Adapted from Annette Chan
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Fixing and Staining Worms on a Slide

1. Use 1%, 1.5%, and 2% PFA with a 5-minute fix. Determine empirically which one works best for your antibody.
   a. Place worms in 5µl 1X sperm salts on a positively-charged glass slide
   b. Using a needle (Precision Glide ® Needle, Becton Dickinson & Co., 22 G 1 ½), make an incision between the pharynx and gonad (closer to the gonad than the pharynx) or between the tip of the tail and the gonad of each worm. To release the embryos, cut at the vulva.
   c. Add 5 □l of 2%, 3%, or 4% PFA (Electron Microscopy Sciences, cat. #15710) in 1X sperm salts, mix a little.

      To prepare 2% PFA:
      125 µl 16% PFA
      200 µl 5X sperm salts
      675 µl water

      To prepare 3% PFA:
      187.5 µl 16% PFA
      200 µl 5X sperm salts
      612.5 µl water

      To prepare 4% PFA:
      200 µl 16% PFA
      200 µl 5X sperm salts
      550 µl water

   d. Place the slide in a humid chamber for 5 minutes without coverslip. Place an 18-mm x 18-mm coverslip on top of the worms. Freeze the slide on a piece of dry ice. Let the slide sit on the dry ice for at least 10 minutes.

2. Remove the slide from the dry ice and quickly pop off the coverslip with a quick downward stroke of a single razor.

3. Optional: Immediately place the slide in 95% ethanol for 1 minutes. Be careful or the worms will fall off. (Ethanol coagulates proteins – can do without this step.)

4. Incubate the slide in a vessel containing PBST for at least 10 minutes (Longer washes can decrease background but reverse fix). Repeat this step twice.

5. Wick off most of the PBST, but do not allow the worms to dry. Add 30 µl primary antibody diluted in PBST. Carefully cover the worms with a 20 x 20-mm piece of parafilm. Place the slide in a humid chamber. Incubate overnight at room temperature (or for a few hours at 37°C).

6. Incubate the slide in a vessel containing PBST for at least ten minutes. Carefully remove the piece of parafilm.

7. Incubate the slide in a vessel containing fresh PBST for at least 10 minutes. Repeat this step once.

8. Wick off most of the PBST, but do not allow the worms to dry. Add 25 □l secondary antibody diluted in PBST. Carefully cover the worms with a 20 x 20-mm piece of parafilm. Place the slide in a humid chamber. Incubate for 4 hours at room temperature (30 min to 1 hr at 37°C). Keep the slides in the dark whenever possible.

9. Incubate the slide in a vessel containing PBST for at least 10 minutes. Carefully remove the piece of parafilm.

10. Incubate the slide in a vessel containing fresh PBST for at least ten minutes. Repeat this step with fresh PBST to which you add 1 µl of 1 mg/mL DAPI.

11. Wick off most of the PBST, but do not allow the worms to dry.

12. Mount the slide with 20 µl Vectashield or other mounting medium and a 22 x 40-mm cover glass. Seal the slide with fingernail polish. Alternatively, use a 22 X 50 mm coverglass and do not seal the slides.

13. Store the slides at -20°C. Keep the slides dark whenever possible.

1X Sperm Salts:
50 mM PIPES, pH7.0
25 mM KCl
1 mM $MgSO_4$
45 mM NaCl
2 mM $CaCl_2$

5X:
250 mM PIPES pH 7.0
125 mM KCl
5 mM $MgSO_4$
225 mM NaCl
10 mM $CaCl_2$

PBST:
100 ml 10X PBS
5 ml Triton X-100
2 ml 0.5 M EDTA, pH 8
ddH_2O to 1L

DABCO (Mounting medium):

5 g DABCO
5 ml PBS
45 ml glycerin