

## Automated Post-Hybridization Processing of FISH Slides

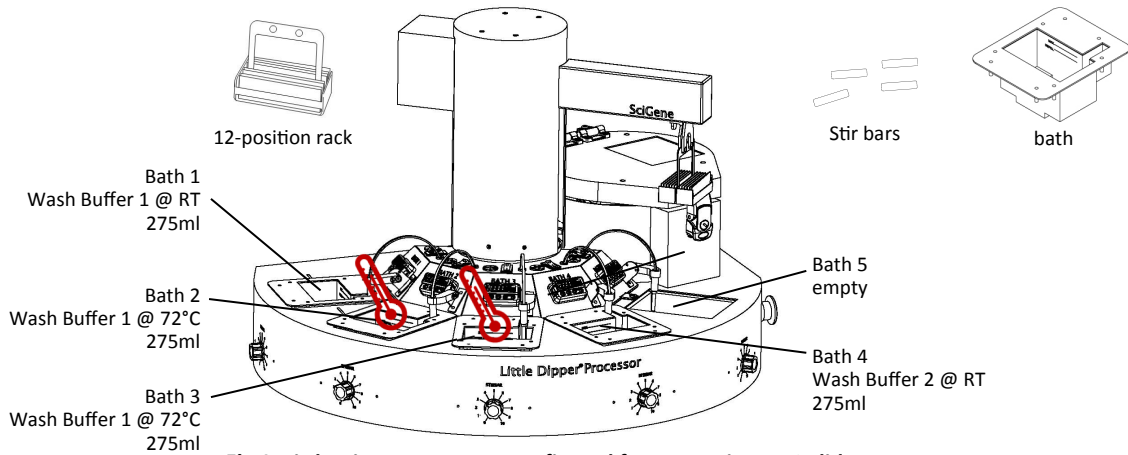
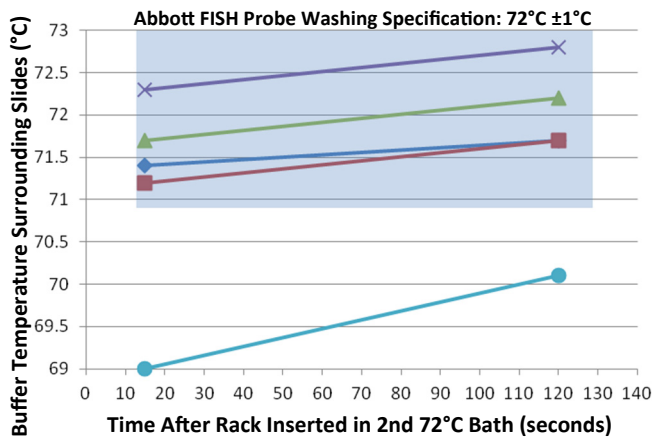


Fig 1. Little Dipper Processor configured for processing 1-12 slides

### Introduction

The Little Dipper Processor provides precise control over time and temperature during the critical slide washing steps after FISH probe hybridization, producing slides ready for counterstaining and imaging. Two Wash Buffer 1 baths are used in sequence to briefly warm slides and then perform a hot wash step for the programmed time. This ensures buffer temperature is maintained regardless of the number of slides processed (Fig 2).

Fig 2. Effects of Slide Preheating on Wash Buffer Temperatures



- Legend
- 15 sec pre-heat STD bath 24-slide rack w/23 slides
  - 15 sec pre-heat STD bath 24-slide rack w/12 slides
  - 15 sec Pre-heat LVH bath 12-slide rack w/11 slides
  - 15sec pre-heat LVH bath 12-slide rack w/6 slides
  - NO PRE-HEAT - STD bath 24-slide rack w/23 slides

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 POSTHYB-1 program, racks were pre-warmed 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.

### Equipment Configuration

- **Little Dipper Processor for FISH.**  
SciGene cat. #1080-70-1 (115V) / 1080-70-2 (230V)
- **4x Low volume, heatable bath, 275 ml with stir bar.**  
SciGene cat. #1080-10-5
- **2x Slide rack, 12 position for 3 inch slides.**  
SciGene cat. #1080-20-1

### Reagents Needed

- FISH Wash Buffer 1, 4L (0.4x SSC/0.3% IGEPAL, pH 7)  
SciGene cat. #2010-00-1
- FISH Wash Buffer 2, 4L (2.0x SSC/0.3% IGEPAL, pH 7)  
SciGene cat. #2010-00-2

### Instrument Setup

1. Turn on main power to the instrument (right side, at back).
2. Create the **POSTHYB-1** program using the touchscreen (Table 1). Consult the **Little Dipper User Manual** for instructions.
3. Insert clean low volume heatable baths in positions 1-4.
4. Insert a 12-position slide rack in the centrifuge balance bucket with the same number of slides to be processed.
5. Fill Baths 1-3 with FISH Wash Buffer 1 (Table 2).
6. Fill Bath 4 with FISH Wash Buffer 2.
7. Set stir bars to achieve a gentle vortex without splashing.

Table 1. Little Dipper **POSTHYB-1** Program

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	15	0	0
4	4	0	60	0	0
5	Centrifuge	n/a	300	0	0

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Table 2. Bath Setup for Post-Hyb Processing

Bath	FISH Wash Buffer	LVH Volume	Temp (°C)
1	Buffer 1	275	RT
2	Buffer 1	275	72°
3	Buffer 1	275	72°
4	Buffer 2	275	RT

### Load Slides / Run Protocol

1. Ensure baths are properly filled and stir bars are providing good mixing action. Verify that baths 2 and 3 are at 72°C.
2. Remove CytoBond sealant (SciGene cat. #2020-00-1) and coverslips from hybridized slides to be processed.
3. Insert slides into Little Dipper slide rack.
4. Start the **POSTHYB-1** program (Table 2) and load the rack in Bath 1. After washing, slides are dried and held away from light in the centrifuge. If “wet” slides are preferred for counterstaining, set the centrifuge time to zero (“0”) to keep the slides in the dark, without spinning.
5. At the completion of the program, remove the slide rack from the centrifuge. Slides are now ready for counterstaining and imaging.

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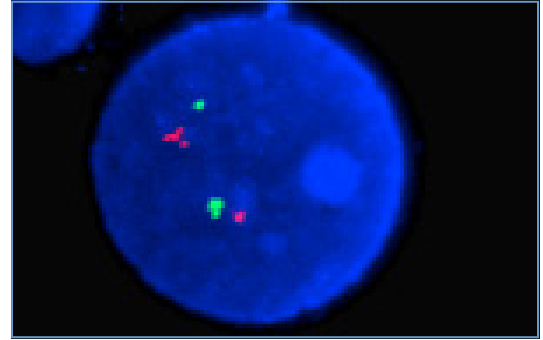
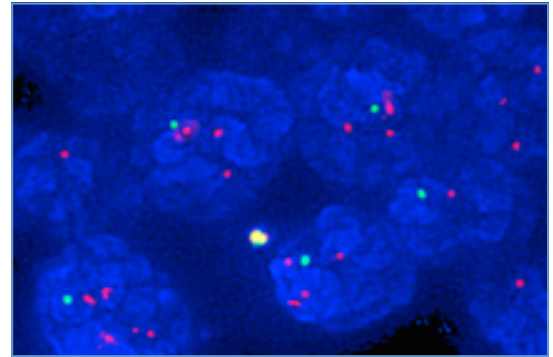
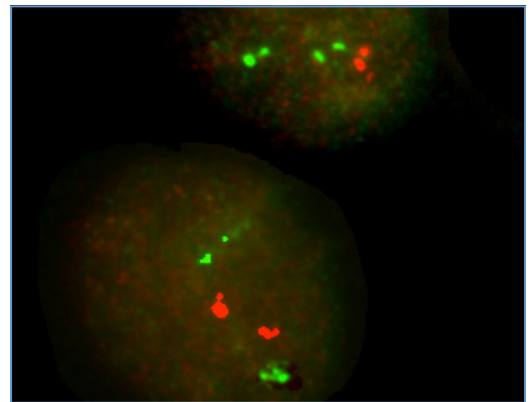


Image of cultured bone marrow cells hybridized with P53 probe (red) and centromeric probe (green) processed on the Little Dipper Processor for FISH.



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.

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