somalogic™

LabThread SLX Automation Equipment

SomaScan 11K Assay Plasma and Serum Kits, 8x12

Experienced User Checklist

ANALYST NAME: _____ **DATE:** _____

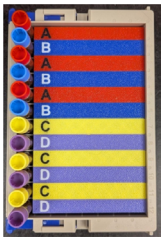
STUDY NAME/ID: _____

SAMPLE TYPE: ☐ **PLASMA** ☐ **SERUM**

General SomaScan™ 11K Assay, 8x12 Preparation	
Label Required Consumables	
Nunc round-bottom plates – 6	**Adapter plates – 4**
☐ Dil 1 (**D1**)	☐ Magnetic Bead (**M-B**)
☐ Extra Dil 1 (**ED1**)	☐ Archive
☐ Dil 2 (**D2**)	☐ Catch 1 Elution (**C1E**)
☐ Extra Dil 2 (**ED2**)	☐ Elution
☐ Dil 3 (**D3**)	**Pyramid-base troughs – 4**
☐ MB Prep Buffer (**M-P**)	☐ Quench Buffer (**1-Q**)
☐ Sample Waste	☐ Photo-Cleavage Buffer (**1-PC**)
V-bottom trough – 1	☐ Elution Buffer (**2-E**)
☐ MB Wash Buffer 20% (**2-W**)	☐ Tag
V-bottom plates – 3	**Black spec plate – 1**
☐ MB Block (**2-B**)	☐ Black spec plate
☐ Hybridization (**Hyb**)	
Obtain Required Consumables	
☐ Clean tip wafers – 2	☐ Lids – 11
☐ 200 µL nested tips – 7 stacks	☐ 50 µL nested tips – 2 stacks
Equipment Check and Preparation	
☐ MCA head attached	☐ Rack thawing station
☐ Inspection of gripper fingers	☐ Centrifuge (1,000 × *g* for 1 min)
☐ Shaker Communication Test	☐ Shaker(s) for binding and hybridization steps (850 RPM, 28 °C, 3 hr 30 min and 300 RPM, 55 °C, 19 hr)
☐ Waste bottle	☐ 4x12 top frame(s)
☐ Gravity-fed trough	☐ 4x bottom frame(s)
☐ Thermal adapters – 7	☐ Torque screwdriver
☐ Water bath (25 °C ±2.0 °C)	☐ 4x12 gasket applicator

SomaScan 11K Assay – Sample Preparation	
Required Reagents and Consumables	
☐ Assay Buffer (**AB**) – **4 °C**	☐ SomaScan 11K or 4K – Kit, 4 °C 8x12 or 4x24 – **4 °C**
☐ SomaScan 11K or 4K – Kit, –20 °C 3-Dilution 8x12 or 4x24 – **–20 °C**	☐ SomaScan 11K or 4K – Kit, Ambient 8x12 or 4x24 – **RT**
☐ Matrix rack with samples and controls – **–80 °C**	☐ 200 µL nested tips – 2 stacks
☐ Sample Diluent (**D-P** or **D-S**) – **–80 °C**	☐ 50 µL nested tips – 1 stack
☐ Dilution plates: **D1, ED1, D2, ED2, D3**	☐ Clean tip wafer – 1

LabThread™ SLX System Startup	Verified
☐ Power strip (Myrius box, computer, Thermal Magnetic Shakers, control box and BioShake shakers) on	
☐ Myrius box (LabThread SLX robot) on	
☐ FluentControl software opened	
☐ MCA head adapter attached	
☐ Inspection of gripper fingers complete	
☐ **Shaker Communication Test SLX** complete	
☐ Waste-collection bottle attached	

Sample Preparation	Verified
☐ Verify Sample Diluent used ☐ **D-P** or ☐ **D-S**	
☐ Thaw **D-P** or **D-S** for at least **20 min** in the **25 °C** water bath	
☐ Place Tag Reagent (**1-T**) and Tag Diluent (**1-D**) in the **25 °C** water bath	
☐ Place tubes in a Matrix rack for 1 mL tubes according to the Leak Detector Tube Placement Aid (410-00027) ☐ Leak detector A (red) is pipetted to columns 1, 3 and 5 ☐ Leak detector B (blue) is pipetted to columns 2, 4 and 6 ☐ Leak detector C (yellow) is pipetted to columns 7, 9 and 11 ☐ Leak detector D (purple) is pipetted to columns 8, 10 and 12 	
☐ Thaw hybridization solution tubes at **RT**. Do not remove the tube caps.	
☐ Thaw samples ☐ Remove the cover from the Matrix sample rack ☐ Place Matrix sample rack on rack thawing station ☐ Turn **ON** rack thawing station ☐ Thaw for at least **15 min**	

☐ During the diluent and sample thaw, begin the LabThread SLX robot setup. Set up gravity-fed trough: ☐ Fill bottle with **1 L** of Assay Buffer (**AB**) ☐ Place bottle base with filled bottle on **left rail pin 7** ☐ Place trough on **deck position 16-4**	
☐ Open and run **SomaScan 11K Sample Prep SLX** method	
LabThread SLX deck setup ☐ When prompted, add: ☐ 2-3: stack of 200 µL tips (8 wafers per stack, cover removed) ☐ 2-4: stack of 200 µL tips (8 wafers per stack, cover removed) ☐ 2-6: stack of 50 µL tips (8 wafers per stack, cover removed) ☐ 23-1: Dil 3 (**D3**) plate ☐ 23-2: extra Dil 2 (**ED2**) plate ☐ 23-3: Dil 2 (**D2**) plate ☐ 23-4: extra Dil 1 (**ED1**) plate ☐ 29-6: clean tip wafer on top of waste trough ☐ TMS-1: 11K SOMAmer™ Bead (Catch-0) plate **Dil 1** in **thermal adapter** (foil seal removed) ☐ TMS-2: 11K SOMAmer Bead (Catch-0) plate **Dil 2** in **thermal adapter** (foil seal removed) ☐ TMS-3: 11K SOMAmer Bead (Catch-0) plate **Dil 3** in **thermal adapter** (foil seal removed) ☐ Confirm LabThread SLX deck setup ☐ With **approximately 7 min** left in sample diluent thaw, select **Continue** to initiate pipetting of AB to dilution plates	
Sample and Dil 1 plate preparation ☐ After **15 min** sample thaw: ☐ Confirm all samples are thawed ☐ Centrifuge sample rack at **1,000 × *g*** for **1 min** ☐ Remove sample tube caps ☐ After **20 min** Sample Diluent thaw: ☐ Pipette **140 µL** into **Dil 1** plate (**CRITICAL VOLUME**) ☐ Centrifuge **Dil 1** plate at **1,000 × *g*** for **1 min** ☐ When prompted, place on LabThread SLX deck: ☐ 23-5: Dil 1 plate ☐ 23-6: sample rack (no lid, lock tabs facing analyst) ☐ Select **Continue** within the method prompt to initiate pipetting samples ☐ Carefully watch for abnormalities during pipetting	

Binding Reaction	Verified
☐ When prompted, remove 11K SOMAmer Bead plates from LabThread SLX deck ☐ Seal each plate with a foil seal ☐ Remove 11K SOMAmer Bead plates from thermal adapters ☐ Place into binding shaker(s): **3 hr 30 min, 28 °C, 850 RPM** Binding start time: _____ Binding end time (estimated): _____ (+ 3 hr 30 min)	

LabThread SLX Robot Cleanup, Workbook Update and Auxiliary Reagent Preparation	
LabThread SLX Robot Cleanup	**Verified**
☐ Remove sample rack ☐ Remove and save remaining tips ☐ Remove and discard sample dilution plates and wafer from the waste trough ☐ Wipe down deck with a disinfectant cloth ☐ Leave **AB** refillable bottle, base and refillable trough on deck	
Workbook Update	**Verified**
☐ Create **Plate Map** in sample workbook ☐ Add all sample-specific notes (if applicable) ☐ Add all sample preparation-related notes to the **Assay Overview** tab (**Tab 1**)	
Auxiliary Reagent Preparation	**Verified**
Prepare Magnetic Bead (M-B) plate ☐ Vortex **M-B** bottle for at least **60 sec** ☐ Obtain Mag Bead **M-B** plate (adapter) ☐ Pipette **75 µL** (**CRITICAL VOLUME**) M-B into **Mag Bead** plate ☐ Foil-seal plate ☐ Store at **+4 °C**	
Prepare MB Prep Buffer (M-P) plate ☐ Obtain MB Prep Buffer (**M-P**) plate (Nunc round-bottom) ☐ Pipette **100 µL** M-P into **MB Prep Buffer** plate ☐ Foil-seal plate ☐ Store at **+4 °C**	
Prepare MB Block (2-B) plate ☐ Obtain MB Block plate (V-bottom) ☐ Pipette **30 µL** 2-B into **MB Block** plate ☐ Foil-seal plate ☐ Store at **+4 °C**	

SomaScan 11K Assay	
Required Reagents and Consumables	
☐ 200 µL nested tips – 5 stacks	☐ Catch 1 Elution (**C1E**) plate (in thermal adapter)
☐ 50 µL nested tips – 1 stack	☐ Magnetic Bead (**M-B**) plate (in thermal adapter) – **4 °C**
☐ Black spec plate – 1	☐ Archive plate (in thermal adapter)
☐ Sample Waste plate – 1	☐ Elution plate (in thermal adapter)
☐ Hybridization plate – 1	☐ Clean tip wafer
☐ Quench Buffer trough (pyramid base)	☐ Quench Buffer (**1-Q**) (85 mL)
☐ Photo-Cleavage Buffer trough (pyramid base)	☐ Photo-Cleavage Buffer (**1-PC**) (40 mL)
☐ Elution Buffer trough (pyramid base)	☐ Elution Buffer (**2-E**) (20 mL)
☐ 20% MB Wash Buffer trough (V-bottom)	☐ 20% MB Wash Buffer (**2-W**) (85 mL)
☐ MB Block (**2-B**) plate – **4 °C**	☐ Tag Reagent (**1-T**) (400 µL)
☐ MB Prep (**M-P**) plate – **4 °C**	☐ Tag Diluent (**1-D**) (40 mL)
☐ Tag trough (pyramid base)	☐ Lids – 11
	☐ Thermal adapters – 7

Buffer Preparation	Verified
☐ Pour buffers into their corresponding troughs and add lids ☐ Quench Buffer (**1-Q**) 85 mL (pyramid base) ☐ Photo-Cleavage Buffer (**1-PC**) 40 mL (pyramid base) ☐ Elution Buffer (**2-E**) 20 mL (pyramid base) ☐ 20% MB Wash Buffer (**2-W**) 85 mL (V-bottom)	
Plate Preparation	**Verified**
☐ Place Catch-1 Elution (**C1E**) plate into thermal adapter and cover with a lid	
☐ Place Magnetic Bead (**M-B**) plate into thermal adapter, remove foil and cover with a lid	
☐ Place Archive plate into thermal adapter and cover with a lid	
☐ Centrifuge plates, remove foil and cover with a lid ☐ MB Block (**2-B**) plate ☐ MB Prep (**M-P**) plate	
SomaScan 11K Assay	**Verified**
☐ Log in to FluentControl software	
☐ Start method: **SomaScan 11K Assay 12_24 SLX**	

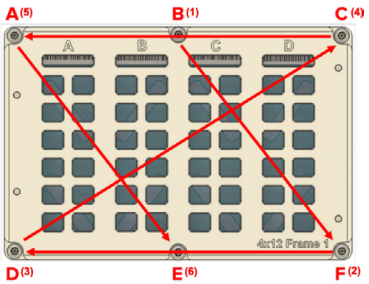
☐ Set up LabThread SLX deck when prompted ☐ 2-2: stack of 200 µL tips (8 wafers per stack, no cover) ☐ 2-3: stack of 200 µL tips (8 wafers per stack, no cover) ☐ 2-4: stack of 200 µL tips (8 wafers per stack, no cover) ☐ 2-6: stack of 50 µL tips (8 wafers per stack, no cover) ☐ 9-1: stack of 200 µL tips (8 wafers per stack, no cover) ☐ 9-2: stack of 200 µL tips (8 wafers per stack, no cover) ☐ 9-5: Hybridization (**Hyb**) plate (no lid) ☐ 9-6: black spec 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) ☐ 16-4: gravity-fed trough [filled with Assay Buffer (**AB**)] ☐ 16-6: Heat Block/MB Wash Buffer (**2-W**) trough (lidded) ☐ 23-2: Magnetic Bead (**M-B**) plate with thermal adapter (lidded) ☐ 23-3: Catch-1 Elution (**C1E**) plate with thermal adapter (lidded) ☐ 23-4: Elution plate with thermal adapter (lidded) ☐ 23-5: Archive plate with thermal adapter (lidded) ☐ 23-6: Tag trough (no lid) ☐ 29-2: MB Prep Buffer (**M-P**) plate (lidded) ☐ 29-3: MB Block (**2-B**) plate (lidded) ☐ 29-4: Sample Waste plate (no lid) ☐ 29-6: liquid waste trough with clean wafer on top	
☐ After the **3.5 hr** binding step, remove 11K SOMAmer Bead plates from shaker and carefully place into thermal adapters	
☐ Remove foil seal from 11K SOMAmer Bead plates and place on LabThread SLX deck ☐ 36-1 (TMS-1): 11K SOMAmer Bead plate **Dil 1** with **thermal adapter** ☐ 36-2 (TMS-2): 11K SOMAmer Bead plate **Dil 2** with **thermal adapter** ☐ 36-3 (TMS-3): 11K SOMAmer Bead plate **Dil 3** with **thermal adapter**	
☐ When prompted, prepare Tag Reagent (**1-T**) ☐ Pipette **400 µL** (**CRITICAL VOLUME**) Tag Reagent (**1-T**) into Tag Diluent (**1-D**) bottle ☐ Mix by inversion ☐ Add prepared reagent into Tag trough on **deck location 23-6**	
☐ Select **Continue** within the method prompt to initiate processing ☐ Carefully watch for abnormalities during pipetting ☐ Watch for possible tip-loading errors ☐ At the start of photocleavage, ensure both UV bulbs on photocleavage station turn on	

Method Completion Tasks	Verified
☐ Remove black spec plate from **deck position 9-6** when prompted	
☐ At the completion of the method, remove and save the following from LabThread SLX deck when prompted: ☐ 9-5: Hybridization (**Hyb**) plate ☐ 36-1 (TMS-1): Archive plate	
LabThread SLX Robot Cleanup	**Verified**
☐ Clear remaining labware from deck and discard	
☐ Wipe down deck with a disinfectant cloth	
☐ Exit FluentControl software	
☐ Remove Assay Buffer (**AB**) gravity bottle and trough, and rinse with tap water then deionized water (DIW)	
☐ Remove waste bottle from waste trough and add bleach	
☐ Turn off LabThread SLX robot (Myrius box)	
☐ Turn off power strip connected to computer, TMS control box and BioShake shakers	
Documentation	**Verified**
☐ Update **Assay Overview** tab (**Tab 1**) of the workbook with notes ☐ Transfer well-specific notes to **Plate Map** tab (**Tab 4**) of the workbook	
Cyanine 3 Readings	**Verified**
☐ Inspect black spec plate for any bubbles. If bubbles are present: ☐ Centrifuge plate (**1,000 x *g*, 1 min**) ☐ Use pipette tips to pop any remaining bubbles ☐ Read black spec plate on a plate reader with settings for measuring the fluorescence of cyanine 3. For example: • Excitation: 535 nm • Emission: 575 nm • Cutoff: 570 nm ☐ Save and export file ☐ Copy data to **Cyanine 3 Analysis** tab (**Tab 5**) of the workbook ☐ Evaluate Cyanine 3 results ☐ Transfer well-specific notes to **Plate Map** tab (**Tab 4**) of the workbook	

Microarray Hybridization

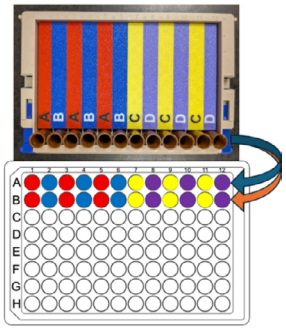
Required Reagents and Consumables

☐ Hyb plate (see above) – **RT**	☐ Bottom frame(s), 4x
☐ Hybridization solution tubes – **RT**	☐ 300 µL tips for Integra (automated) ☐ N/A
☐ Microarray slides, 12 array – **RT** Slide IDs: 2587544–2587550, 2587738	☐ 300 µL tips for spanning pipettor (manual) ☐ N/A ☐ Hybridization foil seals
☐ Gasket(s), 4x12 – **RT**	☐ Gasket applicator, 4x12
☐ Top frame 1, 4x12	☐ Compression screws
☐ Top frame 2, 4x12 (if applicable)	☐ Torque screwdriver

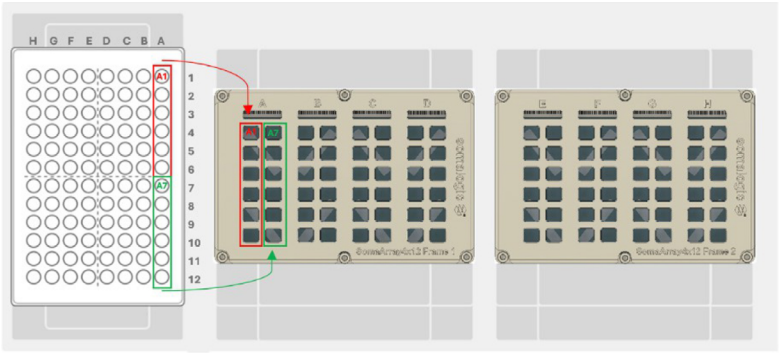
Hybridization Frame Assembly	Verified
☐ Place **clean gloves** on prior to handling gaskets ☐ Assemble top frame(s) • Apply gasket to gasket applicator, thin side down with pinholes facing up • Place top frame 1 on top of gasket and applicator • Press down firmly on the top frame to seat pins in pinholes • Lift top frame and gasket from gasket applicator • Remove gasket applicator from gasket by gently lifting at 1 corner • Ensure the gasket remains fully seated in the top frame ☐ Assemble bottom frame(s) • Screw in alignment pins to bottom frame finger-tight • Place Agilent 8x12 microarray slides into all 8 slide slots on 2 bottom frames • Place slide Agilent side up, barcodes aligned with barcode window on frame • Inspect slides to ensure each slide is seated flat and flush with seating rails ☐ Assemble top and bottom frame(s) • Place top frame(s) on top of bottom frame(s), aligning barcode window in top frame with barcodes on slides • Place gasket applicator face-down onto the holes of the top frame to protect the wells during assembly • Remove alignment pins from bottom frame • Place compression screws into the screw holes on the top frame(s) • Loosely turn screws using torque screwdriver in pattern (B, F, D, C, A, E) until each screw engages a few threads of the bottom frame • Turn screws in the same pattern until the torque screwdriver clicks, indicating that the desired 1.1 Nm of torque has been met and all screws are fully tightened  ☐ Document slide barcode numbers in **Tab 4 – Plate Map** of the workbook	

<table>
<tr><th colspan="2">8x12 Slide Hybridization – Automated</th><th>Verified</th></tr>
<tr><td colspan="2">☐ Skip to the next section, 8x12 Slide Hybridization – Manual, if performing the manual 8x12 sample loading procedure</td><td>☐ N/A</td><td></td></tr>
<tr><td colspan="2">☐ Mix hybridization solution tubes by inversion 10 times and centrifuge in the rack at 1,000 x g for 1 min</td><td></td></tr>
<tr><td colspan="2">☐ Add the following to the Integra deck:
<table><tr><th>Deck Position</th><th>Labware</th></tr><tr><td>Tip segment</td><td>300 µL Integra LONG filter GRIPTIPS (portrait orientation)</td></tr><tr><td>Segment A</td><td>Hyb plate</td></tr><tr><td>Segment B</td><td>Hybridization solution tubes</td></tr><tr><td>Segment C</td><td>N/A</td></tr></table>☐ Power on Integra Assist Plus base unit
☐ Mount Voyager 300 µL 6-channel pipette onto Integra Assist Plus and connect</td><td></td></tr>
<tr><td colspan="2">☐ Under VIALAB Programs on the Voyager pipette, select appropriate script for number of frames being used
☐ 8x12_1Fr_v3.0.0
OR
☐ 8x12_2Fr_v4.0.0
☐ Select RUN on pipette
• Verify new tip in row field corresponds with tip box on deck
☐ Select RUN on pipette
• Select Edit within selection of Integra script and setup of Integra deck
☐ Press Start/Pause on Integra base unit to start script
☐ After hybridization solution is added to all samples in the Hyb plate and script is paused, remove the Matrix rack from the deck
☐ Add the following to the Integra deck:
<table><tr><th>Deck Position</th><th>Labware</th></tr><tr><td>Tip segment</td><td>300 µL Integra LONG filter GRIPTIPS (if necessary)</td></tr><tr><td>Segment A</td><td>Hyb plate (keep in same position)</td></tr><tr><td>Segment B</td><td>Frame 1 (cover removed)</td></tr><tr><td>Segment C</td><td>Frame 2 (cover removed), if applicable</td></tr></table>☐ Press Start/Pause on Integra base unit to continue script
☐ Note any abnormalities (for example, low volume or leaks) under Assay Notes on Tab 4 – Plate Map
☐ Once script is complete, seal each frame with a hybridization foil seal. Ensure foil seal is thoroughly applied over the surface of each chamber.</td><td></td></tr>
</table>

8x12 Slide Hybridization – Manual	☐ N/A	Verified



- ☐ Mix hybridization solution tubes by inversion 10 times and centrifuge in the rack at 1,000 x *g* for 1 min
- ☐ Uncap tubes carefully
- ☐ To 22 µL samples in the Hyb plate taken from the Fluent deck, add **104.5 µL** (**CRITICAL VOLUME**) of hybridization solution, 1 row at a time
  - Mix by pipetting up and down **slowly** 3 times using the same pipette tips. Discard tips after transfer and mixing.
- ☐ Verify the following settings on the 6-channel spanning P300 pipette:
  - Volume is set at **100 µL**
  - Spanning knob is set to **10**
- ☐ From the Hyb plate, load **100 µL** (**CRITICAL VOLUME**) of each sample onto the 8x12 slide
  - Ensure that pipette tips do not touch the 8x12 slide



- ☐ Verify the microarray slide number against **Tab 4 – Plate Map**
- ☐ Note any abnormalities under Assay Notes on **Tab 4 – Plate Map**
  - Repeat for remaining samples
- ☐ Once manual sample transfer is complete, seal each frame with a **hybridization foil seal**

Hybridization	Verified

- ☐ Verify Hybridization plate shaker settings:

Parameter	Setting
Temperature	**55 ±2.0 °C**
Rotator speed	**300 RPM**
Timer	**19 hr**

- ☐ Load 4x12 frames into the plate shaker
- ☐ Record hybridization start time and calculated end time (start time + **19 hr**)

☐ Hyb start time: _____ Hyb end time (est.): _____

Cleanup	Verified
☐ Store unused microarray slides in nitrogen-purged desiccator cabinet ☐ Seal Archive plate and store at **–20 °C** ☐ Clean Integra Plus unit with a lint-free cloth and 70% ethanol, if used	

Documentation	Verified
☐ Ensure well-specific notes have been recorded on plate map ☐ Update Assay Overview tab of the workbook ☐ Save the workbook file	

Microarray Post-Hybridization Processing

Required Reagents and Equipment	
☐ 10X Wash Buffer 1 (**Wash 1**) – **RT**	☐ Gasket applicator, 4x12
☐ 10X Wash Buffer 2 (**Wash 2**) – **RT**	☐ 4x slide ejector tool
☐ Acetonitrile (**ACN**) – **RT**	☐ 4x slide release tool
☐ Disassembly basin	☐ Wash basins for wash 1, wash 2 and ACN
☐ Disassembly screwdriver	☐ Graduated cylinder

Dilute Wash Buffers	Verified

☐ Using a graduated cylinder, measure 4.5 L of DIW (DiH2O)
☐ Pour 4.5 L of DiH2O into a clean, labeled container
☐ Add 500 mL of 10X Wash Buffer 1 to the same container
  ☐ Mix by inversion >10 times to ensure complete dispersion of 10X wash buffer 1
  OR
  ☐ Mix by placing container to **RT** magnetic stir plate and mixing for 10 min
☐ Using a graduated cylinder, measure 900 mL of DiH2O
☐ Pour 900 mL of DiH2O into clean, labeled container	

☐ Add 100 mL of 10X Wash Buffer 2 directly to the same container
  ☐ Mix by inversion >10 times to ensure complete dispersion of 10X wash buffer 1
  OR
  ☐ Mix by placing container to **RT** magnetic stir plate and mixing for 10 min	

Slide Washing Preparation – Automated		Verified
☐ Skip to the next section, Slide Washing Preparation – Manual, if performing the manual slide washing procedure	☐ N/A	

☐ Add 1 stir bar to each SciGene Wash Bath ☐ Place baths into slide washer base slots 1, 2 and 3 and fill: ☐ Bath 1: **wash 1** ☐ Bath 2: **wash 2** ☐ Bath 3: **ACN** ☐ Increase the stir dial until a small dimple forms on the surface (approximately 5–7) ☐ Lower temperature probe for bath 2 and ensure it is set to **37 °C** (**critical temp**)		
Slide Washing Preparation – Manual	**☐ N/A**	**Verified**
☐ Add 1 stir bar to each wash bath ☐ Place bath 1 on the **RT** stir plate and bath 2 on the **37 °C** stir plate and fill: ☐ Bath 1: **wash 1** ☐ Bath 2: **wash 2** ☐ Bath 3: **ACN** ☐ Set stir dial approximately halfway for each bath (until small dimple forms on surface of buffer) ☐ Lower temperature probe for bath 2, and ensure it is set to **37 °C** (**critical temp**)		
Hybridization Chamber Disassembly		**Verified**
☐ Set up disassembly basin ☐ In the basin, place a slide rack, 4x slide ejector tool, 4x12 gasket applicator and 4x slide release tool ☐ Add **Wash Buffer 1** into disassembly basin with enough volume to cover slides in slide rack by at least 1 inch ☐ When 19 hr incubation is complete: • Visually confirm hybridization shaker temperature is 55.0 °C ±2.0 °C • Visually confirm hybridization shaker speed is set to 300 RPM • Remove both 4x12 hybridization chambers from hybridization shaker ☐ Slowly remove foil seal from hybridization chamber ☐ Visually inspect wells for liquid covering the bottom. Ensure well-specific notes have been recorded on plate map. ☐ Place entire 4x12 hybridization chamber into the disassembly basin ☐ Place gasket applicator onto top frame ☐ Using disassembly T10 screwdriver, remove each of the 6 screws on the chamber and remove from wash basin		
☐ Insert 4x slide release tool into barcode holes on top frame. Lift top frame while fully inserting slide release tool so that slides are “unstuck” from gasket and remain seated on bottom frame. ☐ Lift top frame and gasket out of water bath ☐ Transfer the 4x bottom frame underwater to above the SomaArray 4x slide ejector tool. Slowly lower the bottom frame onto the slide ejector so that diagonal ejector pedestals support and lift slides out of bottom frame. ☐ Transfer slides to the slide rack without lifting slides above the wash buffer ☐ Remove bottom frame 1 from basin ☐ Add 4x12 hybridization chamber 2 to basin. Repeat steps above to remove and transfer slides. ☐ Ensure all slides are level in the slide rack		

Washing of Microarray Slides – Automated		Verified
☐ Skip to the next section, Washing of Microarray Slides – Manual, if performing the manual slide washing procedure	☐ N/A	
☐ Start protocol **Wash 1+2** on SciGene Little Dipper: • Quickly transfer the slide rack to bath 1 and align with the instrument paddle		
☐ After the slide rack is transferred to bath 2, remove bath 1 from its base		
☐ Once the **Wash 1+2** protocol is complete: • Manually remove the slide rack from the instrument paddle • Start protocol **ACN** ◦ Place slide rack into empty base 1 position to reload the paddle		
☐ Once the **ACN** protocol is complete: • Manually remove the slide rack from the instrument paddle • Continue to Scanning of Microarray Slides section before completing General Slide Washing Cleanup section		
Washing of Microarray Slides – Manual	**☐ N/A**	**Verified**
☐ Quickly transfer the slide rack to bath 1 and start **5 min** timer: • Ensure stir bar is spinning freely below the slide rack		
☐ After the **5 min** timer is complete, quickly transfer the slide rack from wash 1 to wash 2		
☐ Start a **5 min** timer for wash 2		
☐ After the slide rack is transferred to wash 2 (and 5 min timer started), remove bath 1 from the **RT** stir plate		
☐ Place the **ACN** bath on the RT stir plate		
☐ After the **5 min** timer for wash 2 is complete, **SLOWLY** remove the slide rack from the 37 °C Wash Buffer 2 bath and transfer to the ACN bath • Aim for a 15 sec removal time from wash 2 • Ensure stir bar is spinning freely below the slide rack in ACN bath		
☐ Start a **5 min** timer for ACN		
☐ After the **5 min** timer for ACN is complete, **SLOWLY** remove the slide rack from the ACN bath • Aim for a 15 sec removal time from ACN		
☐ Once the **ACN** wash is complete • Continue to Scanning of Microarray Slides section before completing General Slide Washing Cleanup section		
Scanning of Microarray Slides		**Verified**
☐ Place slides in scanning chambers with the Agilent label facing up		
☐ In the Agilent Microarray Scan Control software, select **Open Door** • Place scanning chambers in Agilent scanner slots		

☐ In the Agilent scanner software: • Select **Close Door** • Ensure the scan protocol for all slides defaults to **AgilentG3_GX_1Color** • Select all slides and navigate to and select the appropriate study output folder • Add all slides to the queue • Select **Start Scan**	
☐ Open Feature Extraction Software ☐ Select **File** > **New** > **On-Time Project** ☐ Update the following fields: • **Operator**: technician's initials • **Incoming Image Folder**: study output folder • **Grid**: Local File Only ☐ Select the **Run Project** icon:  ☐ Save project as **Study ID** + **Set_FE** ☐ Select **All to Extract**	
General Slide Washing Cleanup	**Verified**
☐ Turn off all stir bar and temperature probe dials ☐ Wash bath 1, bath 2 and disassembling dish ☐ Dispose of ACN in bath 3 according to institutional guidelines ☐ Turn off SciGene Little Dipper or stir plates ☐ Discard gaskets	
Frame Washing	**Verified**
☐ Prepare a wash basin with 1 of the following soaps (or similar) at a working concentration: ☐ RBS 35 Concentrate (Thermo Fisher Scientific, 27952) diluted in water to a working concentration of 2% (v/v) OR ☐ Alconox Detergent 8 (VWR, 21839-066) diluted in water to a working concentration of 3–5% (v/v) ☐ Prepare two 2 L plastic beakers with 2 L of ultrafiltered DIW. Label 1 **Rinse A** and the other **Rinse B**. ☐ Soak used frame components and accessories for 2–60 min in diluted soap ☐ Lightly scrub each item on all sides with soft-bristled lab brush ☐ Rinse each item thoroughly with DIW in Rinse A beaker then in Rinse B beaker ☐ Allow frames and accessories to dry until all water has evaporated before reuse	
QC and Data Upload – Sending Data to SomaLogic	**Verified**
☐ Skip to the next section, QC and Data Upload – DataDelve™ Normalization Tool, if performing normalization independently ☐ N/A	

☐ After Feature Extraction is complete: • QC all PDF files ☐ Create study folder containing the completed workbook and all TXT files from the Feature Extraction output ☐ Compress this folder to a ZIP file ☐ Upload the ZIP file to box.com ☐ Email bi-services@somalogic.com about the data upload and include all additional observations from below	

QC and Data Upload – DataDelve Normalization Tool	☐ N/A	Verified
☐ After Feature Extraction is complete: • QC all PDF files ☐ Create study folder containing the completed workbook and all TXT files from the Feature Extraction output ☐ Go to normalization.somalogic.com (for DDN Cloud) or open the DataDelve Normalization Application (for DDN Desktop) ☐ Fill out appropriate study information ☐ Upload study workbook ☐ Upload Agilent TXT files ☐ Process study and review results: • Review SQS • Download and review ADAT files		

Additional Observations (attach additional pages if required)
☐ N/A this section

Document Finalization	
Analyst signature	Date: