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G-Banding of Metaphase Chromosomes

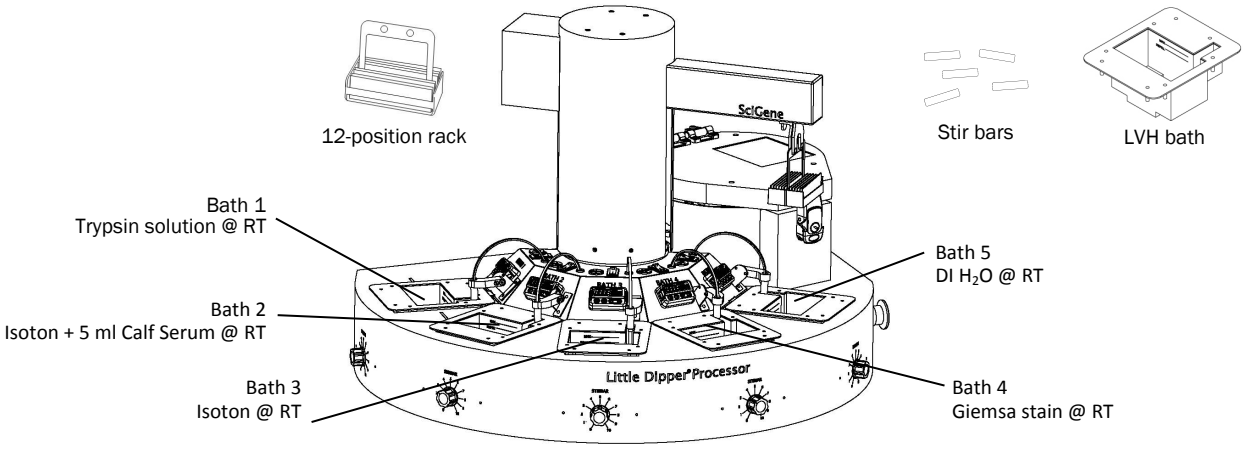

Fig 1. Little Dipper Processor configured for processing 1-12 slides

Equipment Configuration

- **Little Dipper Processor for FISH.**
  SciGene cat. #1080-70-1 (115V) / 1080-70-2 (230V)
- **5x 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

— OR —

- **5x Standard bath, 670 ml with stir bar. (STD)**
  SciGene cat. #1080-10-2

  ⇒ **2x Slide rack, 24 position for 3 inch slides.**
  SciGene cat. #1080-20-1

Reagents Needed

1. **2.5% Trypsin**
   Gibco cat. # 15090046 (or similar)
2. **Isoton / Phosphate Buffered Saline, pH 7.0-7.2**
   ThermoFisher cat. # 8504 (or similar)
3. **Newborn Calf Serum**
   Gibco cat. # 26010066 (or similar)
4. **Giemsa Stain**
   EM Science - Harleco Cat. # 620g/75
5. **Gurr Phosphate Buffer, pH 6.8.** *Dissolve one tablet in 1L DI H_2O*
6. **Deionized Water** — DI H_2O

Table 1. Bath Setup for G-Banding

Bath	Solutions and Reagents	Total Bath Volume	
		LVH	STD
1	Dilute trypsin with Isoton to working concentration.	275	670
2	Add 5 ml Calf Serum and fill with Isoton.	275	670
3	Isoton	275	670
4	Dilute Giemsa in Gurr buffer to working concentration .	275	670
5	DI H_2O	275	670

Instrument Setup

1. Turn on main power to the instrument (right side, at back).
2. Insert clean baths in positions 1-5.
3. Check centrifuge buckets to ensure they are sized to match your slide rack. Insert a balance rack with the same number of slides to be processed.
4. Fill baths per Table 1.

Load Slides / Run Protocol

1. Create the **GBAND** program using the touchscreen (Table 2). Create a unique program for different sample types as needed. Consult the **Little Dipper User Manual** for instructions.
2. Prepare slides for banding following your standard protocol. Select a few test slides for checking trypsin digestion time
3. Place one slide from the batch in the slide rack using either the 12 or 24 position rack depending on instrument set up.
4. Load the rack and run the **GBAND** protocol. At the completion of the run, remove the rack from the centrifuge.
5. Check the quality of banding on the slide. If needed, adjust the trypsin digestion time and run another test slide.
6. Process the remaining slides using the optimum digestion time.

Table 2. GBAND Protocol for the Little Dipper Processor

Step	Bath	Agitation (cpm)	Time (sec)	Pause (sec)	Drip Time (sec)
1	1	0	*per Lab Method*	0	0
2	2	450	10	0	0
3	3	450	10	0	0
4	4	0	*per Lab Method*	0	0
5	5	450	10	0	0
6	Centrifuge	n/a	30	0	0