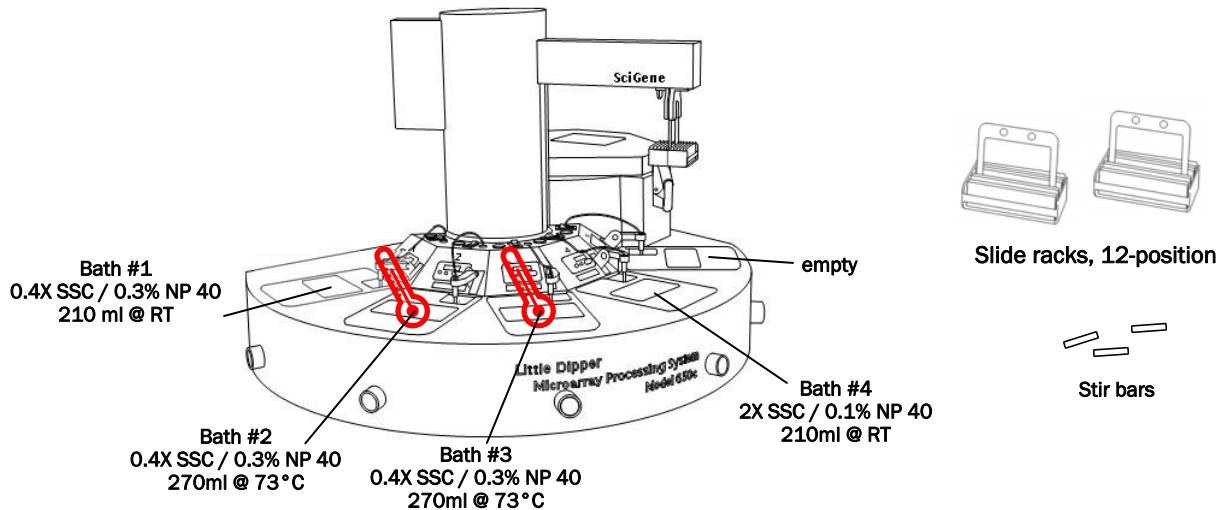


Post-Hybridization Processing of FISH Assays for Vysis TelVysion™, AneuVysion™, CEP® and LSI® Probes (1-12 slides)

Day 2



Equipment Configuration

- Little Dipper Processor for FISH, 115v/230v. (SciGene cat. #1080-70-1/1080-70-2)
- 2x Low volume, temperature controlled baths. (SciGene cat. #1080-10-5) – for Baths #2 & 3
- 2x Bath cover. (SciGene cat. #1080-12-0) – for Baths #2 & 3
- 2x Low volume baths. (SciGene cat. #1080-10-2)
- 2x Slide racks, 12 position for 3 inch slides. (SciGene cat. #1080-20-1)
- 3x Stir bars for baths. (SciGene cat. #1080-21-1, 25/pk)

Buffer Preparation

- 0.4x SSC / 0.3% NP 40 solution

Nuclease-free water	950 ml
20x SSC, pH 5.3	20 ml
NP40	3 ml

Mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust pH to 7.0 ±0.2 with 1N NaOH. Store at ambient temperature up to 6 months until use.

- 2x SSC / 0.1% NP 40 solution

Nuclease-free water	255 ml
20x SSC, pH 5.3	30 ml
NP40	0.3 ml

Mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust pH to 7.0 ±0.2 with 1N NaOH. Store at ambient temperature up to 6 months until use.

Instrument Setup

1. Rinse the removable baths, stir bars and the processing racks with 100% ethanol, then with de-ionized water three times, and dry with lint-free towels. Do not use detergent.
2. Place clean low volume baths into positions 1 and 4. Place clean low volume temperature controlled baths into positions 2 and 3. Add stir bars to Baths #2, 3 and 4. Rotate all temperature sensors down.
3. Using the touch screen, create a protocol named 'Vysis12' or similar and enter the agitation rates and times for the baths as shown in **Table 1**. Consult the Little Dipper User Manual for details on creating and editing protocols.
4. Fill baths with the buffers and volumes shown in **Table 1** and place covers over Baths #2 and 3.

Table 1. Little Dipper Program for Vysis FISH Protocols.

Bath Position	Buffer	Volume (ml)	Temp (°C)	Agitation (cpm)	Time (sec)
1	0.4X SSC / 0.3% NP 40*	210	RT	0	0
2	0.4X SSC / 0.3% NP 40	270	73°	0	15
3	0.4X SSC / 0.3% NP 40	270	73°	125	120
4	2X SSC / 0.1% NP 40	210	RT	125	60
C	—	—	—	—	300

*Bath #1 provides a location to load slides onto racks, keeping them wet while disassembling and loading multiple hybridizations. Some users have obtained good results when loading slides without buffer in Bath #1, however, prolonged exposure to air should be avoided.

Instrument setup continued on next page...

Instrument Setup (continued)

5. Turn on main power to the instrument and the individual power switches to Bath #2 and 3 only and set the temperature on the controller to 73 °C. Activate and set rotation speed of stir bars in baths 2, 3 and 4 so that a vigorous vortex is formed without splashing. Wait approximately 25 minutes for the temperature of the buffer to stabilize.
6. Set a 12-position slide rack into empty Bath #1
7. Assemble another 12-position slide rack with the same number of plain slides as the number being processed, and set it in the balancing rack position of centrifuge.

Load Arrays / Run Protocol

1. Remove covers from Baths #2 and 3.
2. Remove the FISH slides from the Vysis® HYBrite™ or other incubation chamber when hybridizations are complete according to Vysis product inserts.
Note: Slides can be processed 1 to 12 at a time. If processing more than 12 slides, divide slides into batch sizes ≤ 12. Maintain slides in hybridization chamber until ready for washing.
3. Working with one slide at a time, remove rubber cement and coverslip using forceps, and immediately place the slide into the slide rack in Bath #1, minimizing the time the slides are exposed to air. Continue inserting all the slides.
4. Start the 'Vysis12' protocol using the touch screen and load the rack on the gripper as described in the **Little Dipper Processor Operations Guide**. The robot will process slides through Baths #2 to 4 and dry them by centrifugation. After centrifugation, slides are held in the dark.
5. Retrieve the slide rack from the centrifuge. Apply counter-stain to each target area and apply a cover slip. Apply gentle pressure to the top of coverslip to remove excess counter-stain. Blot with paper towel. When all slides have been processed, proceed to visualization on an appropriate fluorescent microscope.
6. If necessary, process additional slides following steps 1 to 4. Verify that Baths #2 and 3 have stabilized at $73 \pm 0.5^\circ\text{C}$ before each use.
7. Dispose of wash buffers immediately after use. Wash the baths, stir bars and processing rack with warm water, rinse 3 times with de-ionized water and dry with lint-free towels. Do not use detergents to clean baths. Store the baths in a dust-free environment ready for the next use.

— End Protocol —