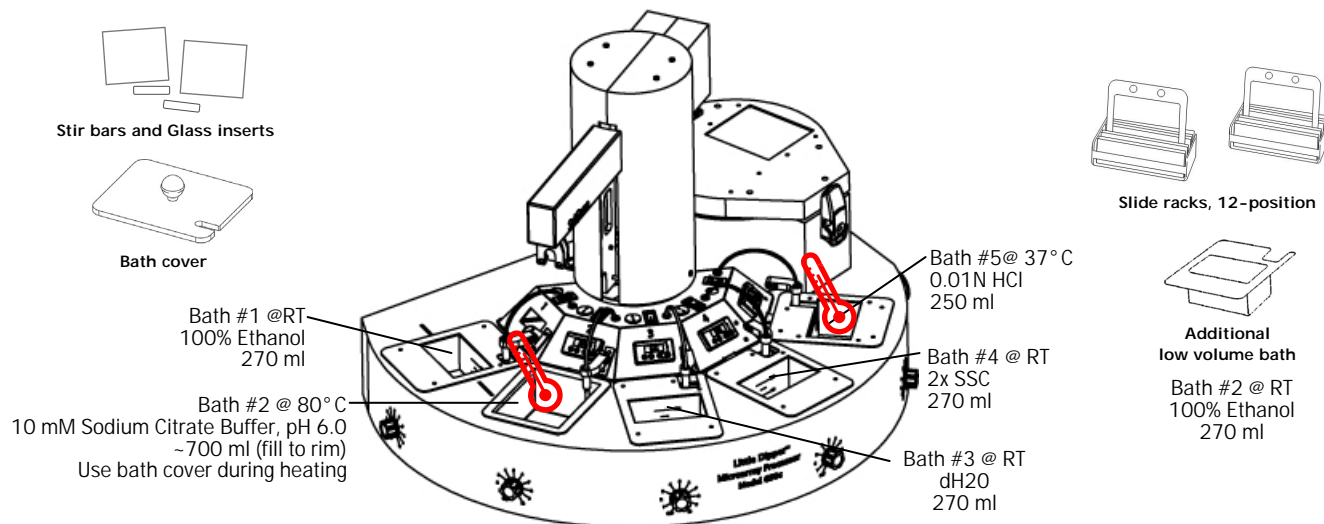


Pre-Hybridization Processing of Formalin-Fixed Paraffin Embedded Samples (FFPE) for FISH

Day 1



Equipment Configuration

- Little Dipper® Processor for FISH, 115v/230v. (SciGene cat. #1080-70-1/1080-70-2)
- 5x Low volume baths. (SciGene cat. #1080-10-2)
- 2x Heatable low volume baths. (SciGene cat. #1080-10-5)
- 1x Standard bath. (SciGene cat. #1080-10-1)
- 1x Bath cover. (SciGene cat. #1080-12-0)
- 2x Slide rack, 12 position for 3 inch slides. (SciGene cat. #1080-20-1)
- 2x Glass Inserts for low volume baths. (SciGene cat. #1080-10-3, 5/pk)
- 2x Stir bars for low volume baths. (SciGene cat. #1080-11-1, 10/pk)

Buffer Preparation

- 100% Ethanol, reagent grade (Sigma cat. #E7023)
Use fresh from container.
- 10mM Sodium Citrate Buffer, pH 6.0 (Sigma cat. #S4641)
Dilute from concentrate with dH₂O. Adjust to pH 6.0.
- 2x SSC (Abbott cat. #32-804850)
Dilute from 20x powder concentrate with dH₂O.
- 0.01N HCl, ACS grade (VWR cat. #3202-2)
Dilute from concentrate with dH₂O.
- Pepsin (Sigma cat. #P-7012)
Add dH₂O to pepsin powder to a concentration of 75000 U/ml. Store 2.5 ml aliquots at -20°C.
- dH₂O

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. Rotate temperature sensors down in positions 1, 3, and 4 and then insert clean low volume baths.
3. Rotate sensors up in positions 2 and 5. Insert a clean standard bath in position 2 and a heatable low volume bath in position 5. Rotate sensors down.
Note: Any sensor remaining in the “up” position will interfere with the movement of the Little Dipper arm.
4. Place glass inserts and stir bars into Baths #2 and 5.
5. Fill the baths with buffer solutions as shown in Table 1.
6. Turn on main power to the instrument and the individual power switches to Baths #2 and 5.
7. Set the temperature for Bath #2 to 80°C and place cover over bath. Set Bath #5 to 37°C. Wait at least 30 minutes for the temperature of the buffers to stabilize.
8. Activate stir bars in Bath #2 and 5.
9. Program the FFPEDAY1 protocol according to Table 2.

Table 1. Bath Setup for FISH FFPE Pre-Hyb Processing

Bath	Bath Type	Buffer	Temp (°C)	Volume (ml)
1	Low volume	100% Ethanol	RT	270
2	Standard	10 mM Sodium Citrate Buffer, pH 6.0	80	~700 (to rim)
3	Low volume	dH ₂ O	RT	270
4	Low volume	2x SSC	RT	270
5	Low volume, heatable	0.01N HCl	37	250
Additional Bath				
2	Low volume	100% Ethanol	RT	270

Continued on next page...

Pre-Hybridization Processing of FFPE Samples for FISH

Deparaffinization

Follow your standard procedure using multiple changes of xylene in a fume hood followed by air drying.

Load Slides / Run Protocol

1. Remove cover from Bath #2. Wait 5 minutes for the temperature to stabilize.
2. Place slides in a 12 position rack for the Little Dipper instrument.
3. Start the 'FFPEDAY1' protocol previously programmed (Table 2) and load the rack containing the slides on the gripper as described in the **Little Dipper Processor Operations Guide**.

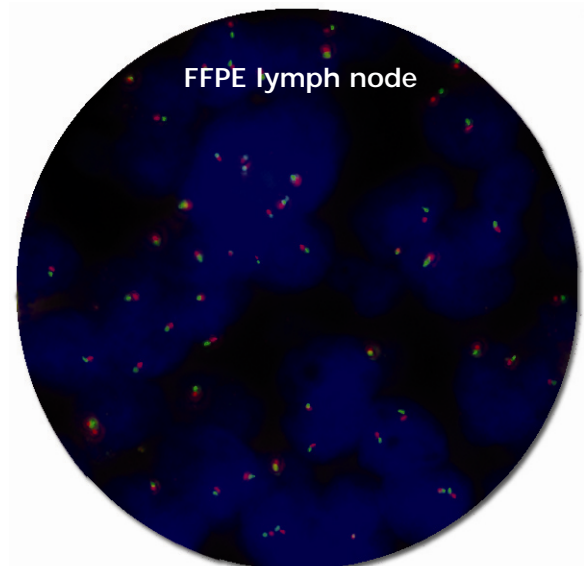
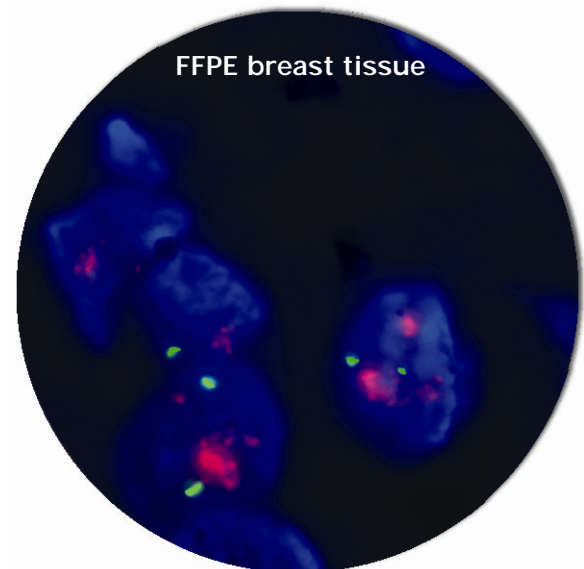
Table 2. Little Dipper Protocol for FISH Pre-Hyb FFPE Processing (FFPEDAY1)

Step	Bath	Buffer	Temp (°C)	Agitation (cpm)	Time (sec)	Drip (sec)
1	1	100% Ethanol	RT	50	300	300
2	2	10 mM Sodium Citrate Buffer, pH 6.0	80	150	5400	10
3	3	dH2O	RT	150	120	10
User programmed pause. Change Bath #2 to additional bath containing 100% EtOH. Add 2.5ml pepsin to Bath #5.						
4	4	2x SSC	RT	150	300	20
5	5	0.01N HCl + Pepsin	37	150	900	10
6	3	dH2O*	RT	150	300	10
7	4	2x SSC*	RT	150	300	20
8	1	100% Ethanol*	RT	50	60	10
9	2	100% Ethanol	RT	50	60	120

*Note: The same bath and buffer is reused from an earlier step.

4. After completion of Step 3, the Little Dipper will pause and activate a beeping user alarm. Turn off the temperature controller on Bath #2 and remove the standard bath using insulated gloves. Replace with the additional low volume bath containing 100% EtOH.
5. Add 2.5 ml of prepared pepsin buffer to Bath #5.
6. Press the touch screen to resume. The instrument will start at Step 4 and continue through completion of the protocol.
7. Dispose of buffers and reagents at the end of the work day. Wash baths and processing racks with warm water and rinse three times with de-ionized water and dry with lint-free towels. Do not use detergents to clean baths. Store baths and racks in a dust free environment ready for next use.

– End Protocol –



*FISH images from slides processed on the Little Dipper Processor.

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