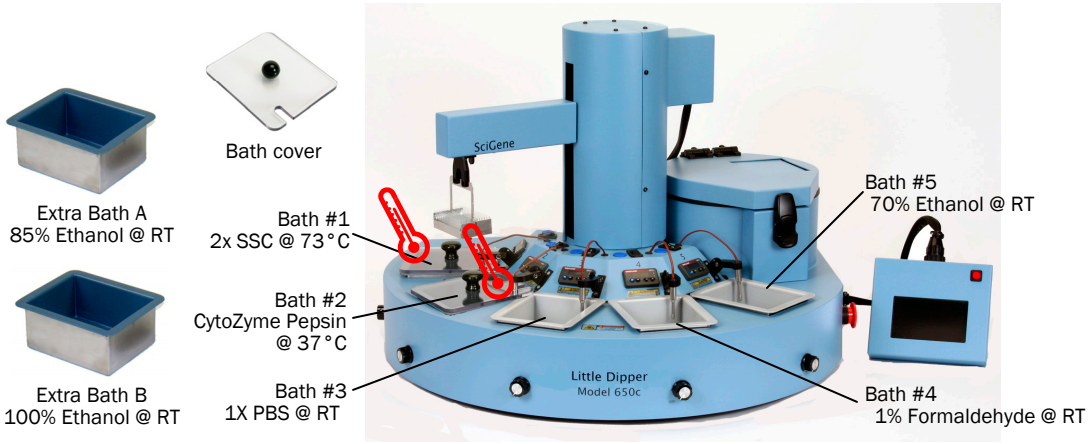
Automated Processing of Fixed Cells for FISH Analysis



NOTE: This method is derived from the manual procedure used with the Abbott FISH Pretreatment Kit. Since many variations are possible, this method should be considered only as an example. Custom protocols for your specific process are easily programmed.

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	5
Low volume, heatable bath with stir bar (LVH)	1080-10-5	2
12-position slide rack*	1080-20-1	2
Bath cover	1080-20-0	2
Items Required for 13 to 24 Slides	**Catalog #**	**Qty**
Standard bath with stir bar (STD)	1080-10-1	7
24-position slide rack*	1080-20-5	2
Bath cover	1080-20-0	2

*Requires matching sized centrifuge buckets

Reagents

- 2X SSC — dilute from 20X concentrate with diH20
- 1X PBS — dilute from 20X concentrate with diH20
- CytoZyme Stabilized Pepsin (SciGene cat. #2022-00-X) Dilute 1:50 v/v in CytoZyme Reaction Buffer (cat. #2022-10-X)
- 1% Formaldehyde — dilute 10% formalin to 1% in 1X PBS
- 70% Ethanol
- 85% Ethanol
- 100% Ethanol

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

Table 2. Bath Setup for Fixed Cells Slide Processing

Bath Position	Reagents for 1 to 12 Slides	Bath Type	Volume	Temp (°C)
1	2X SSC	LVH	290	73°
2	CytoZyme Pepsin	LVH	290	37°
3	1X PBS	LV	270	RT
4	1% Formaldehyde	LV	270	RT
5	70% Ethanol	LV	270	RT
Extra A	85% Ethanol	LV	270	RT
Extra B	100% Ethanol	LV	270	RT
Bath Position	**Reagents for 13 to 24 Slides**	**Bath Type**	**Volume**	**Temp (°C)**
1	2X SSC	STD	670	73°
2	CytoZyme Pepsin	STD	670	37°
3	1X PBS	STD	670	RT
4	1% Formaldehyde	STD	670	RT
5	70% Ethanol	STD	670	RT
Extra A	85% Ethanol	STD	670	RT
Extra B	100% Ethanol	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 with a user controlled programmed pause.
2. Referencing Table 2, place required baths in the indicated positions on the instrument. Two extra baths are kept ready to replace the baths #3 and #4 for the final two ethanol dehydration steps.

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Table 3. Sample Protocol for FFPE Slide Processing (FIXED-1)

Step #	Bath #	Agitation (cpm)	Time (sec)	Pause (sec)	Drip Time (sec)
1	#1	150	120	0	0
2	#2	150	600	0	0
3	#3	150	300	0	0
4	#4	150	300	0	0
5	#3	150	300	0	0
6	#5	150	60	9999	0
— Programmed Pause —					
6	#3	150	60	0	0
7	#4	150	60	0	0

3. Fill each bath with its specified reagent to the max fill line and set stir bar rotation speed to achieve a gentle vortex. Place bath covers on baths #1 and #2 to prevent evaporation.
4. Turn on power to heat the baths in positions 1 and 2. Set the temperature of bath #1 to 73°C and #2 to 37°C.

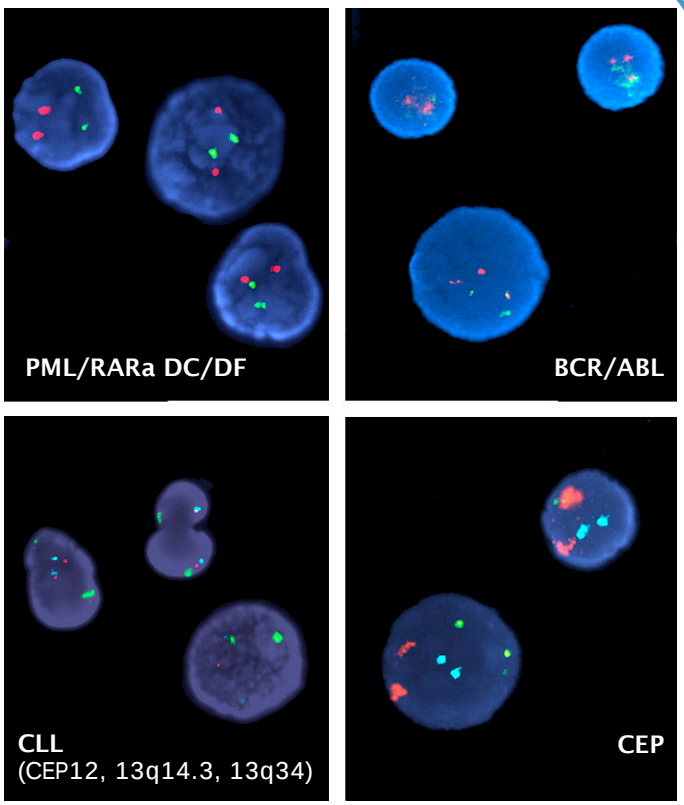
Cell Fixation

Fix cells on slides using Carnoy's solution according to standard methods.

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 bath #1 is 73°C and bath #2 is 37°C.
2. Remove the bath covers on baths #1 and #2.
3. Load the slide rack containing the fixed slides and start the protocol.
4. At the completion of step 5, the instrument will pause and provide an audible signal. Replace bath #3 with extra bath A containing 85% ethanol and bath #4 with extra bath B containing 100% ethanol.
5. Release the pause to resume the protocol by pressing the touch screen.
6. At the completion of the program, remove the slide rack from gripper on the instrument and air dry the slides.

— **Proceed to probe hybridization**. —



FISH images of bone marrow cells using multiple probes. Slides processed (pre & post-hyb) on the Little Dipper Processor for FISH.

SciGene

Automating FISH and CMA Workflows