

## BDA lineage tracing on sections - Hiroki Kuroda

Kuroda et al., PLoS 2, 0623-0634 (2004)

BDA: Biotin Dextran Amine

One of the strongest way for lineage tracing on sections is to use biotin dextran amine. For example, fluoresceine or Texas-Red Dextran amines are used by many people. However, as you know, fluoresceine is temporary. lacZ staining is very good for whole embryos but not so nice on section. This method using Biotin dextran amine can very easily provide you with permanent and very strong staining on sections.

### (Preparation of Sample)

**Note. Total 16 nl of 1% BDA per embryo is the maximum.**  
e.g. If you inject 2 nl of 0.5% BDA into one animal blastomere at 8-cell stage, you can get very strong staining later.

### (Sectioning)

**Time courses are different in sample types and stages.**

**(Time1/Time2/Time3/Time4) means (stages less than 20/stages 20-30/stages greater than 30/explants)**

- Fix sample in MEMFA at room temperature (5h/2h/2h/30m)
- 70% EtOH (3h/1h/1h/10m) Note. You can store sample in 70% EtOH forever at 4°C
- 100% EtOH (2h/30m/30m/10m)
- 100% EtOH (2h/1h/30m/10m)
- Isopropanol (2h/1h/30m/10m)
- Xylene (overnight/5h/30m/10m)
- Xylene (1h/1h/15m/5m)
- Paraffin at 60 °C (overnight/2h/30m/10m)
- Paraffin at 60 °C (2h/1h/15m/5m)
- Embedding Note. You can keep sample in paraffin forever.
- Section at 10-20 µm thickness

### (Staining)

For sections

- Xylene for 5 min
- Xylene for 5 min
- 100% EtOH for 1 min
- 100% EtOH for 1 min
- 70% EtOH for 1 min
- (Wash with 1 x wash buffer for 5 min) x 2 (in a plastic slide mailer Coplin jar).
- Treat with Streptavidin-AP solution for 3h at room temperature (in a Coplin jar).
- (Wash with 1x Wash Buffer for 5 min) x 2 (in a Coplin jar).
- Wash with AP buffer for 5 min (in a Coplin jar).
- Treat with 10-fold diluted BM purple solution with AP buffer (in a plastic Coplin jar).
- Incubate until appearance of signal at 4°C in the dark. Note: Usually it takes overnight.



### Solutions:

**Dextran amine BDA:** biotin-dextran amine  
Molecular Probes #D1956

**FDA:** Fluorescein-dextran amine  
Molecular Probes #D1820

**TDA:** Texas red-dextran amine  
Molecular Probes #D1863

**10 x Wash Buffer:** 1M Tris-HCl, 1.5M NaCl, pH 7.5.

**AP buffer:** 50 ml of Tris solution (1M Tris-HCl, 1M NaCl, pH 9.5), 50 ml of 0.5M MgCl<sub>2</sub> solution, adjust volume to 500 ml with DDW.

**Streptavidin-AP solution:** 15 µl of Streptavidin-AP with 300 ml of 1x wash buffer. Store at 4°C for less than 2 weeks or until used 5 times.

**Streptavidin-AP (Roche:** #1089161: 1000U, 1 ml)  
**BM purple (Roche)**