Fixation of embryos for *Crepidula fornicata*

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To fix embryos for in situ hybridization

1) Transfer embryos with seawater solution into a 2mL-tube and remove seawater.

- If drug-treated, add 100% FSW and remove it again.
- Make sure to remove as much seawater as possible *without exposing the embryos—they will burst*.

2) Fix embryos in 4% PFA/FSW

- Under hood:
    - With 16% PFA on ice, make 4% solution by combining 10mL 16% PFA with 30mL 100% FSW. *Add 0.38g of Instant Ocean.*
        - Keep in fridge for use in about 1 week, or store in the freezer.

Option 1: 1 hour in 4% PFA/FSW at room temperature

- Transfer 4% PFA to embryo tube with enough space left at the top to comfortably close the tube.
- Spin tube to mix and leave tube on its side.
- Incubate for 60 minutes at room temperature.
    - Flip over about every 15 minutes.
    - Stand tube up with 10 minutes to go.

Option 2: overnight in 4% PFA/FSW at 4°C*

- Transfer 4% PFA to embryo tube with enough space left at the top to comfortably close the tube.
- Put samples in fridge immediately.

3) Wash 3 times, 5 min with 1X PBS

- First wash takes place under hood:
    - Remove solution. Add PBS to tube with enough space left at the top to comfortably close the tube.
    - Put on shaker for 5 minutes, 300 rpm, 23°C
- Repeat two times, not under hood.
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* These washes are the time at which the embryos are vulnerable to RNAses.

4) Wash 2 times, briefly, with 100% ice cold methanol; then add methanol again and store at -80°C.

*Option 2 may be a better fix for younger embryos (pre-gastrulation)