Technical Note

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Performing the QuantiGene® ViewRNA ISH Tissue Assay Using Little Dipper® Processor, Dry Oven and a Humidified Incubator

About this Technical Note

Guidelines and procedure are provided for running the QuantiGene ViewRNA ISH Tissue Assay using the Little Dipper Processor for Affymetrix from SciGene for batch slide processing, a dry oven and a humidified incubator. This technical note should be used in conjunction with the *QuantiGene ViewRNA ISH Tissue Assay User Manual* and the *Little Dipper Processor Operations Guide*.

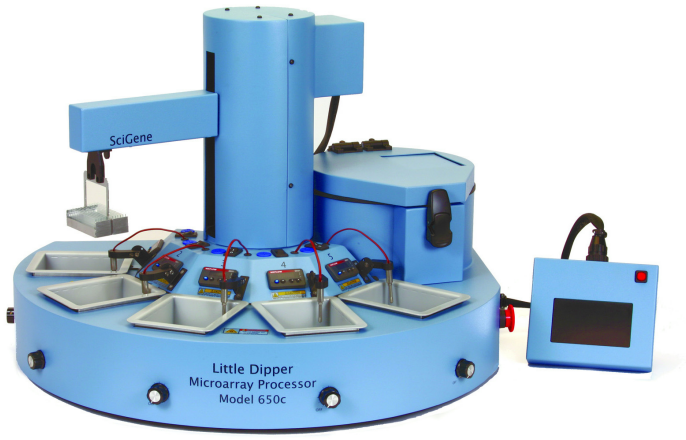
Automated Steps for Batch Slide Processing using the Little Dipper Processor:

- **Part 2**. Slide De-Paraffinization (De-Wax)
- **Part 4**. Slide Pretreatment (Pretreat)
- **Part 6**. Slide Post-Hybridization Washing (QGV Wash)
- **Part 9**. Slide Counterstain (Gills)

Before you start, decide if you choose to run all of the above steps using the Little Dipper Processor for batch slide processing or just some of the steps. Please refer to the appropriate part in this technical note for information on how to run that step using the Little Dipper Processor. All of the other steps are to be run in manual mode following the protocol described in this application note. For complete materials required, guidelines and instructions, please refer to the *QuantiGene ViewRNA ISH Tissue Assay User Manual* and the *Little Dipper Processor Operations Guide*.

About the Little Dipper Processor

The Little Dipper Processor can be used for the automated processing of slides for QuantiGene ViewRNA ISH Tissue Assay. The instrument controls wash time, agitation and buffer temperatures. A batch of 6 to 12 slides can be processed at one time using the protocol below. It should be advised that running 2 batches or more will increase the processing time in both manual and automated portions of Part 1–5 in the QuantiGene ViewRNA ISH Tissue Assay as described in this technical note. Stacking of slides in batch mode processing is possible in Automated Post Hybridization Washing of Part 6 in the QuantiGene ViewRNA ISH Tissue Assay as described in this technical note. Slides can be preloaded in a slide rack and submerged in wash buffer bath for 10 minutes in queue for the next protocol run. The number of batches processed would be at the discretion of the user, but additional baths and slide racks will be needed.



- Automates four main steps in the QuantiGene ViewRNA ISH Tissue Assay
- Produces slides with uniform signal and low background
- Reduces the user variability of day to day processing
- "Load and go" walk-away automation
- Simple temperature validation

Required Materials from Affymetrix

Table 1 Required Materials from Affymetrix for Running a QuantiGene ViewRNA ISH Tissue Assay using the Little Dipper Processor

Item	Size	Catalog #
QuantiGene ViewRNA ISH Tissue Assay Kit	24 assays 96 assays	QVT0050 QVT0051
QuantiGene ViewRNA Chromogenic Signal Amplification Kit	24 assays 96 assays	QVT0200 QVT0201
QuantiGene ViewRNA TYPE 1 Probe Set – Catalog and "By Request"	15 assays (120 µL)* 55 assays (0.44 mL) 180 assays (1.44 mL) 900 assays (7.2 mL) 1,800 assays (14.4 mL)	TBD†
100X Pretreatment Solution (for Part 4: Slide Pretreatment)	21 mL	QVT0500

** For Catalog Probe Sets ONLY. "By Request" Probe Sets are not available in the 15 arrays size.*

† TBD – to be determined – based on ordering catalog or "By Request" probes, size, species and target sequence.

Required Materials from SciGene

Table 2 Required Materials from SciGene for Running a QuantiGene ViewRNA ISH Tissue Assay using the Little Dipper Processor

Item	Size	Catalog #
Little Dipper Processor for Affymetrix. (Programmed with optimized protocols. Includes all required accessories.)	EA	1080-65-1 (115V) 1080-65-2 (230V)

Optional Items for the Little Dipper Processor from SciGene

Table 3 Other Optional Items and Accessories from SciGene for Running a QuantiGene ViewRNA ISH Tissue Assay Using the Little Dipper Processor

Item	Size	Catalog #
On-site Operator Training and Certification (USA only)*	EA	1080-90-0
Extended Warranty – Additional 12 Months (USA only)	EA	1080-01-1
Extended Warranty – Additional 24 Months (USA only)	EA	1080-01-2
Extended Warranty – Additional 36 Months (USA only)	EA	1080-01-3
Bath, Standard (670 ml)	EA	1080-10-1
Bath, Low Volume, Non Heatable (210 ml)	EA	1080-10-2
Bath, Low Volume, Heatable (275 ml)	EA	1080-10-5
Bath Cover, Slotted for 12-Position Racks for 3 Inch Slides	EA	1080-12-2
Slide Rack, 12-Position for Standard 3 Inch Slides, with Handle	EA	1080-20-1
Digital Thermometer with NIST Certificate	EA	1051-52-0

** On-site installation and training on the Little Dipper Processor for Affymetrix. Training will not cover QuantiGene ViewRNA ISH Assay procedure.*

Other Required/Optional Materials and Equipment

For a complete list of other required/optional materials and equipment for running the QuantiGene ViewRNA ISH Tissue Assay, please refer to the *QuantiGene ViewRNA ISH Tissue Assay User Manual*.

QuantiGene ViewRNA ISH Tissue Assay Procedure Using Little Dipper Processor, a Dry Oven and a Humidified Incubator

Important Procedural Notes and Guidelines

- Validate the temperature of the dry oven incubator and the humidified incubator using the QuantiGene View Temperature Validation Kit (Affymetrix P/N QV0523)
- Before beginning the procedure, know the pretreatment boiling time and protease digestion time optimized for your sample type. If you do not know these optimized conditions, refer to *QuantiGene ViewRNA ISH Tissue Assay User Manual – Appendix A: Pretreatment Assay Optimization Procedures and Typical Results*.
- After Step 9 — Do not let slides dry out during processing. Prolonged exposure may result in low signal and high background.
- Little Dipper Processor — it is critical that slides are aligned properly in the slide rack. Misalignment may cause slides to come loose during agitation.
- Little Dipper Processor — when the procedure calls for the temperature sensors for all five baths to be rotated to the "down" position, please ensure they are. Sensors remaining in the "up" position may interfere with the movement of the Little Dipper arm.

Part 1: Sample Preparation — Manual Mode

Part 1 Procedure — Manual Processing

Step	Action
Step 1. Bake Slides **35 min**	**A.** Use a pencil to label the slides. **B.** Set dry oven at 60 ± 1 °C, insert slides into slide rack, and bake the slides for 30 min. This increases tissue attachment to the slide.
Step 2. Prepare Buffers and Reagents (while slides bake)	**A.** Prepare 3 L of 1X PBS: To a 3 L container add 300 mL of 10X PBS and 2.7 L ddH_2O. **B.** Prepare 10% formaldehyde in 1X PBS under a fume hood. To a 200 mL capacity container add 146 mL 1X PBS and 54 mL of 37% formaldehyde and mix well. **WARNING: Formaldehyde is a poison and irritant. Avoid contact with skin and mucous membranes.** **C.** Prepare 4% formaldehyde in 1X PBS under a fume hood: To a 200 mL capacity container add 22 mL of 37% formaldehyde to 178 mL 1X PBS and mix well. **D.** Prepare 3 L of Wash Buffer: To a 3 L capacity container add components in the following order to prevent precipitation from forming and then mix well: 2.5 L ddH_2O, 27 mL Wash Comp 1, 7.5 mL Wash Comp 2, and ddH_2O to 3 L. **E.** Ensure availability of: ▪ 400 mL 95% ethanol ▪ 400 mL ddH_2O ▪ 200 mL HistoClear **F.** Prewarm 40 mL of 1X PBS and Probe Set Diluent QF to 40 ± 1 °C. **G.** Thaw Probe Set(s). Place on ice until use. **H.** Prepare 200 mL of Storage Buffer (for Optional Stop Point): To a 200 mL container add 60 mL of Wash Comp 2 to 140 mL ddH_2O and mix well.
Step 3. Fix Slides **1 hr 5 min**	**A.** Under a fume hood, insert slides into an empty slide rack and submerge into a clear staining dish containing 10% formaldehyde. Incubate for 1 hour at room temperature (RT). **B.** Under a fume hood, remove the slide rack from the 10% formaldehyde and submerge it into a clear staining dish containing 200 mL of 1X PBS. Incubate for 1 min with frequent agitation. **C.** Decant the 1X PBS, refill with 200 mL of fresh 1X PBS and incubate for 1 min with frequent agitation. **D.** Remove each slide and decant the 1X PBS by flicking and placing it on its edge on a laboratory wipe. Place the slides flat face up on a paper towel to air dry. Make sure the slides are completely dry before going to the next step.

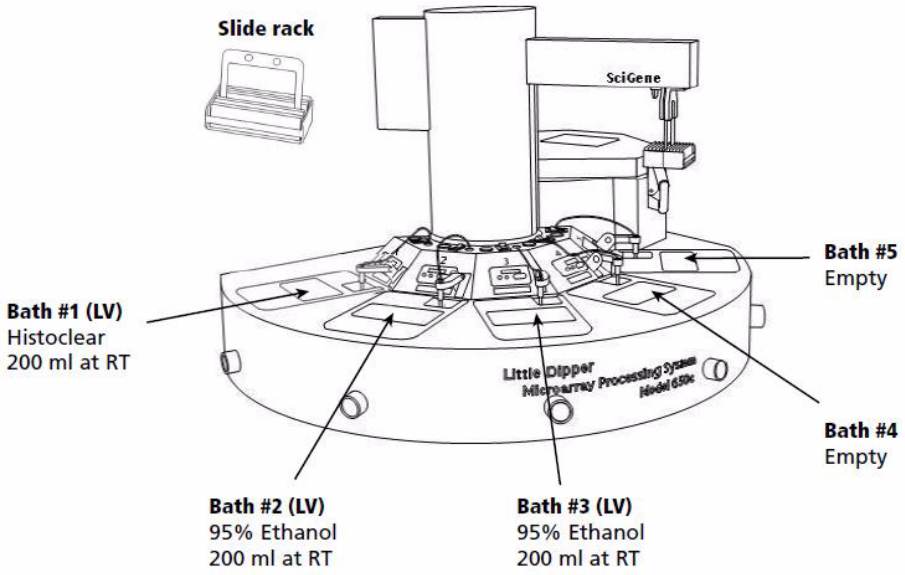
Part 2: Slide De-Paraffinization (De-Wax) — Automated Processing Using Little Dipper Processor

Little Dipper Configuration for Slide De-Paraffinization

For this section, use the following materials provided with the instrument Little Dipper Processor for Affymetrix, 115v/230v (SciGene cat. #1080-65-1 / 1080-65-2):

Table 4 Required Materials from SciGene for Slide De-Paraffinization using the Little Dipper Processor

Required Material	Source	Part Number
Little Dipper Processor for Affymetrix, 115v/230v	SciGene	1080-65-1 / 1080-65-2
3x Low volume baths (LV)	SciGene	1080-10-2
1x Slide racks, 12 position for 3 inch slides	SciGene	1080-20-1



Part 2 Procedure — Automated Processing Using Little Dipper Processor

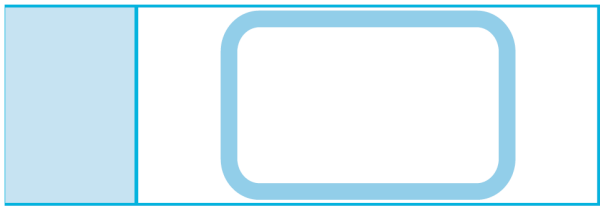
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 4. Instrument Setup

5 min</td><td>
A. Rinse the removable baths and the 12-position slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergent.
B. Turn on the main power to the instrument. Ensure the temperature sensors for all five baths are rotated to the “down” position.
C. Place low volume baths (LV) into positions 1, 2 and 3. Fill baths with the corresponding reagents and volumes shown in table below.
<table>
<tr><th colspan="9">Program for QuantiGene ViewRNA ISH Tissue Assay Slide De-paraffinization (De-Wax)</th></tr>
<tr><th>Step</th><th>Bath Position</th><th>Reagent</th><th>Volume (mL)</th><th>Temp. (°C)</th><th>Agitation (cpm)</th><th>Stroke</th><th>Time (sec)</th><th>Pause</th></tr>
<tr><td>1</td><td>1</td><td>HistoClear</td><td>200</td><td>RT</td><td>400</td><td>Long</td><td>120</td><td>0</td></tr>
<tr><td>2</td><td>1</td><td>HistoClear</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>120</td><td>0</td></tr>
<tr><td>3</td><td>1</td><td>HistoClear</td><td>200</td><td>RT</td><td>400</td><td>Long</td><td>120</td><td>0</td></tr>
<tr><td>4</td><td>1</td><td>HistoClear</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>120</td><td>0</td></tr>
<tr><td>5</td><td>1</td><td>HistoClear</td><td>200</td><td>RT</td><td>400</td><td>Long</td><td>120</td><td>0</td></tr>
<tr><td>6</td><td>2</td><td>95% EtOH</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>30</td><td>0</td></tr>
<tr><td>7</td><td>2</td><td>95% EtOH</td><td>200</td><td>RT</td><td>350</td><td>Long</td><td>30</td><td>0</td></tr>
<tr><td>8</td><td>3</td><td>95% EtOH</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>30</td><td>0</td></tr>
<tr><td>9</td><td>3</td><td>95% EtOH</td><td>200</td><td>RT</td><td>350</td><td>Long</td><td>30</td><td>0</td></tr>
</table>
D. Set a dry oven to 80 ± 1 °C and allow the temperature to reach 80 ± 1 °C before starting the protocol.
</td></tr>
<tr><td>Step 5. Load Slides/Run Protocol

20 min</td><td>
WARNING: During the baking process, the slide rack is extremely HOT! Please exercise caution and use protective equipment when handling the rack.
A. Load slides into an empty 12-position slide rack and bake in the dry oven for 5 minutes at 80 ± 1 °C.
B. Once the incubation is complete, immediately submerge the rack into Bath #1 containing 200 mL of HistoClear.
C. Start the “De-Wax” protocol using the touch screen. When the arm reaches the end of its travel above Bath #1, carefully load the rack onto the gripper as described in the Little Dipper Processor Operations Guide on page 13 – E4-E5. The “De-Wax” protocol will process slides from Bath #1 through Bath #3.
D. When the protocol is complete, the arm will raise the slide rack above Bath #3. Apply thumb pressure onto the black thumb pad on the back of the gripper paddle. Once the gripper releases the slide rack, retrieve the slide rack and proceed to Part 3, Step 6 below.
E. Dispose of HistoClear and 95% Ethanol according to Health and Safety Regulations. Any residual HistoClear in Bath #1 can be removed by rinsing with 95% Ethanol. Then wash the baths and slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergents.
F. Store the baths and rack in a dust-free environment ready for the next use.
</td></tr>
</table>

Part 3: Draw Hydrophobic Barrier — Manual Mode

Part 3 Procedure — Manual Processing

Step	Action
Step 6. Draw Hydrophobic Barrier **1 hr**	**IMPORTANT:** If using positively-charged Gold Plus slides, refer to Appendix F of the *QuantiGene ViewRNA ISH Tissue User Manual* for alternative procedural steps. **A.** Dab the hydrophobic pen on a paper towel several times before use to ensure proper flow of the hydrophobic solution. **B.** To create a hydrophobic barrier, place the slide over the template image below, tissue sections should fall inside blue rectangle, and lightly trace the blue rectangle 2 to 4 times with the Hydrophobic Barrier Pen to ensure a solid seal. It may be necessary to draw the hydrophobic barrier over tissue edges for larger sections or sections mounted close to the edge of the slide. Allow for barrier to dry at RT for 20–30 min. **IMPORTANT:** Consistently draw hydrophobic barrier size as indicated in template, even if using smaller tissue sections. 

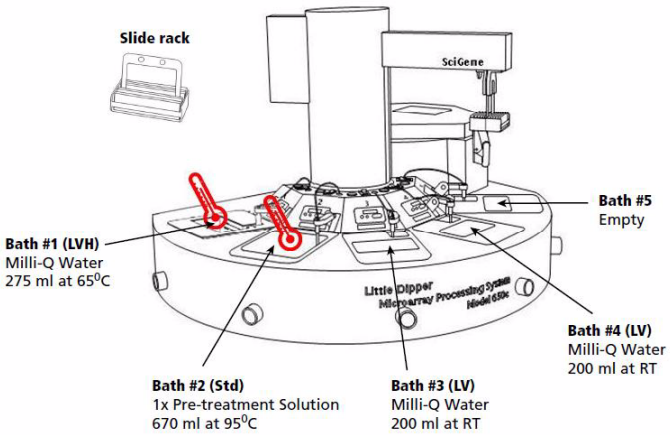
Part 4: Slide Pretreatment (Pretreat) — Automated Processing Using Little Dipper Processor

Little Dipper Configuration for Slide Pretreatment

For this section, use the following materials provided with the instrument Little Dipper Processor for Affymetrix, 115v/230v (SciGene cat. #1080-65-1 / 1080-65-2):

Table 5 Required Materials from SciGene for Slide Pretreatment using the Little Dipper Processor

Required Material	Source	Part Number
2x Low volume baths (LV)	SciGene	1080-10-2
1x Low volume baths, heatable bath (LVH)	SciGene	1080-10-5
1x Standard bath (Std)	SciGene	1080-10-1
2x Bath cover, slotted	SciGene	1080-12-2
1x Slide rack, 12 position for 3 inch slides	SciGene	1080-20-1



Part 4 Procedure — Automated Processing Using Little Dipper Processor

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 7. Reagent Preparation

3 min</td><td>A. Prepare 670 mL of 1X Pretreatment Solution.
In a clean standard bath (std) add components in the following order and mix well:
663.3 mL of Milli-Q water and 6.7 mL of 100X Pretreatment Solution.</td></tr>
<tr><td>Step 8. Instrument Setup

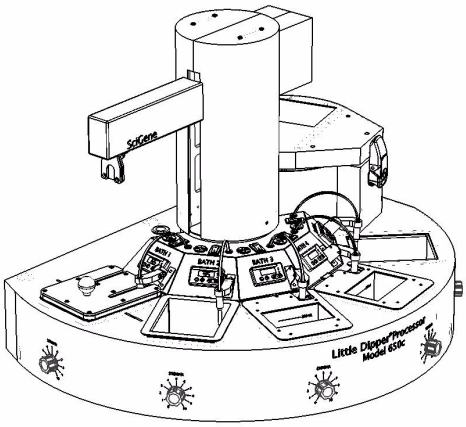
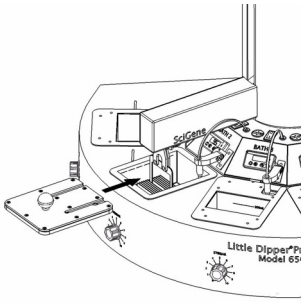
30 min</td><td>A. Rinse the removable baths and the 12-position slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergent.
B. Turn on the main power to the instrument.
C. Place a low volume, heatable bath (LVH) into position 1, low volume baths (LV) into positions 3 and 4. Fill the baths with the reagents and volumes shown in table below.

Program for QuantiGene ViewRNA ISH Tissue Assay Slide Pretreatment (Pretreat)
<table><tr><th>Step</th><th>Bath Position</th><th>Reagent</th><th>Volume (mL)</th><th>Temp. (°C)</th><th>Agitation (cpm)</th><th>Stroke</th><th>Time (sec)</th><th>Pause</th></tr><tr><td>1</td><td>1</td><td>Milli-Q Water</td><td>275</td><td>65</td><td>0</td><td>Std</td><td>120</td><td>User</td></tr><tr><td>2</td><td>2</td><td>1X Pretreatment Solution</td><td>670</td><td>95</td><td>0</td><td>Std</td><td>XX*</td><td>User</td></tr><tr><td>3</td><td>3</td><td>Milli-Q Water</td><td>200</td><td>RT</td><td>300</td><td>Long</td><td>30</td><td>0</td></tr><tr><td>4</td><td>3</td><td>Milli-Q Water</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>30</td><td>0</td></tr><tr><td>5</td><td>4</td><td>Milli-Q Water</td><td>200</td><td>RT</td><td>300</td><td>Long</td><td>30</td><td>0</td></tr><tr><td>6</td><td>4</td><td>Milli-Q Water</td><td>200</td><td>RT</td><td>0</td><td>Std</td><td>30</td><td>User</td></tr></table>* The optimal incubation time in Bath #2 needs to be determined by performing the Appendix A: Pretreatment Assay Optimization Procedures and Typical Results in the Affymetrix QuantiGene ViewRNA ISH Tissue Assay User Manual before starting the protocol.

D. Insert the standard bath (std) containing 670 mL of 1X Pretreatment Solution into position 2, then cover Bath #1 and Bath #2 with slotted lids.
E. Ensure the temperature sensors for all five baths are rotated to the “down” position.
F. Activate and set the temperature for Bath #1 to 65 °C and Bath #2 to 95 °C. Allow 25 minutes for the temperature in Bath #2 to reach 95 °C before starting the protocol.</td></tr>
<tr><td>Step 9. Load Slides/Run Protocol

15–50 min, depending on optimized time</td><td>A. Load slides into an empty 12-position slide rack, remove the slotted bath cover from Bath #1 and submerge the rack into the Bath #1.
B. Start the “Pretreat” protocol using the touch screen. When the arm reaches the end of its travel above Bath #1, carefully load the rack onto the gripper as described in the Little Dipper Processor Operations Guide on page 13 – E4-E5.

WARNING: During the loading process the slide rack will be hot. Please exercise caution and use protective equipment when handling the rack.</td></tr>
</table>

Step	Action
Step 9. Load Slides/Run Protocol...*continued* **15–50 min, depending on optimized time**	**C.** Slide the slotted cover into place on Bath #1 (see illustration). After 120 sec, an alert will sound from the touch screen indicating the protocol has paused. Remove the slotted covers from Bath #1 and Bath #2.  Warming step without rack using the slotted cover on Bath #1  Insert cover over slide rack **D.** Press the touch screen to silence the alert and resume the protocol. The rack will now move from Bath #1 to Bath #2, once the rack is submerged in Bath #2 slide a slotted bath cover into place. **E.** Once incubation step is complete, an alert will sound from the touch screen indicating the protocol has paused. **IMPORTANT:** **Pretreatment incubations that exceed the determined optimal conditions may result in high background.** **F.** Remove the slotted cover from Bath #2. Press the touch screen to silence the alert and resume the protocol to process slides from Baths #3 through Bath #4. **G.** At the end of the "Pretreat" protocol, the slide rack will remain submerged in Bath #4. An alert will sound from the touch screen indicating the protocol has been completed. **H.** Holding the slide rack, apply thumb pressure onto the black thumb pad on the back of the gripper paddle. Once the gripper releases the rack, place it into Bath #4. Press the touch screen to silence the alert. **I.** Remove the fully loaded bath from position 4 on the Little Dipper Processor to the hybridization station. Proceed to the next hybridization step in Part 5, Step 10 below. **J.** Dispose of the Pretreatment Solution and Milli-Q water after each use. Wash the baths and 12-position slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergents. **K.** Store the baths and slide rack in a dust-free environment ready for the next use.

Part 5: Protease Digestion and Target Probe Set Hybridization — Manual Mode

Part 5 Procedure — Manual Processing

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 10. Protease Digestion

25–45 min, depending on optimized time</td><td>
A. Set the humidified Incubator to 40 ± 1 °C and verify the bottom tray is filled with dd$H_2$0.
B. Using the table below as a guide, prepare the Working Protease Solution by diluting the Protease QF 1:100 in prewarmed 1X PBS. Scale reagents according to the number of assays to be run. Include one slide volume overage.
<table>
<tr><th colspan="2">Working Protease Solution per Slide</th></tr>
<tr><th>Reagent</th><th>Volume</th></tr>
<tr><td>Protease QF</td><td>4 µL</td></tr>
<tr><td>1X PBS (prewarmed to 40 °C)</td><td>396 µL</td></tr>
<tr><td>Total volume</td><td>400 µL</td></tr>
</table>
C. Remove slides from slide rack, flick off excess ddH_2O and place them face up onto an aluminum slide rack.
D. Add 500 µL of prewarmed 1X PBS to each slide and incubate in the humidified Incubator for 3 min at 40 ± 1 °C.
E. Working with one slide at a time, decant the 1X PBS, place the slide onto the aluminum slide rack, and then add 400 µL of the Working Protease Solution onto the tissue section. Repeat for the rest of the slide.
IMPORTANT: Process ONE slide at a time to avoid drying.
F. Place the aluminum slide rack into the humidified incubator and incubate at 40 ± 1 °C for the optimal time as determined in the QuantiGene ViewRNA ISH Tissue Assay User Manual – Appendix A: Pretreatment Assay Optimization Procedures and Typical Results.
G. After incubation, remove the slides from the incubator, decant the Working Protease Solution from the slides, insert them into the slide rack and transfer slide rack into clear staining dish with 200 mL 1X PBS and gently wash by moving slide rack up and down for 1 min.
H. Decant the 1X PBS, refill with 200 mL of fresh 1X PBS and gently wash by moving slide rack up and down for 1 min.
</td></tr>
<tr><td>Step 11. Fixation

7 min</td><td>
A. Under a fume hood, transfer the slide rack into the clear staining dish containing 4% formaldehyde and incubate for 5 min at RT.
B. Decant the clear staining dish containing 1X PBS and refill with 200 mL of fresh 1X PBS.
C. Under a fume hood, transfer the slide rack from the 4% formaldehyde solution to the clear staining dish containing 1X PBS, and incubate for 1 min with frequent agitation.
D. Decant the 1X PBS, refill with 200 mL of fresh 1X PBS and gently wash by moving slide rack up and down for 1 min.
E. Under a fume hood, transfer the 4% formaldehyde solution to a 200 mL capacity container, keep for later use.
</td></tr>
</table>

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 12. Target Probe Set Hybridization

3 hr 10 min</td><td>
A. Set the humidified incubator to 40 ± 1 °C and verify the bottom tray is filled with dd$H_2$0.
B. Using the table below as a guide, prepare the Working Probe Set Solutions by diluting the QuantiGene ViewRNA Probe Set(s) 1:50 in prewarmed Probe Set Diluent QF and briefly vortex. Scale reagents according to the number of assays to be run. Include one slide volume overage.
IMPORTANT: We recommend running assay controls, 1 positive and 1 negative control slide.
<table>
<tr><th colspan="4">Working Probe Set Solution per Slide</th></tr>
<tr><th></th><th>Target Sample</th><th>Negative Control</th><th>Positive Control</th></tr>
<tr><th>Reagent</th><th></th><th>Volume</th><th></th></tr>
<tr><td>Probe Set Diluent (prewarmed to 40 °C)</td><td>392 µL</td><td>400 µL</td><td>392 µL</td></tr>
<tr><td>QuantiGene ViewRNA TYPE 1 Probe Set</td><td>8 µL*</td><td>0†</td><td>8 µL‡</td></tr>
<tr><td>Total volume</td><td>400 µL</td><td>400 µL</td><td>400 µL</td></tr>
</table>
* Use target Probe Set.
C. Remove each slide from 1X PBS and decant the solution by flicking and briefly placing the slide on its edge on a laboratory wipe.
D. Place the slides flat face up on the aluminum slide rack and immediately add 400 µL Working Probe Set Solution to each tissue section.
E. Place the aluminum slide rack, with slides face up into the humidified incubator and incubate at 40 ± 1 °C for 3 hr.
</td></tr>
</table>

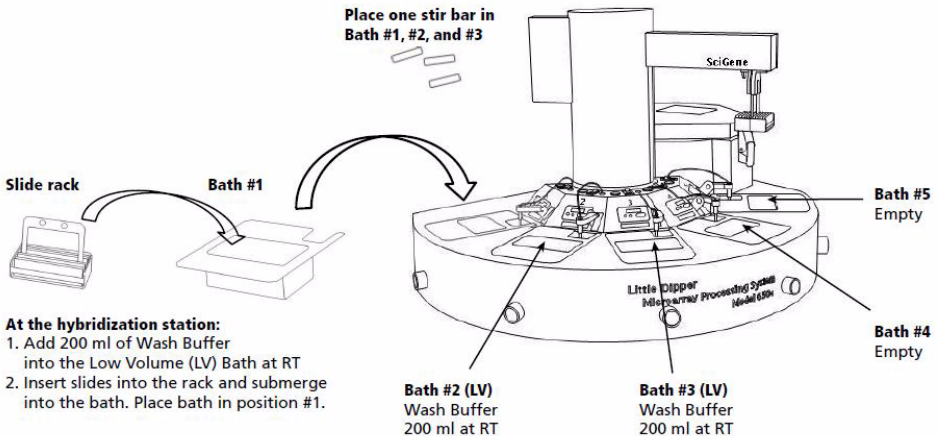
Part 6: Slide Post-Hybridization Washing (QGV Wash) — Automated Processing Using Little Dipper Processor

Little Dipper Configuration for Slide Post Hybridization Washing

For this section, use the following materials provided with the instrument Little Dipper Processor for Affymetrix, 115v/230v (SciGene cat. #1080-65-1 / 1080-65-2):

Table 6 Required Materials from SciGene for Slide Post-Hybridization Washing (QGV Wash) using the Little Dipper Processor

Required Material	Source	Part Number
3x Low volume baths (LV)	SciGene	1080-10-2
1x Slide racks, 12 position for 3 inch slides	SciGene	1080-20-1
3x Stir bars for low volume baths	SciGene	1080-11-1



Part 6 Procedure — Automated Processing Using Little Dipper Processor

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 13. Instrument Setup

5 min</td><td>
A. Rinse the removable baths, stir bars and the 12-position slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergent.
B. Turn on the main power to the instrument. Ensure the temperature sensors for all five baths positions are rotated to the “down” position.
C. Insert low volume baths (LV) into positions 2 and 3, place a stir bar in each bath and fill with 200 mL of Wash Buffer as shown in table below.

<table>
<tr><th colspan="10">Program for QuantiGene ViewRNA ISH Tissue Assay Slide Washing (QGV Wash)</th></tr>
<tr><th>Step</th><th>Bath Position</th><th>Reagent</th><th>Volume (mL)</th><th>Temp. (°C)</th><th>Agitation (cpm)</th><th>Stroke</th><th>Time (sec)</th><th>Stir Bar</th><th>Pause</th></tr>
<tr><td>1</td><td>1</td><td>Wash Buffer</td><td>200</td><td>RT</td><td>400</td><td>Long</td><td>50</td><td>Yes</td><td>0</td></tr>
<tr><td>2</td><td>1</td><td>Wash Buffer</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>10</td><td>Yes</td><td>0</td></tr>
<tr><td>3</td><td>2</td><td>Wash Buffer</td><td>200</td><td>RT</td><td>400</td><td>Long</td><td>50</td><td>Yes</td><td>0</td></tr>
<tr><td>4</td><td>2</td><td>Wash Buffer</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>10</td><td>Yes</td><td>0</td></tr>
<tr><td>5</td><td>3</td><td>Wash Buffer</td><td>200</td><td>RT</td><td>400</td><td>Long</td><td>50</td><td>Yes</td><td>0</td></tr>
<tr><td>6</td><td>3</td><td>Wash Buffer</td><td>200</td><td>RT</td><td>0</td><td>Std</td><td>10</td><td>Yes</td><td>User</td></tr>
</table>
D. Activate and set the rotation of stir bars in Baths #2 and #3, so a vigorous vortex is formed without splashing.
</td></tr>
<tr><td>Step 14. Load Slides/ Run Protocol

5 min</td><td>
A. Take a stir bar, an empty 12-position slide rack and a low volume bath (LV) to the hybridization station.
B. Fill the bath with 200 mL of Wash Buffer, add a stir bar and an empty 12-position slide rack into the bath.
C. Once the hybridization is complete, remove one slide at a time and decant the hybridization solution. Immediately insert the slide into the 12-position slide rack and submerge it into the bath containing 200 mL of Wash Buffer.
D. Repeat this process until all the slides are loaded into the 12-position slide rack. The slides should remain submerged in the bath of Wash Buffer during the loading process.
E. Insert the fully loaded bath into position 1 on the Little Dipper Processor. Activate and set the rotation of the stir bar, so a vigorous vortex is formed without splashing.
F. Start the “QGV Wash” protocol using the touch screen. When the arm reaches the end of its travel above Bath #1, carefully load the rack onto the gripper as described in the Little Dipper Processor Operations Guide on page 13 – E4-E5. The protocol will process slides from Baths #1 through Bath #3.
G. At the end of the “QGV Wash” protocol, the slide rack will remain submerged in Bath #3. An alert will sound from the touch screen indicating the completion of the “QGV Wash” protocol.
H. Holding the slide rack, apply thumb pressure onto the black thumb pad on the back of the gripper paddle. Once the gripper releases the slide rack, deposit the rack into Bath #3. Press the touch screen to silence the alert.
I. Remove the fully loaded bath from position 3 on the Little Dipper Processor. Proceed to the next step in Part 7, Step 15 on page 12.
J. Dispose of wash buffers after each use. Wash the baths, stir bars and 12-position slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergents to clean baths.
K. Store the baths in a dust-free environment ready for the next use.
</td></tr>
</table>

Part 7: Optional Stopping Point — Manual Mode

Part 7 Procedure — Manual Processing

Step	Action
Step 15. Optional Stopping Point **1 min**	**A.** Store slides in a clear staining dish containing 200 mL of Storage Buffer for up to 24 hours at RT. **B.** The following reagent preparations should be stored at RT for use in Part 2: ▪ 4% formaldehyde ▪ 1X PBS ▪ Wash Buffer **C.** All other reagent and solution preparations should be discarded. **D.** When you are ready to continue the assay, proceed to Part 8, Step 16 below.

Part 8: Signal Amplification and Detection — Manual Mode and Automated Processing Using Little Dipper Processor

Part 8 Procedure — Manual Processing and Automated Slide Washing

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 16. Preparation

5 min</td><td>If you paused the assay after Part 6, complete this step, otherwise go to Step 17 below.
A. Remove the slides from Storage Buffer, transfer slide rack to clear staining dish containing Wash Buffer, and incubate for 1 min with frequent agitation.
B. Decant Wash Buffer, refill with 200 mL fresh Wash Buffer, and incubate for 1 min with frequent agitation.</td></tr>
<tr><td>Step 17. Prepare Additional Buffers and Reagents

10 min</td><td>A. Prepare 1 L of 0.01% ammonium hydroxide in ddH_2O: In a fume hood, add 0.33 mL 30% ammonium hydroxide to 999.67 mL ddH_2O and mix well.
B. Ensure availability of 200 mL Gill's Hematoxylin.
C. If you plan on using fluorescent detection, prepare 200 mL DAPI. The final dilution of DAPI should be 0.5 µg/mL in 1X PBS. Store at 4 °C, protected from light, until ready to use.
D. Prewarm Amplifier Diluent QF and Label Probe Diluent QF buffers to 40 °C.
E. Thaw PreAmp1 QF and Amp1 QF. Place on ice until use.
F. Place Label Probe-AP on ice.
G. Bring Fast Red Tablets, Naphthol Buffer and AP Enhancer Solution to RT.</td></tr>
<tr><td>Step 18. PreAmplifier Hybridization

35 min</td><td>A. Set the humidified incubator to 40 ± 1 °C.
B. Using the table below as a guide, prepare the Working PreAmp1 Solution by diluting PreAmp1 QF 1:100 in prewarmed Amplifier Diluent QF and briefly vortex. Scale reagents according to the number of assays to be run. Include one slide volume overage.
<table><tr><th colspan="2">Working PreAmp1 Solution per Slide</th></tr><tr><th>Reagent</th><th>Volume</th></tr><tr><td>Amplifier Diluent QF (prewarmed to 40 °C)</td><td>396 µL</td></tr><tr><td>PreAmp1 QF</td><td>4 µL</td></tr><tr><td>Total volume</td><td>400 µL</td></tr></table>C. Remove each slide and decant the Wash Buffer by flicking and briefly placing the slide on its edge on a laboratory wipe. Place slides flat face up on aluminum slide rack and immediately add 400 µL of Working PreAmp1 Solution to each tissue section.
D. Place the aluminum slide rack with slides into the humidified incubator and incubate at 40 ± 1 °C for 25 min.

IMPORTANT: Incubation time should not exceed 25 min.</td></tr>
<tr><td>Step 19. Wash Slides Using Little Dipper

10 min</td><td>A. Follow entire procedure described in Part 6: Slide Post-Hybridization Washing (QGV Wash) — Automated Processing Using Little Dipper Processor Steps 13A – Step 14H.
B. Remove the fully loaded bath from position 3 on the Little Dipper Processor to the hybridization station.</td></tr>
</table>

Part 8 Procedure — Manual Processing and Automated Slide Washing

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 20. Amplifier Hybridization

20 min</td><td>
A. Using the table below as a guide, prepare the Working Amp1 Solution by diluting Amp1 QF 1:100 in prewarmed Amplifier Diluent QF and briefly vortex. Scale reagents according to the number of assays to be run. Include one slide volume overage.
<table>
<tr><th colspan="2">Working Amp1 Solution per Slide</th></tr>
<tr><th>Reagent</th><th>Volume</th></tr>
<tr><td>Amplifier Diluent QF (prewarmed to 40 °C)</td><td>396 µL</td></tr>
<tr><td>Amp1 QF</td><td>4 µL</td></tr>
<tr><td>Total volume</td><td>400 µL</td></tr>
</table>
B. Remove each slide from the Wash Buffer and decant the solution by flicking and briefly placing the slide on its edge on a laboratory wipe. Place slides flat face up on an aluminum rack and immediately add 400 µL of Working Amp Solution to each tissue section.
C. Place the aluminum slide rack with the slides in the humidified incubator and incubate at 40 ±1 °C for 15 min.
IMPORTANT: Incubation time should not exceed 15 min.
</td></tr>
<tr><td>Step 21. Wash Slides Using Little Dipper

10 min</td><td>
A. Follow entire procedure described in Part 6: Slide Post-Hybridization Washing (QGV Wash) — Automated Processing Using Little Dipper Processor Steps 13A–Step 14H.
B. Remove the fully loaded bath from position 3 on the Little Dipper Processor to the hybridization station.
</td></tr>
<tr><td>Step 22. Label Probe-AP Hybridization

25 min</td><td>
A. Using the table below (left side) as a guide, prepare 1:10 Working Label Probe-AP Solution by diluting Label Probe-AP 1:10 in prewarmed Label Probe Diluent QF and briefly vortexing to mix.
B. Using the table below (right side) as a guide, prepare 1:1,000 Working Label Probe-AP Solution by diluting the 1:10 Working Label Probe-AP Solution to 1:1,000 in prewarmed Label Probe Diluent QF and briefly vortexing to mix. Scale reagents according to the number of assays to be run. Include one slide volume overage.
<table>
<tr><th colspan="2">1:10 Working Label Probe-AP Solution per Slide</th><th colspan="2">1:1,000 Working Label Probe-AP Solution per Slide</th></tr>
<tr><th>Reagent</th><th>Volume</th><th>Reagent</th><th>Volume</th></tr>
<tr><td>Label Probe Diluent QF (prewarmed to 40 °C)</td><td>54 µL</td><td>Label Probe Diluent QF (prewarmed to 40 °C)</td><td>396 µL</td></tr>
<tr><td>Label Probe-AP</td><td>6 µL</td><td>1:10 Working Label Probe-AP (from 1:10 dilution)</td><td>4 µL</td></tr>
<tr><td>Total volume</td><td>60 µL</td><td></td><td>400 µL</td></tr>
</table>
C. Discard any remainder of 1:10 Working Label Probe-AP Solution.
D. Remove each slide from the Wash Buffer and decant the solution by flicking and briefly placing the slide on its edge on a laboratory wipe. Place slides flat face up on an aluminum slide rack and immediately add 400 µL of 1:1,000 Working Label Probe-AP Solution to each tissue section.
E. Place the aluminum slide rack with the slides in the humidified incubator and incubate at 40 ±1 °C for 15 min.
IMPORTANT: Incubation time should not exceed 15 min.
</td></tr>
<tr><td>Step 23. Wash Slides Using Little Dipper

10 min</td><td>
A. Follow entire procedure described in Part 6: Slide Post-Hybridization Washing (QGV Wash) — Automated Processing Using Little Dipper Processor Steps 13A–Step 14H.
B. Remove the fully loaded bath from position 3 on the Little Dipper Processor to the hybridization station.
</td></tr>
</table>

Step	Action
Step 24. Apply Fast Red Substrate **1 hr**	**A.** Remove each slide from the Wash Buffer and decant the solution by flicking and briefly placing the slide on its edge on a laboratory wipe. Place slides flat on the lab bench. **B.** Immediately add 400 µL of the AP-Enhancer Solution to each tissue section (pipet directly from bottle) and incubate at RT for 5 min. while preparing the Fast Red Substrate. **C.** Prepare the Fast Red Substrate: in a 15-mL conical tube, add 5 mL of Naphthol Buffer and one Fast Red Tablet. Vortex at high speed to completely dissolve the tablet. **D.** Decant the AP Enhancer Solution by flicking and briefly placing the slide on its edge on a laboratory wipe. Place the slides flat face up on an aluminum rack and immediately add 400 µL of Fast Red Substrate onto each tissue section. **E.** Place the aluminum rack with the slides into the humidified incubator and incubate at 40 ±1 °C for 30 min. **F.** Insert an empty slide rack into a clear staining dish containing 200 mL of 1X PBS. **G.** After incubation remove the aluminum rack with slides from the incubator, decant the Fast Red Substrate from the slides and insert them into the slide rack. **H.** Move the slide rack up and down several times for 1 min to rinse off the Fast Red Substrate. **I.** Retrieve 200 mL of 4% formaldehyde (used previously) and under a fume hood, pour in the clear staining dish labeled for formaldehyde. **J.** Under a fume hood, move the slide rack to the clear staining dish containing 200 mL of 4% formaldehyde and incubate for 5 min. **K.** Under a fume hood, rinse off the residual formaldehyde by transferring the slide rack to a clear staining dish containing fresh 1X PBS. Move the slide rack up and down several times for 1 min.

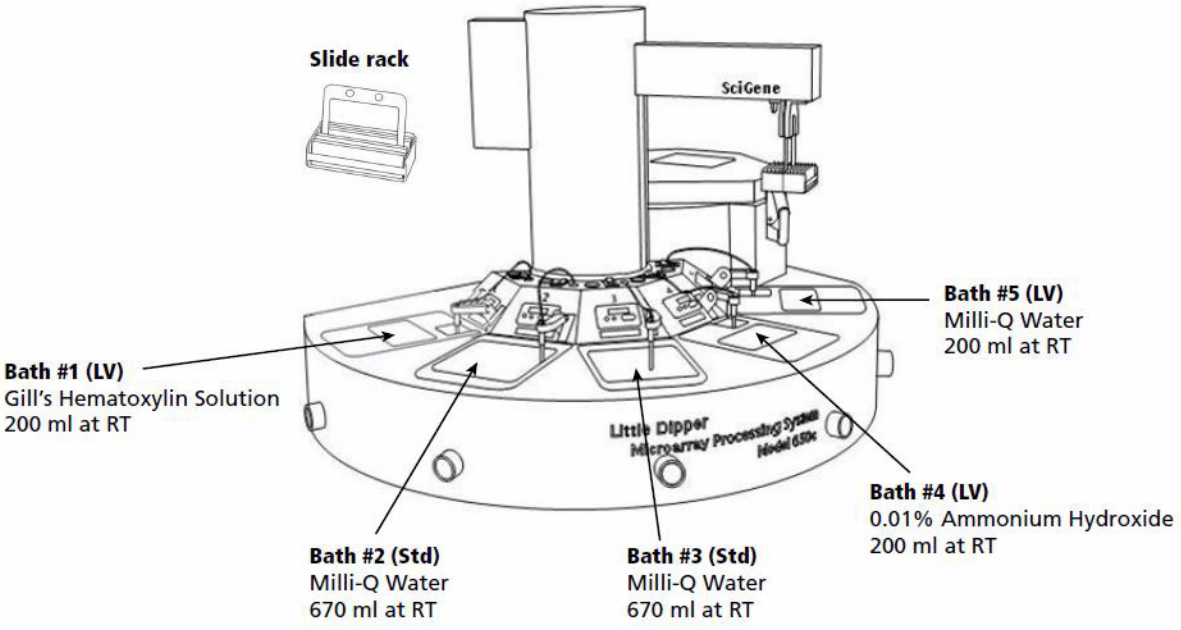
Part 9: Slide Counterstain (Gills) — Automated Processing Using Little Dipper Processor

Little Dipper Configuration for Slide Counterstain

For this section, use the following materials provided with the instrument Little Dipper Processor for Affymetrix, 115v/230v (SciGene cat. #1080-65-1 / 1080-65-2):

Table 7 Required Materials from SciGene for Slide Counterstain (Gills) using the Little Dipper Processor

Required Material	Source	Part Number
3x Low volume baths (LV)	SciGene	1080-10-2
2x Standard baths (Std)	SciGene	1080-10-1
1x Slide rack, 12 position for 3 inch slides	SciGene	1080-20-1



Part 9 Procedure — Automated Processing Using Little Dipper Processor

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>Step 25. Instrument Setup

5 min</td><td>
A. Rinse the removable baths and the 12-position slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergent.
B. Turn on the main power to the instrument.
C. Place low volume baths (LV) into positions 1, 4 and 5 and standard baths (Std) into position 2 and 3. Fill baths with the corresponding reagents and volumes shown in table below.
<table>
<tr><th colspan="9">Program for QuantiGene ViewRNA ISH Tissue Assay Slide Gills Counterstain (Gills)</th></tr>
<tr><th>Step</th><th>Bath Position</th><th>Reagent</th><th>Volume (mL)</th><th>Temp. (°C)</th><th>Agitation (cpm)</th><th>Stroke</th><th>Time (sec)</th><th>Pause</th></tr>
<tr><td>1</td><td>1</td><td>Gill's Hematoxylin Solution</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>420</td><td>0</td></tr>
<tr><td>2</td><td>2</td><td>Milli-Q Water</td><td>670</td><td>RT</td><td>400</td><td>Long</td><td>20</td><td>0</td></tr>
<tr><td>3</td><td>2</td><td>Milli-Q Water</td><td>670</td><td>RT</td><td>0</td><td>Long</td><td>10</td><td>0</td></tr>
<tr><td>4</td><td>3</td><td>Milli-Q Water</td><td>670</td><td>RT</td><td>400</td><td>Long</td><td>20</td><td>0</td></tr>
<tr><td>5</td><td>3</td><td>Milli-Q Water</td><td>670</td><td>RT</td><td>0</td><td>Long</td><td>10</td><td>0</td></tr>
<tr><td>6</td><td>4</td><td>0.01% Ammonium Hydroxide</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>10</td><td>0</td></tr>
<tr><td>7</td><td>5</td><td>Milli-Q Water</td><td>200</td><td>RT</td><td>400</td><td>Long</td><td>20</td><td>0</td></tr>
<tr><td>8</td><td>5</td><td>Milli-Q Water</td><td>200</td><td>RT</td><td>0</td><td>Long</td><td>10</td><td>0</td></tr>
</table>
D. Ensure the temperature sensors for all five baths are rotated to the “down” position.
</td></tr>
<tr><td>Step 26. Load Slides/ Run Protocol

15 min</td><td>
A. Remove one slide at a time from 1X PBS and immediately insert the slide into an empty 12-position slide rack.
B. Once all slides are loaded into the 12-position slide rack, submerge the rack into Bath #1 containing 200 mL of Gill’s Hematoxylin I.
C. Start the “Gills” protocol using the touch screen. When the arm reaches the end of its travel above Bath #1, carefully load the slide rack onto the gripper as described in the Little Dipper Operations Guide on page 13 – E4-E5. The protocol will process slides in Bath #1 through Bath #5.
D. When the “Gills” protocol is complete, the arm will raise the slide rack above Bath #5. Holding the slide rack, apply thumb pressure onto the black thumb pad on the back of the gripper paddle. Once the gripper releases the slide rack, retrieve the slide rack and proceed to the next step in Part 10, Step 27.
E. Dispose of Gill’s Hematoxylin I and 0.01% Ammonium Hydroxide according to Health and Safety Regulations. Wash the baths and slide rack with Milli-Q water three times and dry with lint-free towels. Do not use detergents.
F. Store the baths and rack in a dust-free environment ready for the next use.
</td></tr>
</table>

Part 10: Add Coverslip and Image — Manual Mode

Part 10 Procedure — Manual Processing

Step	Action
Step 27. Add Coverslip and Image **20 min**	**A.** Add a minimum of two drops of DAKO Ultra Mount mounting medium to tissue section without making any bubbles. Cover the slide section with a 24 mm x 55 mm cover glass. **B.** Dab off the excess mounting medium and image the result under a bright-field microscope and/or fluorescence microscope. **IMPORTANT:** **Fluorescent signals will fade over time. However, chromogenic signal will remain stable.**

NOTE: **Remaining reagents sent in the kit can be stored as recommended for up to six months from the date of delivery. 0.01% ammonium hydroxide solution can be stored at RT for up to one month. Discard all other buffers and working reagents used during the procedure.**

Technical Help for Affymetrix

For technical support regarding the QuantiGene ViewRNA ISH Tissue Assay Procedure, contact the appropriate resource provided below based on your geographical location. For an updated list of FAQs and product support literature, visit our website at www.affymetrix.com/panomics.

Table 8 Technical Support Contacts

Location	Contact Information
North America	1.877.726.6642 option 1, then option 3; pqbhelp@affymetrix.com
Europe	+44 1628-552550; techsupport_europe@affymetrix.com
Asia	+81 3 6430 430; techsupport_asia@affymetrix.com

Technical Help for SciGene

For technical support regarding the Little Dipper Processor, contact SciGene.

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