**Staining Protocol for MCF-10A Frozen Sections**

**Notes:**
- Nuclear fast red provides better staining when complete dehydration is desired
- Toluidine blue can provide better contrast for hydrated sections
- Neither stain will bind Matrigel tightly (compared to eosin, for example)
- Other stains have not been tested but might work well (methylene blue, for example)

1) Fix 8 µm frozen sections in 75% ethanol for 30 sec. Move slides directly from –80°C to ethanol (do not allow slides to warm to room temperature).
2) Transfer to distilled water for 30 sec.
3) Stain with a few drops of either nuclear fast red (Vector Laboratories #H-3403) or toluidine blue (VWR #34172-176; 0.01% (w/v) in PBS dropped to pH 5.5 with HCl) for 1 min.
4) Transfer to distilled water for 1 min.
5) Repeat Step 4.
6) For toluidine blue, stop and visualize.
   - For nuclear fast red, dehydrate:
     a. 75% ethanol for 30 sec.
     b. 95% ethanol for 30 sec.
     c. 100% ethanol for 30 sec.
7) For nuclear fast red, remove ethanol with a xylene dip for 1 min.
8) For nuclear fast red, air dry 5–10 min.