ÖZPOLAT LAB PROTOCOL

fixing juvenile platynereis dumerilii

Caution:

* PFA and methanol should be used under the hood.
* For the 3 washes following PFA, liquid waste from changing over solutions should go into the PFA waste container. After 3 washes, PFA is considered effectively absent.

Collection:

* Set worms aside for 2-3 days without food to minimize gut content.
* Ensure that the worms have enough space that they don’t fight each other. This could cause damage to tissues of interest, or change important biological processes.
* One worm per well is the most cautious option. Following our culturing norms, up to 5 worms can be kept in each tiny box, 10-15 in each mini box, and up to 30 in each low density box.
* Keep on the shelves for normal culture conditions (besides feeding).

Anesthetize & manipulate:

* Anesthetize worms in 1:1 0.22um FNSW: 7% MgCl2 for ~15 minutes.
* Do any amputations, manipulations, or segment counts.
* Cut off anterior and posterior cirri to minimize downstream tangling.
* Return them to 0.22um FNSW.

Fixation:

* The final proportions of the fixation solution will be ¼ 16% PFA, ½ 2X PBSt, and ¼ FNSW. To start, put the worms in ¼ volume FNSW with MgCl2 and anesthetize for ~15 minutes.
* Prepare the other components of the fixation solution: ¼ 16% PFA for a working concentration of 4%, and ½ 2X PBSt for a working concentration of 1X.
* Once the worms are fully anesthetized, switch out the MgCl2 FNSW for ¼ FNSW. It’s important to minimize MgCl2, since it may increase background in downstream applications.
* Add in the remaining fixation solution.
* Gently rotate (if fixing in tubes) or shake (if fixing in plates) for 2hrs at room temperature, or overnight at 4°C.

Dehydration:

* If fixing samples for in situ hybridization, HCR, or anything RNA-related, do all post-fixation steps on ice to preserve RNA.
* Wash with 1X PBSt once and rotate/shake for 5 minutes.
* Wash with 25% MeOH, 75% PBSt and rotate/shake for 15 minutes.
* Wash with 50% MeOH, 50% PBSt and rotate/shake for 15 minutes.
* Wash with 75% MeOH, 25% PBSt and rotate/shake for 15 minutes.
* Wash with 100% MeOH and rotate/shake for 15-60 minutes.
* Change over with fresh 100% MeOH, seal with parafilm, and store at -20°C.

Fixation solution volumes and sample sizes for different containers:

* Overall, the minimum recommended solution volume should be 10X the sample volume.
* 1.5mL tubes: 800uL, suited to batches of embryos or single juveniles.
* 15mL falcon tubes: 6-8mL, suited to ~5-10 juveniles. Store horizontally in foam tube rack to reduce packing/tangling of worms.
* 50mL falcon tubes: 20-30mL, suited to ~10-20 juveniles. Store horizontally in foam tube rack to reduce packing/tangling of worms.
* 6 well plates: 4-5mL, suited to up to 5 large juveniles per well. 4 seems optimal.
* 12 well plates: 2-3mL, suited to up to 3 juveniles per well. 2 seems optimal.
* 24 well plates: 1mL, suited to individual tails or worms.

Alternatives and adjustments for segment counts:

* Note for segment counts: to avoid anesthetizing & manipulating worms twice, once to acquire worms of a specific size and once to cut off cirri, you can set aside a large number of worms that are likely to have enough of the size you need, and count & cut after the starvation period. Return remaining unused worms to normal culture conditions.
  + Alternatively, you *could* count, cut, and fix in serial batches of ~5-10 worms, since the worms have to anesthetize in MgCl2 for 15 minutes to start out.