50 µL Nested Tips	6 wafers	8 wafers
200 µL Nested Tips	24 wafers	48 wafers
Pyramid Base Trough	4 each	4 each
V-Bottom Trough	1 each	1 each
Lid	10 each	10 each
Abgene Plate	3 each	3 each
Nunc Round-Bottom Plate	2 each	2 each
Nunc V-Bottom Plate	3 each	3 each
Black Spec Plate	1 each	1 each
Foil Seals	10 each	10 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

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Appendix 2: Instructions for Hybridization Plate Preparation

1. Reagent and consumables preparation
    - Obtain the 10x Slide Block (**3-B**) plate saved during the end of assay tasks (**Step 3.4.1**)
        - If the 10x Slide Block (**3-B**) plate was discarded:
            - ➢ Obtain an aliquot of 10x Slide Block (**3-B**) from -20 °C storage
                - Document lot number and expiration date in Reagents' tab of the workbook
                - Place into a 25 °C water bath and thaw for at least 15 minutes
                - Retrieve from 25 °C water bath and vortex for approximately 15 seconds
                - Quick spin in a microfuge
    - Retrieve the 2x hybridization buffer (**2× Hyb Buffer**)
    - Obtain the archive plate
        - If the archive plate is frozen:
            - ➢ Place at 37 °C for 15 minutes
            - ➢ Shake at 850 rpm, 25 °C for 10 minutes
    - Obtain One V-bottom plate
        - Label on the front and right-hand side, where the notches are towards the analyst
            - ➢ Hybridization (**Hyb**)

2. Hybridization Plate Preparation
    - Obtain the Hybridization (**Hyb**) Plate
    - Add Sample Eluate
        - Carefully remove foil seal from archive plate
        - Place archive plate on a ring magnet
        - Pipette **20 µL** (**CRITICAL VOLUME**) sample from archive plate into Hybridization (**Hyb**) Plate
    - Add the 10x Slide Block (**3-B**) Reagent
        - Pipette **5 µL** (**CRITICAL VOLUME**) 10x Slide Block (**3-B**) into all tested wells of the Hybridization (**Hyb**) Plate
    - Follow **Step 3.5.4** Above to Continue with Microarray Hybridization

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