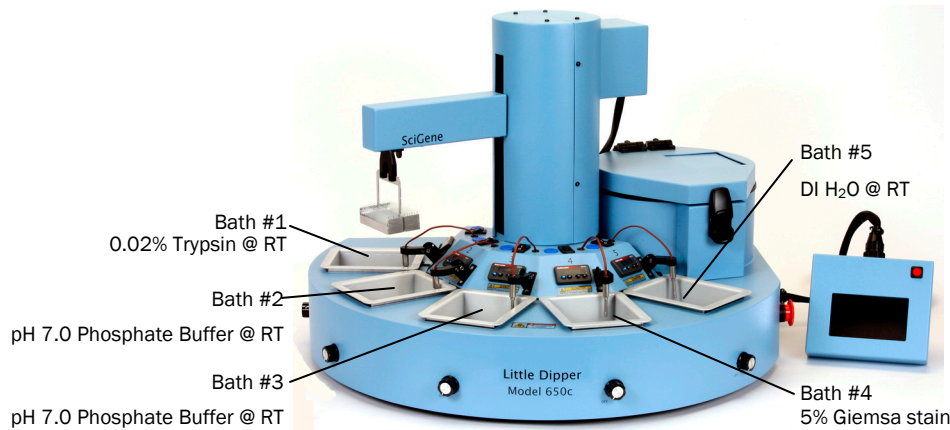


G Banding of Chromosomes on Slides



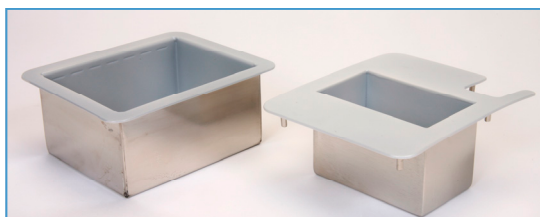
Equipment Configuration

- **Little Dipper® Processor for FISH**, 115v/230v. (SciGene cat. #1080-70-1/1080-70-2)
- **5x Standard volume baths or Low volume baths.** (SciGene cat. #1080-10-1 or 1080-10-2)
- **2x Slide racks, 12 position for 3 inch slides.** (SciGene cat. #1080-20-1)
- **Cytoseal® – 4 oz** (Cole-Parmer cat. #EW-48802-01 or similar)

Buffer and Reagent Preparation

Prepare the following reagents for standard baths according to Table 1 or for low volume baths according to Table 2.

- 5% Giemsa stain**
Mix filtered Giemsa stain (VWR cat. #350862M) with pH 7.0 phosphate buffer (see below). Mix well. Prepare fresh daily.
- 0.02% Trypsin**
Thaw stock 0.25% trypsin (Gibco cat. #15050-065) and mix with pH 7.0 phosphate buffer (see below).
- pH 7.0 Phosphate Buffer**
Dissolve Gurr's buffer pH 7.2 tablets (Biomed Spec cat. #331942F and NaCl into distilled water. Adjust the pH to 7.0 using 1N HCl or 1N NaOH. Store remaining buffer at room temperature for up to one month.
- dI H₂O – 700 ml**



Use this method with either standard or low volume baths.

Table 1. Reagent Preparation for Standard Volume Baths

Reagent	Total Volume	Bath Volume	Ingredients	Amount
5% Giemsa stain	700 ml	670 ml	Giemsa	35.0 ml
			pH 7.0 phosphate buffer	665 ml
0.02% Trypsin	700 ml	670 ml	0.25% trypsin	56 ml
			pH 7.0 phosphate buffer	644 ml
pH 7.0 Phosphate Buffer	2000 ml	670 ml	Gurr's buffer pH 7.2	20 tablets
			NaCl	18.0 g
			dI H ₂ O	2L
			1N HCL -or- 1N NaOH	As needed for pH 7.0
DI H ₂ O	700 ml	670 ml	dI H ₂ O	700 ml

Table 2. Reagent Preparation for Low Volume Baths

Reagent	Total Volume	Bath Volume	Ingredients	Amount
5% Giemsa stain	200 ml	200 ml	Giemsa	10.0 ml
			pH 7.0 phosphate buffer	190 ml
0.02% Trypsin	200 ml	200 ml	0.25% trypsin	16 ml
			pH 7.0 phosphate buffer	184 ml
pH 7.0 Phosphate Buffer	1000 ml	200 ml	Gurr's buffer pH 7.2	10 tablets
			NaCl	9 g
			DI H ₂ O	1L
			1N HCL -or- 1N NaOH	As needed for pH 7.0
DI H ₂ O	200 ml	200 ml	DI H ₂ O	200 ml

Continued on next page...

G Banding of Chromosomes on Slides

Instrument Setup

1. Place baths into positions 1 through 5..
2. Using the touch screen, create a protocol named 'GTGBand' or similar and enter the agitation rates and times for the baths as shown in Table 3. Consult the **Little Dipper User Manual** for details on creating and editing protocols.
3. Fill each bath with reagents as shown in **Table 3**.

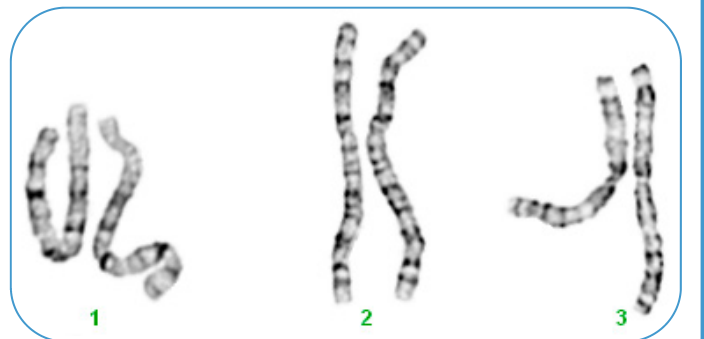
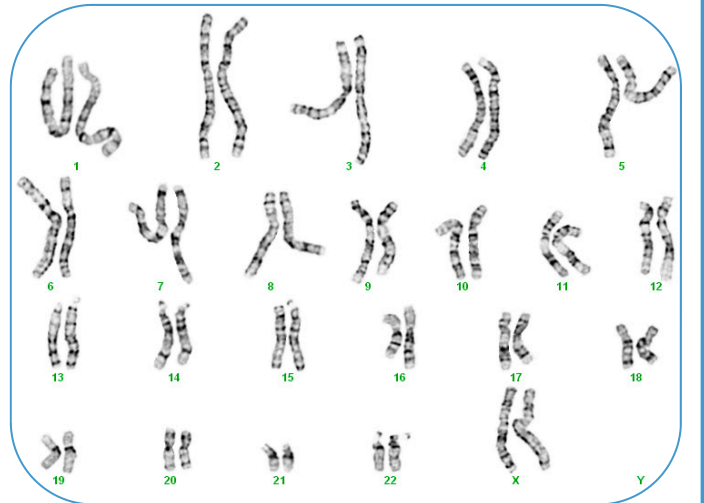
Table 3. Little Dipper Protocol for Chromosome GTG Banding (GTGBand)

Step	Position	Reagent	Bath Volume (ml)		Agitation (cpm)	Time (sec)
			Standard	Low Volume		
1	Bath 1	0.02% Trypsin	670	200	0	90
2	Bath 2	pH 7.0 Phosphate Buffer	670	200	250	15
3	Bath 3	pH 7.0 Phosphate Buffer	670	200	0	60
4	Bath 4	5% Giemsa stain	670	200	0	240
5	Bath 5	DI H ₂ O	670	200	250	60
6	C	—	—	—	—	300

Load Slides / Run Protocol

1. Dry slides containing blood or bone marrow samples prepared according to the **AGT Cytogenetics Laboratory Manual**¹ at 90 °C for one hour before staining.
2. Place slides in a 12 position rack or a 24 position rack (standard volume baths only) for the Little Dipper instrument. It is recommended that a test slide be processed to ensure all solutions are working properly.
3. Start the 'GTGBand' protocol previously programmed (Table 1) and load the rack containing the slides on the gripper. The instrument will process the slides in Bath #1 through 5, dry them in the centrifuge and hold them in the dark.
4. Coverslip the samples with Cytoseal® and visualize.
5. Process additional slides using the same reagents and buffers. Working solutions are good for up to one day.
6. 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.
7. Use a paper towel to dry and clean the centrifuge.

— End Protocol —



*GTG Banding images from slides processed on the Little Dipper® Processor. Compliments of the UMassMemorial Medical Center Cytogenetics Laboratory, Worcester, MA.

SciGene

Automating FISH and CMA Workflows

(1) The AGT Cytogenetics Laboratory Manual, 3rd Ed., Edited by M. Barch, T. Knutsen, J. Spurbeck, 1997, pgs: 77-171