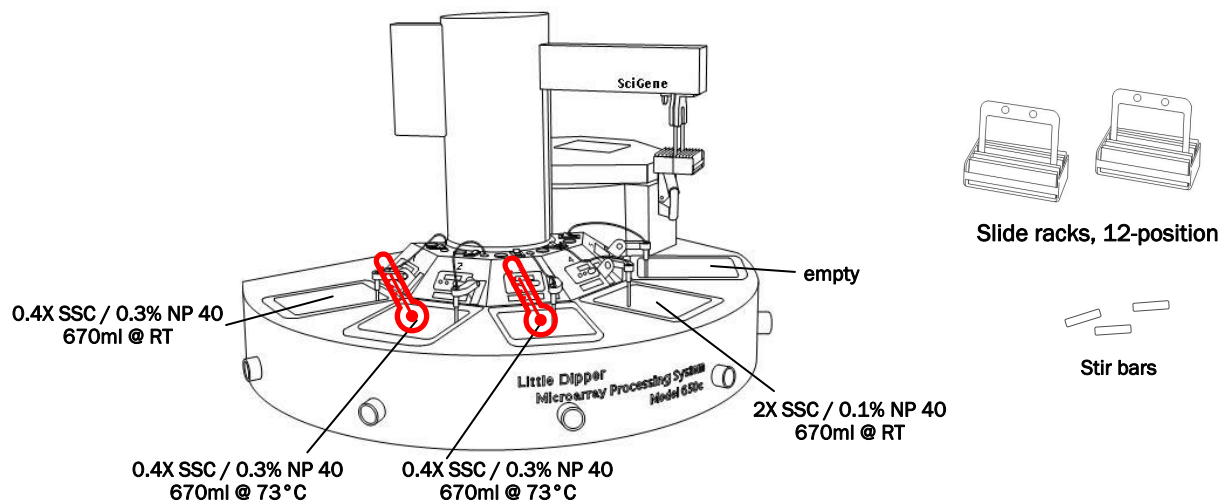


Post-Hybridization Processing of FISH Assays for Vysis TelVysion™, AneuVysion™, CEP® and LSI® Probes

Day 2



Equipment Configuration

- Little Dipper Processor for FISH, 115v/230v. (SciGene cat. #1080-70-1/1080-70-2)
- 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 – 3 liters

Nuclease-free water	2850 mL
20x SSC, pH 5.3	60 mL
NP40	9 mL

Mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust pH to 7.0±0.2 with 1N NaOH. Add nuclease-free water to 3 liters. Store at ambient temperature up to 6 months until use.

- 2x SSC / 0.1% NP 40 solution – 1 liter

Nuclease-free water	850 mL
20x SSC, pH 5.3	100 mL
NP40	1 mL

Mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust pH to 7.0±0.2 with 1N NaOH. Add nuclease-free water to 1 liter. 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 baths into positions 1 to 4 on the unit and add a stir bar in Baths #2, 3 and 4. Rotate all temperature sensors down. **Note:** Any sensor remaining in the “up” position will interfere with the movement of the Little Dipper arm.
3. Using the touch screen, create a protocol named ‘Vysis’ 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**.

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 ¹	670	RT	0	0
2	0.4X SSC / 0.3% NP 40	670	73°	0	15
3	0.4X SSC / 0.3% NP 40	670	73°	125	120
4	2X SSC / 0.1% NP 40	670	RT	125	60
C	–	–	–	–	300

¹ 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 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.
2. 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.
3. Start the 'Vysis' 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.
4. 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.
5. 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.
6. 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 —

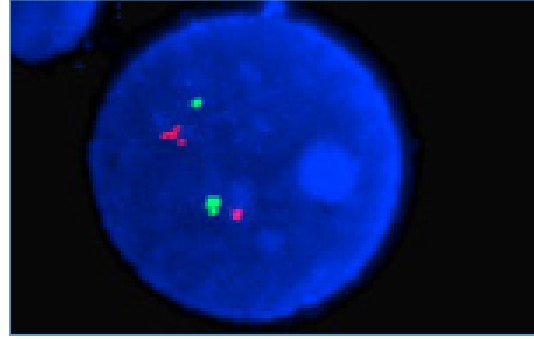


Figure 1. Image of FISH of cultured bone marrow cells hybridized with P53 probe (red) and centromeric probe (green) processed on the Little Dipper Processor.

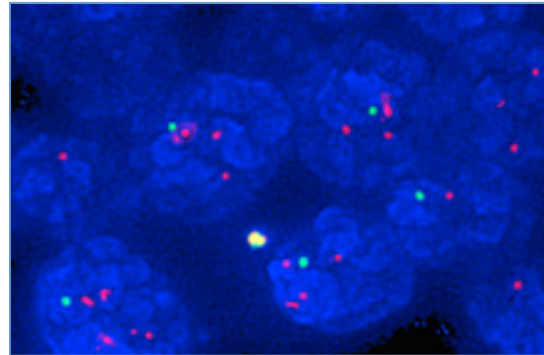


Figure 2. Image of fluorescent *in situ* hybridization (FISH) of formalin-fixed paraffin-embedded (FFPE) breast tissue hybridized with HER-2 probe (red) and centromeric probe (green) and processed on the Little Dipper Processor.

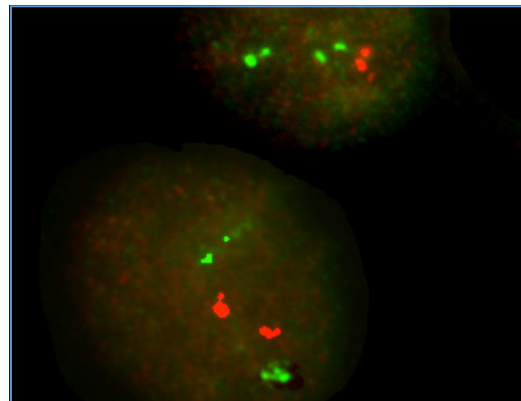


Figure 3. Image of FISH from slides stored at -20 for 3 months and prepared by standard cytogenetic dropping method, then processed on the Little Dipper Processor.

Courtesy of the Cytogenetics Laboratory Shared Resource, University of Minnesota Masonic Cancer Center

SciGene
Automating Array Workflows

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