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Automated Processing of FFPE Tissues for FISH Analysis

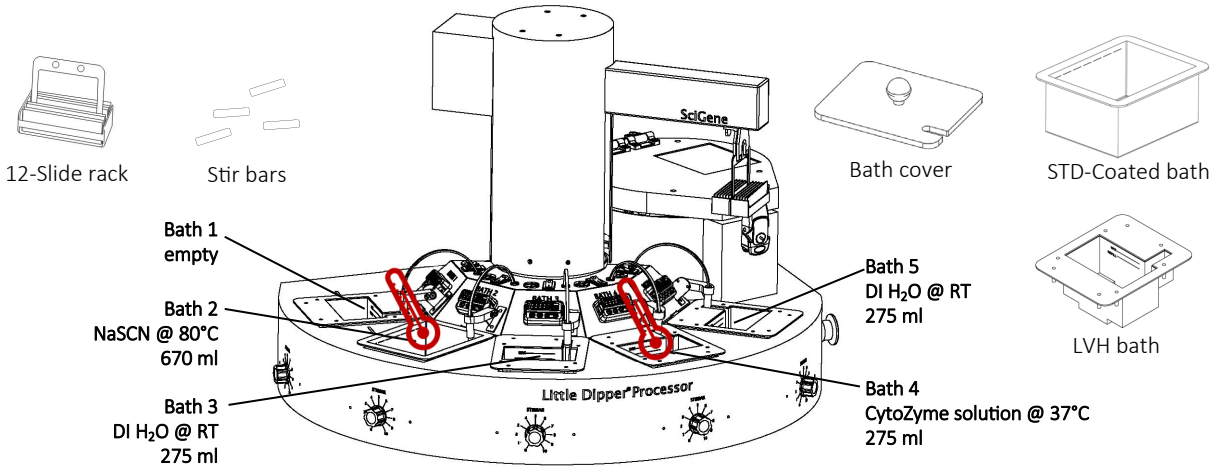

Fig 1. Little Dipper instrument configured for batches of 1 to 12 slides

Equipment Configuration

- Little Dipper Processor for FISH.
  SciGene cat. #1080-70-1 (115V) / 1080-70-2 (230V)
- 1x Standard, coated bath (STD-C) for NaSCN with stir bar.
  SciGene cat. #1080-10-7
- 4x Low volume heatable bath, 275 ml with stir bar. (LVH)
  SciGene cat. #1080-10-5
- 2x Slide rack, 12 position for 3 inch slides.
  SciGene cat. #1080-20-1
- 1x Bath cover — for pre-heating NaSCN only.
  SciGene cat. #1080-12-1

Reagents Needed

- CytoZyme Stabilized Pepsin (SciGene cat. #2022-00-3)
- CytoZyme Stabilized Pepsin HC [high concentration] (SciGene cat. #2022-03-3)
- CytoZyme Reaction Buffer (SciGene cat. #2022-10-3)
- 1M NaSCN — (SciGene cat. #2030-00-2)

Table 1. CytoZyme Dilutions Equivalent to Abbott Kits.

Abbott Kit	CytoZyme Type	Pepsin (ml)	Buffer (ml)	Total Bath Volume (ml)
I	CytoZyme Pepsin *-OR-*	5	270	275
	CytoZyme HC Pepsin	0.5	274.5	
II	CytoZyme HC Pepsin	5	270	275
IV	CytoZyme HC Pepsin	1.5	268.5	275

Note: Adjust CytoZyme concentration or digestion times for variations in sample thickness and other factors. Run test slides to determine optimum conditions.

Instrument Setup

1. Turn on main power to the instrument (right side, at back).
2. Insert baths as shown in Figure 1. Place stir bars in Baths 2-5.
3. Fill Bath 2 (STD-coated bath) with 670ml of NaSCN.
4. Turn on Bath 2 controller and set temperature to 80°C. Adjust stir bar rotation until a gentle vortex is formed.
5. Place a cover over Bath 2 to minimize evaporation.
6. Prepare a CytoZyme solution by adding pepsin and buffer equivalent to your Abbott kit in Bath 4 (Table 1).
7. Turn on Bath 4 controller and set temperature to 37°C. Adjust stir bar rotation until a gentle vortex is formed.
8. Add DI H_20 to fill line in Baths 3 and 5. Adjust stir bar rotation until a gentle vortex is formed.
9. Wait for bath temperatures to stabilize (15-20 min).

Table 2. Bath Setup for FFPE Tissue Processing.

Bath	Reagents	Bath Type	Volume (ml)	Temp (°C)
1	none	LVH	—	RT
2	NaSCN	STD-C	670	80°
3	DI H2O	LVH	275	RT
4	CytoZyme + Buffer	LVH	275	37°
5	DI H2O	LVH	275	RT

Deparaffinization

Perform deparaffinization of the slides to be processed on the Little Dipper using your laboratory's standard procedure.

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Table 3. Little Dipper FFPE Protocol for DeWaxed Samples

Step	Bath	Agiltation (cpm)	Time (sec)	Pause (sec)	Drip (sec)
1	1	0	0	0	0
2	2	0	720	0	5
3	3	0	180	0	5
4	4	0	1200	0	5
5	5	0	180	0	0

Load Slides / Run Protocol

1. Using the Little Dipper Processor touch screen, create the FFPE protocol in Table 3. Consult the Little Dipper User Manual for instructions.
2. After temperature has stabilized in Baths 2 and 4, load a 12-position rack with dewaxed slides and place near Bath 1.
3. Remove the cover on Bath 2 and set aside.
4. Start the FFPE protocol from the touchscreen and load the rack in Bath 1. The instrument will perform the protocol and stop with the rack above Bath 5.
5. Push the tab on the gripper paddle -OR- use the Advance Settings | Open Gripper command on the touch screen to release the rack. Slides are now ready for probe hybridization.

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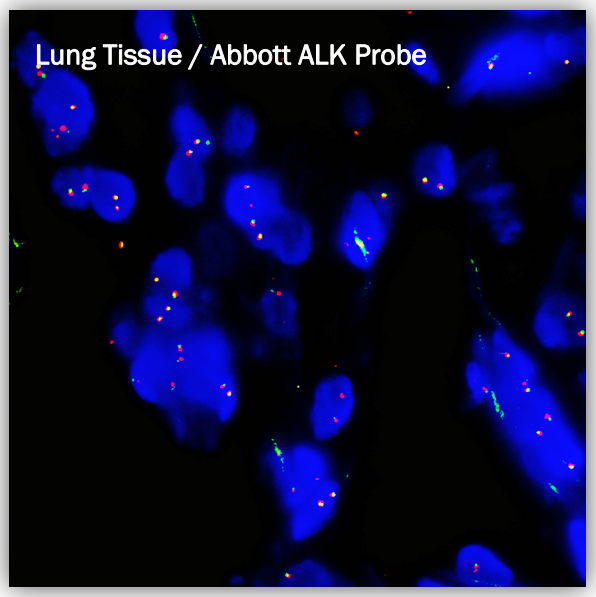

Fig 2. FISH analysis of FFPE tissue processed on the Little Dipper instrument using SciGene CytoZyme Stabilized Pepsin and pretreatment reagents.

SciGene