Pub. 153 Rev.J 0220

Automated G-Banding of Metaphase Chromosomes

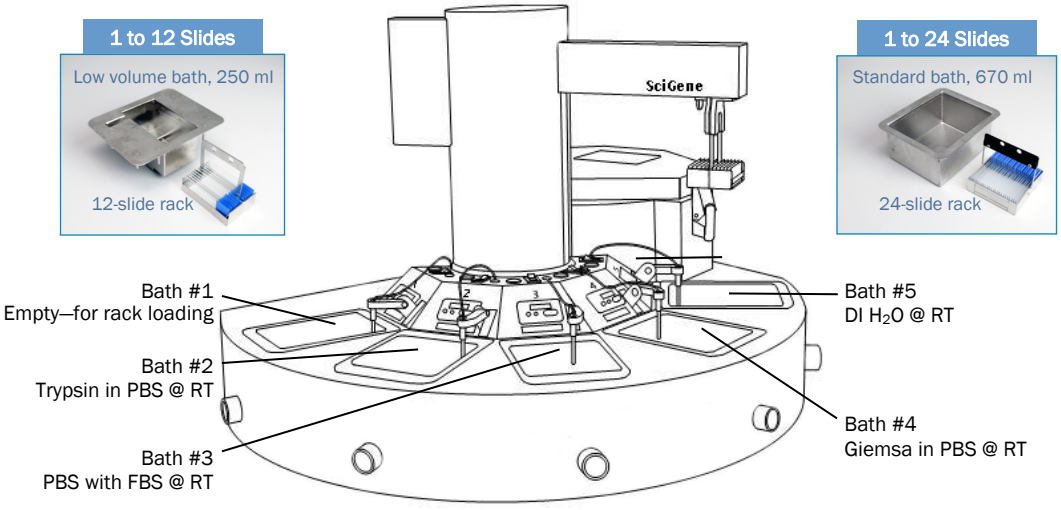

Fig. 1 Little Dipper Processor Setup for G-Banding

Equipment Configuration

Little Dipper Processor for FISH and G-Banding
SciGene cat. #1080-70-1 (115V) / 1080-70-2 (230V)

For 1-24 Slides:

4x Standard bath with stir bars, 670ml (cat. #1080-10-1)
2x 24-position slide racks (cat. # 1080-20-5)

For 1-12 Slides:

4x Low volume baths with stir bars, 250ml (cat. #1080-10-5)
2x 12 position racks (cat. # 1080-20-2)

Stock Reagents

1. **Trypsin** – Gibco 2.5% Trypsin (No phenol red)
   ThermoFisher cat. #15090046
2. **Phosphate Buffered Saline (PBS), pH 7.0-7.2**
   ThermoFisher cat. #8505 or similar
3. **Giemsa** – KaryoMAX® Giemsa Stain Solution
   ThermoFisher cat. #10092013
4. **Fetal Bovine Serum (FBS)** – various suppliers
5. **DI H_2O**

Program the Processor

Create a program for processing metaphase chromosome slides prepared from blood named **GBand-Bld** using the agitate speeds and times shown in Table 1.

Separate programs can be created for different sample types. Consult the *Little Dipper Processor User Manual* for instructions.

Table 1. GBand-Bld Protocol for Little Dipper Processor

Step	Bath	Agitation (cpm)	Time (sec)	Pause (sec)	Drip Time (sec)
1	1	0	5	0	0
2	2	450	15	0	0
3	3	450	30	0	0
4	4	450	300	0	0
5	5	450	30	0	0
6	Centrifuge	–	30	0	0

Instrument Set Up

1. Using lab tape, label the four removable baths and place in positions #2 through 5. Bath #1 remains empty for slide rack loading only.
   - Bath #1: empty
   - Bath #2 Trypsin in PBS
   - Bath #3 PBS with FBS
   - Bath #4 Giemsa in PBS
   - Bath #5 DI H_2O
2. Turn on main power to the instrument. Do not use heaters.
3. Check that the centrifuge has buckets correctly sized for the slide racks:
   - Large for the 24 position slide rack – or –
   - Small for the 12 position slide rack
4. Load a balance slide rack, with the same number of slides to be processed, in the red bucket.

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Reagent Setup for Baths

1. Add reagents in the order shown to standard baths (i.e. PBS in Baths #2,3,4 then trypsin in Bath #2, etc.) in Table 2 or to low volume baths in Table 3.
2. Activate stir bars to achieve a gentle vortex in each bath (speed setting 8-9).

Table 2. Reagent Setup for Standard Baths (1-24 Slides)

Reagent	Bath 1	Bath 2	Bath 3	Bath 4	Bath 5
PBS	—	640 ml	660 ml	615 ml	—
Trypsin 2.5%	—	30 ml	—	—	—
FBS	—	—	10 ml	—	—
Giemsa Concentrate	—	—	—	40 ml	—
DI H_2O	—	—	—	—	670 ml

Table 3. Reagent Setup for Low Volume Baths (1-12 Slides)

Reagent	Bath 1	Bath 2	Bath 3	Bath 4	Bath 5
PBS	—	238 ml	245 ml	230 ml	—
Trypsin 2.5%	—	12 ml	—	—	—
FBS	—	—	5 ml	—	—
Giemsa Concentrate	—	—	—	15 ml	—
DI H_2O	—	—	—	—	250 ml

Run Protocol

1. Place a test slide containing metaphase chromosomes, derived from blood, into the slide rack. Place one slide in the balance slide rack.
2. Load the test rack and run the **GBand-Bld** protocol.
3. After processing, check the quality of banding. Adjust trypsin and/or staining times, if needed, to achieve desired results running additional test slides.
4. After optimum digestion/staining times have been established, process multiple batches of slides. Remember to use the same number of slides in the balance rack.
5. Replace the DI H_2O in the Bath #5 after every 3 batches of slides.

— End Protocol —

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