

Friedemann's Parhyale fixation protocol 1 (12 AUG 09):			
		SOLUTION	INTENTION
1	collect embryos in FASW		
2	drop embryos (with pasteur pipette) into 65°C preheated 3.7% Formaldehyde/PBS	<i>100µl 10xPBS(high salt), 100µl 37% Formaldehyde (MeOH stabilized), 800µl DEPC H2O</i>	heating facilitates the later removal of the chorion
2.2	incubate 2 minutes @ 65°C		fixes embryos to stay in shape during dissection
3	drop embryos into 4°C 3.7% Form./PBS kept on ice.		
3.2	>10' on ice, until all embryos are dissected		embryos get fixed slowly so embryonic cuticle stays soft enough to be removed easily from S14-S19 'bros
4	drop/wash batches of embryos in 1xPBS(high salt) aliquots kept on ice	<i>100µl 10xPBS(high salt), 900µl DEPC H2O</i>	no need to inhale formaldehyde, but temperature and short stay in PBS keeps possible exposure to active RNAses low
5	dissect batch by batch with tungsten needles on sylgard plate		
6	drop into 7.4% Formaldehyde/PBS on ice until all embryos are dissected	<i>100µl 10xPBS(high salt), 200µl 37% Formaldehyde (MeOH stabilized), 700µl DEPC H2O</i>	kept on ice to prevent having large differences in strength of fixation, probably unnecessary
6.2	2 h @ 37°C		works fine, different fixation time ore temp. might be better adapted from other protocols
7	wash in 1xPTX(high salt), a couple of minutes	1x PBS(high salt)(made with DEPC dH2O), 0.2% Triton X-100	
8	50%MeOH/PBS		
9	75%MeOH/PBS		
10	90%MeOH/ddH2O		
11	100%MeOH		
12	100%MeOH		
13	100%MeOH		
13.2	label tube and store @ -20°C		