WHOLE CELL IMMUNOFLUORESCENCE

I. 2% Formaldehyde preparation

1. Combine in a glass flask

    - Formaldehyde powder 0.4 g
    - Millipore water 18 mL
    - 1 N NaOH 50 µL

2. Cover, heat (low heat), and stir on the hot plate in the fume hood until the solution becomes clear. Make sure that it does not boil.

3. Allow to cool. Add 2 mL of 10X PBS.

4. Adjust the pH to ~7. This should require ~5 µL of 6N HCl

5. Filter and add 0.1% of Triton X-100.

Note: Can be stored at 4°C for up to two weeks or at -20°C for longer periods of time.

II. Fixation

1. Add 500 µL of fresh 2% Formaldehyde in a concave 9-well glass plate. Using a mouth-pipette, slowly transfer the oocytes or embryos and allow the cells to fix for a minimum of 15 minutes. Incubate at RT for at least 15 min (30 min max).

2. Add 500 µL of 3% blocking buffer (PBS, 3% BSA, 0.1% Triton X-100) in an adjacent well and transfer oocytes to that well.

Note: Fixed oocytes can be stored at 4°C for 2 weeks in blocking buffer.

III. Staining

1. Prepare primary antibody solution by diluting the antibody in blocking buffer (stored at -20°C in 50% glycerol). A typical dilution is 1:100, but different antibodies may require less dilution or greater dilution to generate specific staining.

2. Add 16 µL of primary antibody solution to a 72-well plate. Transfer the cells to the primary antibody solution. Cover the plate and put it in a humid chamber (e.g. pipette tip box with a wet paper towel). Incubate over night at 4°C on a shaker.

3. Add 16 µL of blocking buffer to two adjacent wells. Transfer oocytes for a 5 min wash in each well on a shaker.

4. Prepare 100 µL of the secondary antibody by diluting the secondary antibody raised against IgG of the host of the primary antibody in blocking buffer (typically 1:100). Add reagents to stain DNA (DAPI 1:100 and DRAQ5 1:1000) or the actin cytoskeleton (Phalloidin1:100).

5. Transfer 16 µL of the secondary antibody solution to a fourth well in the 72-well plate. Cover the plate and put it in a humid chamber. Incubate the cells for 1 hour in secondary antibody at room temperature with gentle agitation.

6. Add 16 µL of blocking buffer to two adjacent wells. Transfer oocytes for a 5 min wash in each well on a shaker.

III. Mounting

1. Stick an adhesive spacer to a slide. Add 1µL of 1xPBS onto the slide in the middle of the circle delineated by the spacer. Add 20 µL of mineral oil on top of the PBS drop.

Note: Make sure the oil completely covers the drop to avoid evaporation and that it spreads throughout the space delineated by the spacer to avoid the drop moving towards the edge.

2. Transfer oocytes into the drop of PBS.

3. Swiftly cover the slide with a cover slip to avoid displacing the drop of PBS.

Note: When looking at the slide under the microscope turn the slide upside down. The cover slip is less thick than the slide and makes it easier to focus on oocytes.