

## **X-GAL Staining for Whole Mounts**

This protocol was adapted from Mombaerts et. Al., 1996

### **Buffer A:**

100 mM phosphate buffer (pH 7.4)  
2 mM MgCl<sub>2</sub>  
5 mM EGTA

### **Buffer B:**

100 mM phosphate buffer (pH 7.4)  
2 mM MgCl<sub>2</sub>  
0.01% sodium desoxycholate  
0.02% Nonidet P40

### **Buffer C:**

Buffer B  
5 mM potassium-ferricyanide  
5 mM potassium-ferrocyanide  
1 mg/mL x-Gal

- Dissect tissue
- Fix on ice for 30 minutes with:
  - 100 mM phosphate buffer (pH 7.4)
  - 4% paraformaldehyde
  - 2 mM MgSO<sub>4</sub>
- Wash with buffer A for 5 minutes at room temperature
- Wash with buffer A for 30 minutes at room temperature
- Wash with buffer B 2 times for 5 minutes each at room temperature
- Expose tissue in dark with Buffer C at 37°C