MicroFISH Slide Preparation and Processing of 12 Patient Samples

Required Equipment

- **CytoBrite Slide Incubation System** with 2x slide trays
  SciGene cat. #2019-00-1
- **CytoBrite Slide Oven** — preheated to 37°C
  SciGene cat. #2019-70-X
- **Little Dipper Processor for FISH**
  SciGene cat. #1080-70-X — as configured in Fig. 7
- **Little Dipper Coverslip Catcher Bath Insert**
  SciGene cat. #1080-10-9
- **MicroFISH Hybridization Chamber**
  SciGene cat. #2040-38-0
- **MicroFISH Hybridization Chamber Absorbent Pads**
  SciGene cat. #2040-32-1
- **Single Channel Pipettors** (2 and 100 µl)
- **Coverslips, 22 x 50 mm**

Required Materials and Reagents

- **12x MicroFISH Assay Slides, 8 well**
  SciGene cat. #2040-01-1
- **FISH Wash Buffer 1 , 4L** (0.4x SSC; 0.3% IGEPAL, pH 5.0)
  SciGene cat. #2010-00-1
- **Carnoy's Solution** (3:1 methanol:acetic acid; prepared fresh)
- **Fixed blood/bone marrow cells** (per your lab method)
- **FISH probes** (prepared per manufacturer instructions)
- **DAPI**

Slide Preparation

1. Create two programs on the CytoBrite System:

10MIN90C	90°C	10 m	00 s
2MIN76C	76°C	02 m	00 s

2. Label a MicroFISH slide for each patient and record the well number for each probe on your standard scoresheet.
3. Prepare cells per your standard lab protocol. On the day of analysis, centrifuge and re-suspend cells in freshly prepared Carnoy's solution and then image cells on a light microscope to verify that the quality and density of each cell preparation meets your lab's quality standards before proceeding.
4. Place patient-labeled MicroFISH slides in CytoBrite slide trays.
5. Pipette 1µl of the patient cell suspension into each well that will receive probe (Fig 1). If necessary, increase humidity around the slide to achieve uniform spreading.
6. Place slide trays into the CytoBrite System and run program **10MIN90C** to age the cells. Slides are heated to 90°C for ten minutes and then cooled to 37°C (Fig 2).
7. Remove the slide trays from the CytoBrite System.
8. Pipet 1µl of each probe solution into the center of assigned wells, changing the pipet tip after each dispense (Fig. 3).
9. Place a 22 x 50 mm coverslip on each MicroFISH slide (Fig 4).

Fig 1. Pipette 1µl fixed cells into wells.

Fig. 2. Age cells in CytoBrite System.

Fig 3. Pipette 1µl probe into wells.

Fig 4. Place one coverslip per slide.

Fig 5. Denature at 76° for 2 min.

Fig 6. Transfer CytoBrite slide trays to hyb chamber and place in oven.

Slide Hybridization

1. Prepare a room temperature MicroFISH Hybridization Chamber by placing dry absorbent pads in the top and bottom sections. Add 50 ml DI water onto each pad. Pour off excess water.
2. Place the CytoBrite trays containing prepared MicroFISH slides into the CytoBrite System and run the **2MIN76C** program. Slides are heated to 76°C for two minutes and then cooled to 37°C (Fig. 5).
3. Place the CytoBrite trays into the MicroFISH Hybridization Chamber with wet absorbent pads. Seal the Chamber and place it on a drawer inside a preheated 37°C CytoBrite Slide Oven (Fig. 6).
4. Incubate slides in the 37°C CytoBrite Oven for 18 to 24 hours.

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Post-Hybridization Slide Processing

1. Create a program named **MFPOSTHYB** on the Little Dipper Processor, as shown in Table 1.

Table 1. Little Dipper MFPOSTHYB Program for MicroFISH

Bath	Temp (°C)	SciGene Reagent	Volume (ml)	Stir Bar	Agitation Speed	Time (sec)
1	RT	FISH Wash Buffer 1	670	5	600	10
2	72°	FISH Wash Buffer 1	275	5	0	15
3	72°	FISH Wash Buffer 1	275	5	0	45
4	RT	FISH Wash Buffer 1	210	5	0	30
C	Centrifuge dry for 30 seconds.					

2. Using clean baths and accessories, configure the Little Dipper Processor as follows (Fig. 7):
   - Bath 1: Insert a standard bath with coverslip catcher.
   - Baths 2 and 3: Insert low volume heatable baths.
   - Bath 4: Insert a low volume bath.
   - Rotate all temperature sensors down. Bath 5 remains empty.
   - Place a 12-slide rack with absorbent pad into the centrifuge balance bucket with an equal number of blank slides as those to be processed.

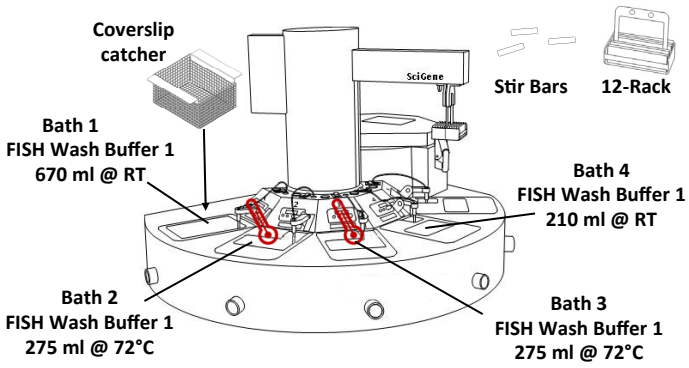

Fig 7. Little Dipper Processor configuration for MicroFISH processing.

3. Fill Baths #1-4 with SciGene FISH Wash Buffer 1, as shown in Table 1, to fill line. Add stir bars to Baths #2-4 only and adjust rotation until a gentle vortex is formed in each.
4. Turn on power to Baths #2 and 3. Wait until temperature stabilizes at 72°C (approx. 15 minutes).
5. Remover the MicroFISH Hybridization Chamber from the CytoBrite Oven, open the chamber and take out the trays.
6. Transfer the MicroFISH slides into a 12-position Little Dipper Processor slide rack (Fig. 8). Coverslips remain attached.
7. Select and start the **MFPOSTHYB** program and load the rack. Coverslips will shake off in Bath #1 (Fig. 9). Slides are washed in the remaining baths and dried in the centrifuge.
8. At completion, remove the rack from the centrifuge (Fig. 10) and return slides to the CytoBrite trays.

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Fig 8. Transfer slides to 12-rack.

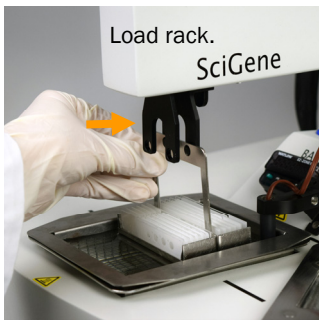

Fig. 9. Load rack onto gripper.

Fig 10. Take rack from centrifuge.

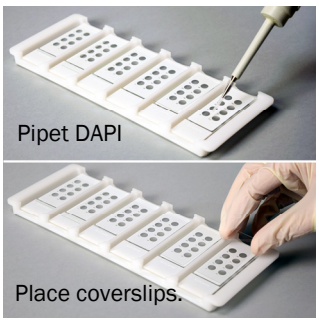

Fig. 11. Pipet DAPI and place coverslip.

9. Using a 100 µl pipettor, dispense a continuous bead of DAPI down the center of each slide between the two rows of wells. Place one 22 x 50 mm coverslip over each slide and gently press down to spread the counterstain over all wells (Fig. 11).

The MicroFISH slides are now ready for imaging.

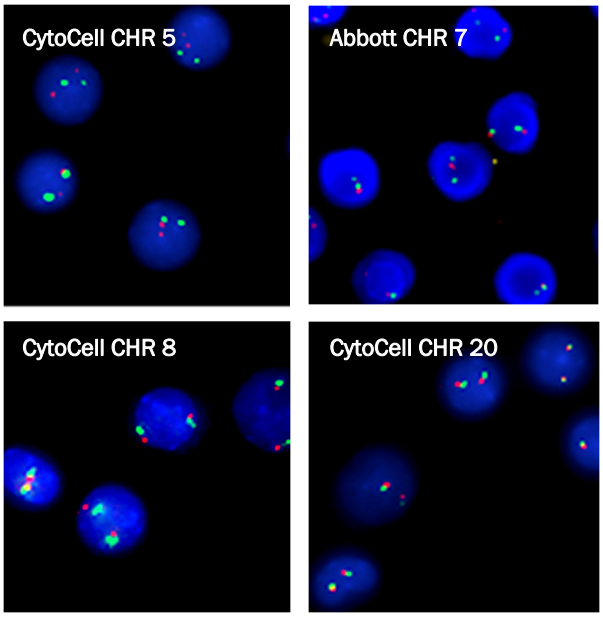

Fig 12. FISH analysis of cells using CytoCell and Abbott probes on MicroFISH slides, processed on SciGene instruments.