BRH JT生命誌研究館
JT Biohistory Research Hall

Fixation of Parasteatoda embryos for conventional whole-mount in situ hybridization (WISH)

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https://www.brh2.jp

1) Put eggs in a dry glass-made dish.
2) Add 1-2ml of 100% bleach and swirl eggs using a Pasteur pipet. Be careful not disrupting eggs. During this step, which we usually take for about 3 min, the surface of each egg should become transparent.
3) Add 1-2 ml of water and swirl eggs to let them sink down.
4) Remove the solution and wash the eggs with water several times.
5) Remove the water from the dish.
6) Add a part of the fixative and heptane, which should be prepared in a glass vial in advance. Eggs must be settled at the interface of the two phases.
7) Transfer the eggs to the glass vial containing 3ml of fixative and 3ml of heptane.
8) Shake the vial at 150 rpm at RT for 2 h or more.
9) Optional: continue to shake it at 4°C overnight (or for several hours).
10) Remove the fixative thoroughly.
11) Add 3ml of PEM and transfer the eggs to a glass-made dish.
12) Remove the PEM as much as possible and add PEM+0.1%tween 20 (PEMt).
13) Dissect out embryos using forceps.
14) Transfer the embryos to a 1.5ml Eppen tube.
15) Incubate the embryos in a graded series of MetOH/PEMt solutions (25% , 50%, 75%, and 95%) and finally in 100% MetOH.
16) Change the solution to 100% EtOH.
17) Store the embryos at -20°C until use.

Alternative method:

13) Incubate the eggs in a graded series of MetOH/PEM solutions (25%, 50%, 75%, and 95%) and finally in 100% MetOH for 10-30 min each.
14) Dissect embryos in MetOH using forceps. [The eggs can be stored at -20°C before dissection.]
15) Transfer the embryos to a 1.5ml Eppen tube.
16) Change the solution to 100% EtOH.
17) Store the embryos at -20°C until use.

2x PEM stock:		Fixative:		
200mM PIPES		2x PEM	1.5mL	
2mM EDTA		Water	1.05mL	
2mM $MgSO_4$		37% Formaldehyde	0.45mL	
pH 6.9		Total	3.0mL	