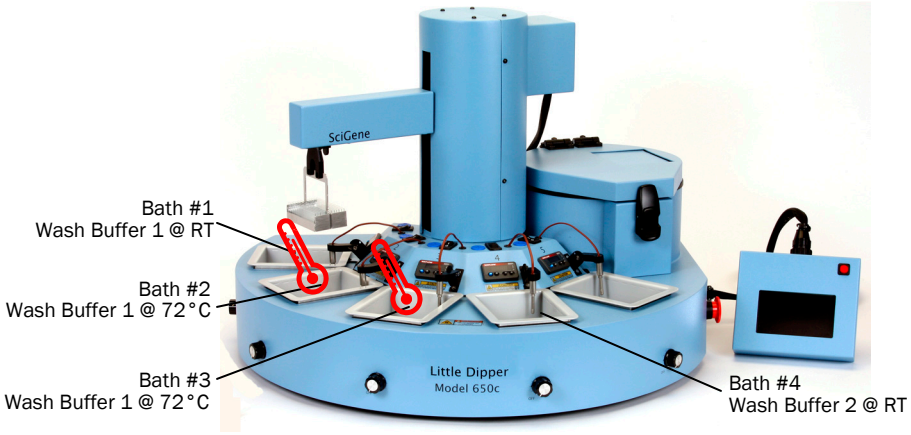
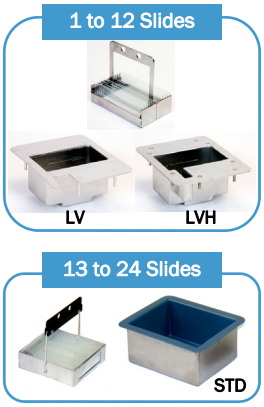
Automated Post-Hybridization Processing of FISH Slides





Summary

The Little Dipper Processor automates the manual processing of FISH slides after probe hybridization providing slides ready for counterstaining and imaging. The instrument provides precise control over time and temperature during the critical slide washing steps using FISH probes.

At the completion of probe hybridization, CytoBond Removable Coverslip Sealant (SciGene cat. #2020-00-1) or rubber cement and coverslips are removed and the slides are inserted into a 12 or 24-position rack which is then loaded onto the instrument. Following the recommended program, slides are briefly pre-warmed in Wash Buffer 1 at 72°C then moved to another bath of Wash Buffer 1 at 72°C for precisely 2 minutes (Fig. 1). After a final wash in Wash Buffer 2, the slide rack is automatically dried in the onboard centrifuge and held safely away from light.

If "wet" slides are preferred for counterstaining, the centrifuge can be programmed not to spin but keep the slides in the dark.

Baths and Accessories

Table 1 lists the number and types of baths, racks and bath covers for setting up the instrument for processing batch sizes of 1-12 and 13-24 slides.

Table 1. Bath and Accessories for Different Batch Sizes

Items Required for 1 to 12 Slides	Catalog #	Qty
Low volume bath with stir bar (LV)	1080-10-2	2
Low volume, heatable bath with stir bar (LVH)	1080-10-5	2
12-position slide rack*	1080-20-1	2
Items Required for 13 to 24 Slides	**Catalog #**	**Qty**
Standard bath with stir bar (STD)	1080-10-1	4
24-position slide rack*	1080-20-5	2

*Requires matching sized centrifuge buckets

Reagents

- FISH Wash Buffer 1 (SciGene cat. #2010-00-1)
  0.4x SSC/0.3% IGEPAL, pH 7; Ready to use
- FISH Wash Buffer 2 (SciGene cat. #2010-00-2)
  2.0x SSC/0.3% IGEPAL, pH 7; Ready to use

Consult Table 2 for the volume of each reagent to be prepared for processing different slide batch sizes.

Table 2. Bath Setup for Post-Hyb Slide Processing

Bath Position	Reagents for 1 to 12 Slides	Bath Type	Volume	Temp (°C)
1	Wash Buffer 1	LV	270	RT
2	Wash Buffer 1	LVH	290	72°
3	Wash Buffer 1	LVH	290	72°
4	Wash Buffer 2	LV	270	RT
Bath Position	**Reagents for 13 to 24 Slides**	**Bath Type**	**Volume**	**Temp (°C)**
1	Wash Buffer 1	STD	670	RT
2	Wash Buffer 1	STD	670	72°
3	Wash Buffer 1	STD	670	72°
4	Wash Buffer 2	STD	670	RT

Instrument Programming and Setup

1. Using the touchscreen, name and create the protocol shown in Table 3. Consult the **Little Dipper User Manual** for instructions on creating protocols.
2. Referencing Table 2, place the specified bath types in the indicated positions in the instrument.
3. Fill the baths with the specified reagent to the max fill lines on the sides of the baths and set the stir bar rotation speed to achieve a gentle vortex.
4. Open the centrifuge and place a slide rack loaded with the same number of slides as being processed in the red colored bucket as a balance.

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Table 3. Sample Protocol for Post-Hyb Processing (POSTHYB-1)

Step #	Bath #	Agitation (cpm)	Time (sec)	Pause (sec)	Drip Time (sec)
1	#1	0	0	0	0
2	#2	0	15	0	0
3	#3	0	120	0	0
4	#4	150	60	0	0
5	Centrifuge	n/a	300	0	0

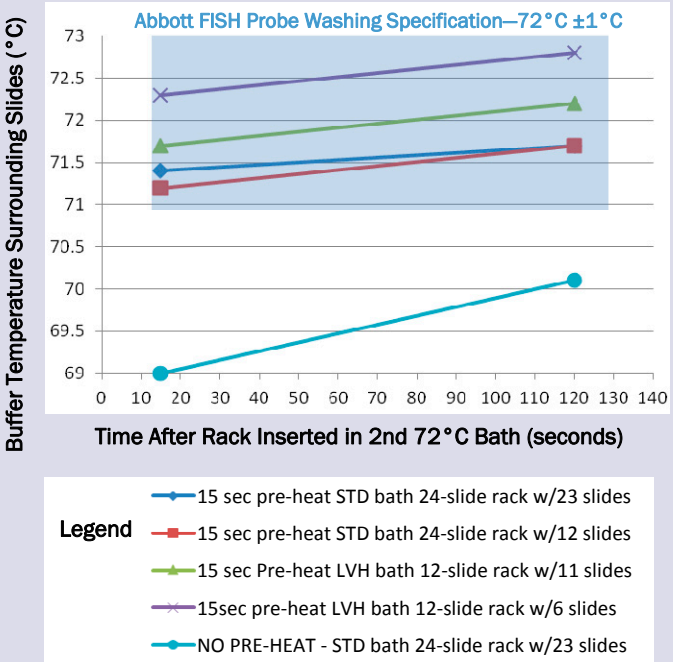
5. Turn on power to heat the baths in positions 2 and 3 and set the temperature of both to 72C.
6. If "wet" slides are desired, set the centrifuge spin time to 0. The slide rack will be inserted into the centrifuge safe from light but will not be spun dry.

Load Slides /Run Protocol

1. Check that all baths on the instrument are filled properly and stir bars are providing good mixing action. Verify that baths #2 and #3 are at 72°C.
2. Load the slide rack containing the slides and start the protocol.
3. At the completion of the program, remove the slide rack from the green centrifuge bucket.

— **Proceed to counterstaining and imaging.** —

Fig. 1: Wash Buffer Temperature During Post-Hyb Slide Processing



Effect of slide preheating on wash buffer temperatures. Wash buffer surrounding the slides was measured using an immersion probe, in the middle of the slide rack, attached to the gripper arm. Using the program shown in Table 3, racks were pre-warmed by immersion in bath #2 at 72°C for 15 seconds then moved to bath #3 at 72°C for 2 minutes. Control racks were not pre-warmed and were placed directly into bath #3 at 72°C.

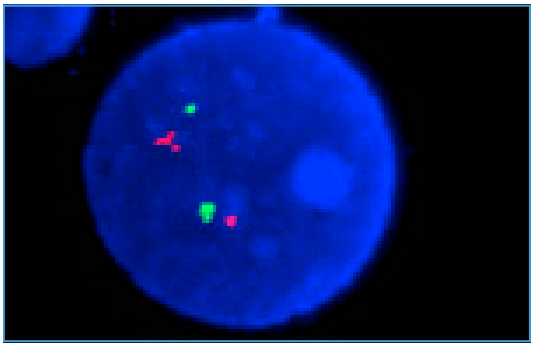
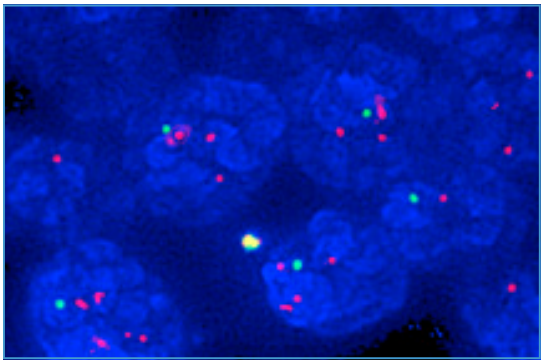
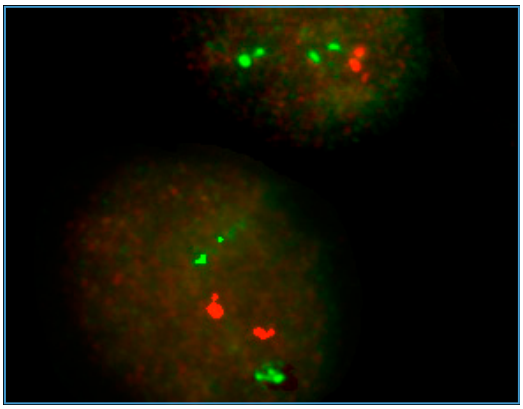


Image of cultured bone marrow cells hybridized with P53 probe (red) and centromeric probe (green) processed on the Little Dipper Processor for FISH.



Formalin-fixed paraffin-embedded (FFPE) breast tissue hybridized with HER-2 probe (red) and centromeric probe (green) and processed on the Little Dipper Processor for FISH.



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

SciGene

Automating FISH and CMA Workflows

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