Immunofluorescence of *N. furzeri* embryos

1. Fix embryos with 4% PFA in PBS for 1-2 overnights at RT. The yolk might still be liquid-ish after 1 overnight fixation at RT. (Preparing 4% PFA in ddH2O would swell the embryos and narrow the perivitelline space significantly. This could cause difficulties in the following steps and also compromise the antibody accessibility to embryos)

2. Break the chorion at the oil-drop area, and then carefully remove all chorion, but leave YSL intact as much as possible. It is possible to keep the whole YSL intact. DO NOT de-yolk. The round yolk provides good support to the embryo, and also makes embryos easier to handle. The embryos can be imaged as early as at the cleavage stages. If needed, the yolk can be removed at the end of staining.

3. Put embryos in 48-well or 96-well plates. Embryos in each well can be well covered by a minimal 100 ul or 50 ul solution, respectively. Use PBST (PBS + 0.1% tx100) and AbDil (PBST + 3% BSA).

[Each of the following steps can be from hours to overnight]
4. Wash embryos with PBST (or PBST with 0.5% tx100 concentration at this step for better permeabilization), ideally overnight.

5. Incubate embryos in AbDil, 6 hrs to overnight.

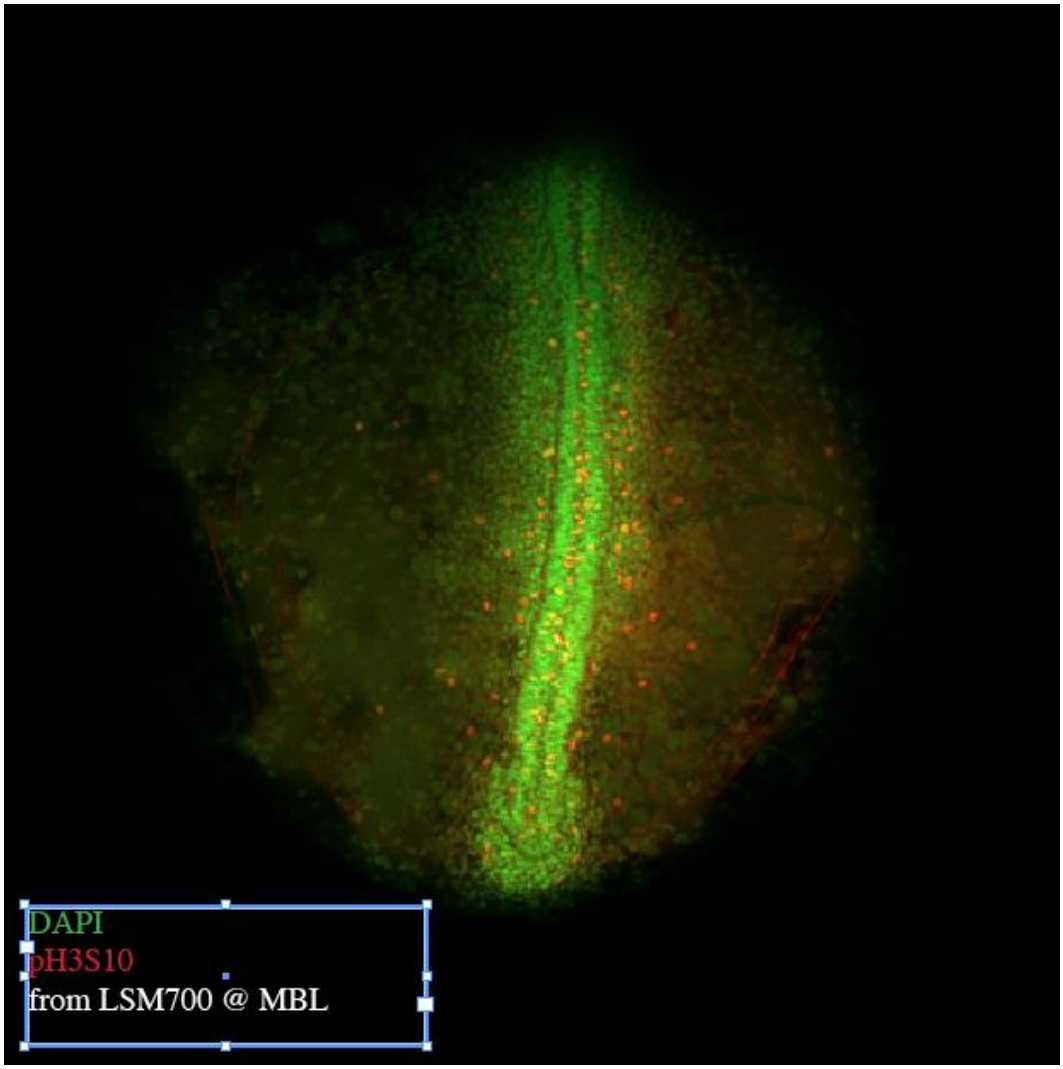
6. Incubate embryos in primary antibodies in AbDil, 6 hrs to overnight, and wash

7. Incubate embryos in secondary antibodies, 6 hrs to overnight, and wash

8. (Optional) Incubate embryos in PBST with 25% and then 50% glycerol

9a. Transfer embryos to Matek glass-bottom plate, either in the same 50% glycerol solution or in 2% or 6% methylcellulose, which allows manually adjusting embryo orientations. Embryos should face toward the glass. Cover embryos in the Matek well with an 18x18mm coverslip to keep embryos in specific orientations.

9b. Transfer embryos to glass slides and manually adjust embryo orientations to face up and away from the slide bottoms. Then add coverslips on top with pressure to flatten embryos for imaging. Embryos might occasionally break into 2 or 3 fragments at junctions between head, trunk, and tail.



Note:

- For embryo transfers, a broader opening pipet should be used (Fisher # 13-678-30); for buffer exchanges, a narrower opening pipet should be used (Fisher #13-678-20). The latter can prevent embryos from accidentally being sucked into pipets and make buffer exchanges easier and faster.
- If the bridging method is used for the sample setup, two #1.5 coverslips will be ideal as bridges.

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