

## Histology Protocol:

### Fix:

1 mL 0.2 M cacodylic acid  
400 uL water  
400 uL 10% ultrapure formaldehyde  
200 uL 25% gluteraldehyde

### Polybed:

9.6 mL of Polybed  
6.2 mL of DDSA  
4.2 mL of NMA (mix well before adding phenol)  
400 uL of T-phenol

Fix fry overnight (O/N) at 4°C (no longer than O/N). If necessary, place fry in 5% sucrose/PBS after one night of fixation until washes.

<u>Washes:</u>	<u>Solution</u>	<u># of washes</u>	<u>Time</u>
1.	0.2M cacodylic acid	3 x	5 minutes each
2.	water	3 x	5 minutes each
3.	50 % EtOH	2 x	5 minutes each
4.	70 % EtOH	2 x	5 minutes each
5.	80 % EtOH	2 x	5 minutes each
6.	95% EtOH	2 x	5 minutes each
7.	100 % EtOH	2 x	10 minutes each
8.	Xylene / EtOH (1:1)	1 x	30 minutes
9.	Xylene	1 x	30 minutes
10.	Polybed / Xylene (1:1)	1 x	60 minutes
11.	Xylene / Polybed (1:2)		O/N
<b>Total Time:</b>			<b>3.5 hours</b>

The next day, place fry in mold and add fresh polybed (100%) for 60 minutes. Transfer mold to 37°C O/N.

The next day, check orientation of larva (you can use a needle and microscope to position larva within mold) and transfer to 42°C for 8 hrs. After 8 hrs, check orientation once more, and transfer mold to 60°C for 48-72 hrs.