**G-Banding of Metaphase Chromosomes**

**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 H₂O
6. **Deionized Water — DI H₂O**

**Table 1. Bath Setup for G-Banding**

<table>
<thead>
<tr>
<th>Bath</th>
<th>Solutions and Reagents</th>
<th>Total Bath Volume</th>
<th>LVH</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dilute trypsin with Isoton to working concentration.</td>
<td>275</td>
<td>670</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Add 5 ml Calf Serum and fill with Isoton.</td>
<td>275</td>
<td>670</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Isoton</td>
<td>275</td>
<td>670</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Dilute Giemsa in Gurr buffer to working concentration</td>
<td>275</td>
<td>670</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DI H₂O</td>
<td>275</td>
<td>670</td>
<td></td>
</tr>
</tbody>
</table>

**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**

<table>
<thead>
<tr>
<th>Step</th>
<th>Bath</th>
<th>Agitation (cpm)</th>
<th>Time (sec)</th>
<th>Pause (sec)</th>
<th>Drip Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>per Lab Method</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>450</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>450</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0</td>
<td>per Lab Method</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>450</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Centrifuge</td>
<td>n/a</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>