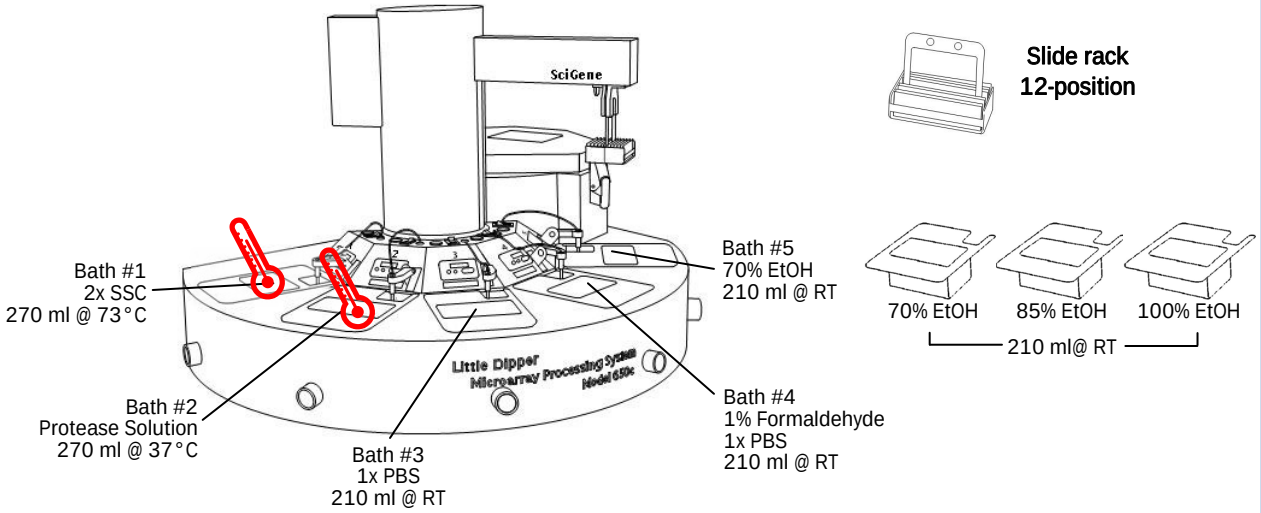
FISH Pre-Hybridization Processing of Blood and Bone Marrow Cells

Day 1



Equipment Configuration

- **Little Dipper® Processor for FISH**, 115v/230v. (SciGene cat. #1080-70-1/1080-70-2)
- **2x Low volume, temperature controlled baths.** (SciGene cat. #1080-10-5) — for Baths #1 & 2
- **2x Bath cover** (SciGene cat. #1080-12-0) — for Baths #1 & 2
- **6x Low volume baths.** (SciGene cat. #1080-10-2)
- **Slide rack, 12 position for 3 inch slides**. (SciGene cat. #1080-20-1)

Buffer and Reagent Preparation

1. **2x SSC – 300 ml**
2. **Protease Solution:**
   - 0.01 N HCL – 300 ml
   - Trypsin – 0.15 g
   - 5% Pepsin – 240 µl*
     *Add to Bath #2 when slides are in Bath #1.
3. **1x PBS – 300 ml**
4. **1% Formaldehyde / 1x PBS – 250 ml**
5. **70% / 85% / 100% EtOH– 250 ml each**

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 through 5 on the unit. Rotate all temperature sensors down.
   Note: Any sensor remaining in the "up" position will interfere with the movement of the Little Dipper arm.

Instrument Setup (continued)

3. Using the touch screen, create a protocol named 'FISHBM1' or similar and enter the bath agitation rates and times and programmed pause as shown in **Table 1**. Consult the **Little Dipper User Manual** for details on creating and editing protocols.
4. Fill each bath to the fill line with buffers shown in **Table 1** and place covers over Baths #1 and 2.
5. Turn on main power to the instrument and individual power switch to Baths #1 and 2 only. Set temperature on Bath #1 to 73°C and Bath #2 to 37°C. Wait 10 minutes for temperature to stabilize.
6. Activate stir bars to all baths. Set stir bar speed to "7" or just below maximum.

Table 1. Little Dipper Protocol for Pre-Hybridization FISH Processing (FISHBM1)

Step	Bath	Reagent	Temp (°C)	Volume (ml)	Agitation (cpm)	Time (sec)
1	1	2x SSC	73	270	150	120
2	2	Protease Solution	37	270	150	600
3	3	1x PBS	RT	210	250	300
4	4	1% Formaldehyde/ 1x PBS	RT	210	150	300
5	3	1x PBS	RT	210	150	300
6	5	70% EtOH	RT	210	50	60
User programmed pause. Replace baths 3-4 with EtOH solutions.						
7	3	85% EtOH	RT	210	50	60
8	4	100% EtOH	RT	210	50	60

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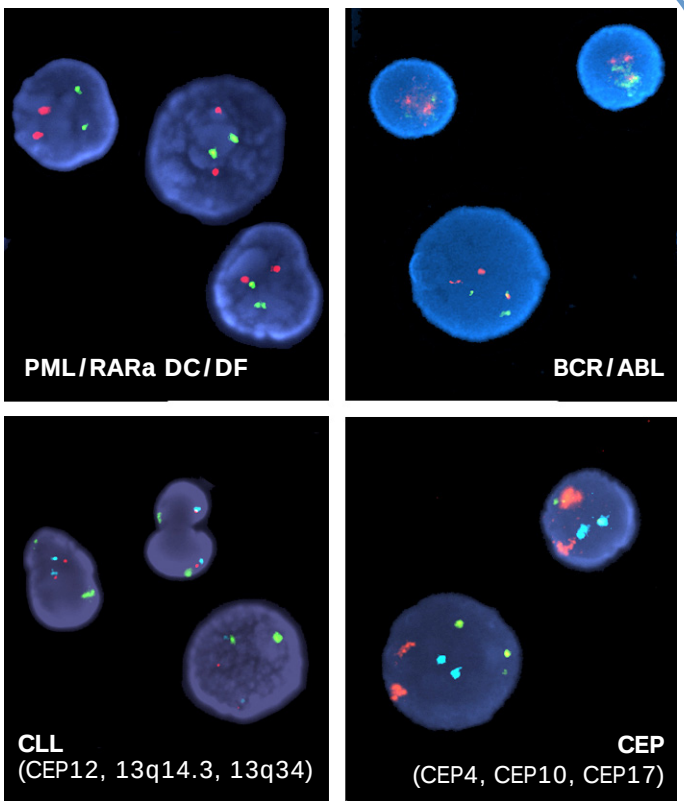
Load Slides / Run Protocol

1. Remove covers from Baths #1 and 2.
2. Place slides in a 12 position rack for the Little Dipper instrument.
3. Start the 'FISHBM1' protocol previously programmed (Table 1) and load the rack containing the slides on the gripper as described in the **Little Dipper Processor Operations Guide**.
4. Add the 240 µl of 5% Pepsin to Bath #2 while slides are being processed in Bath #1.
5. Turn off power to Baths #1 and 2 after steps 1 and 2 (Table 1) are completed.
6. After completion of Step 5, the Little Dipper will pause and activate a beeping user alarm. Replace baths in positions 3-4 with the EtOH solutions as indicated in Table 1.
7. Resume protocol. The instrument will complete the protocol through Step 8 (Table 1), slowly withdrawing the rack, presenting the slides for hybridization.
8. 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 of bone marrow cells using multiple probes. Slides were processed following **Day 1** (pre-hybridization) and **Day 2** (post-hybridization) protocols on the **Little Dipper® Processor for FISH**. The Day 1 protocol is described here and the Day 2 protocol is described separately in a SciGene Method document for post-hybridization of FISH assays using Vysis® Probes. Images compliments of Dr. Teresa Smolarek, Director of Cytogenetics, Cincinnati Children's Hospital Medical Center.*

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