Fly whole mount immunostaining

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Caging and feeding of flies

- Cages consist of 100ml plastic cups with holes poked at the bottom and capped with a 60x15mm grape-agar plate
- To feed and promote egg laying use a mixture of bakers yeast and water that has the consistency of a paste
- Place a small dot of food on grape-agar plate
- Add flies

Preparation for embryo collection

- Use 20ml disposable glass scintillation vials to fix and collect embryos
- Add to each vial: 9ml PEM, 10ml Heptane, 1ml 37% Formaldehyde (50% Heptane/50% PEMFA= PEM and 4% Formaldehyde)
- Shake each vial to mix and allow phases to separate
- Wash collecting sieves (Costar, 74µm polyester mesh) and paintbrush with soap and set aside for embryo collection.

Collect, dechorionate and fix embryos

- Carefully add water to plate and using the paintbrush gently dislodge embryos from the grape plate and food
- Pour the water and embryos through the sieve and repeat until all embryos are off plate
- To dechorionate, place each sieve in the lid of agar plate, add 7-8 ml of 50% bleach to the bottom of the plate and gently pipette 1ml into the sieve
- Allow to sit for 1 min, add 1ml to sieve again and let sit for one more minute
- Pour out bleach and gently rinse sieve with distilled water
- Allow embryos, sieve and brush to dry completely before moving on to the fixation step
- In a fume hood, transfer embryos from the sieves to the scintillation vials using the brush
- Allow embryos to collect at the interface between the liquids
- Rock vials gently for 10-20min
- Using a 1ml pipette, carefully remove lower PEMFA layer avoiding embryos
- Add an equal amount of methanol to the remaining Heptane solution and shake vigorously for 15 seconds
- Allow embryos to settle to the bottom. Dechorionated and fixed embryos will sink to the bottom while others will remain at the interface
- Aspirate top layer including embryos that did not settle
- Wash 3x with 5-6 ml 100 % methanol
- Transfer embryos in methanol to eppendorf tubes using a pipette tip whose very tip has been cut off
- Store at -20°C for at least 1 hour up to several months

Rehydrate and permeabilize embryos for staining

- Rehydrate and permeabilize embryos stepwise as follows:
  - Wash 5 minutes with 75% methanol and PBST
  - Wash 5 minutes with 50% methanol and PBST
  - Wash 5 minutes with 25% methanol and PBST
  - Wash 1hour 30 minutes with PBST (changing PBST every 20 minutes)
- Optional SDS treatment to make antigen more accessible: incubate embryos for 1 min in 0.5% SDS and rinse in PBS/0.2% Triton X-100 for 5 min
- Remove PBST and block with PAXD by rocking for 1 hour at RT
- Remove PAXD and add primary antibody in PAXD and incubate by rocking O/N at 4°C (concentration will vary for each antibody)
- Wash embryos 3x for 20 minutes in PBST while rocking
- Add secondary antibody in PAXD and incubate for 1 hour at RT while rocking
- Wash embryos 3x in PBST for 20 minutes each
- Add two drops of DAPI-containing Vectashield (Vector laboratories) to each tube and wait for ~30 minutes
- Transfer the embryos to a glass slide using a pipette tip with the very end cut off
- Place broken glass or a spacer at each end of the slide to prevent flattening of the embryo and coverslip

Solutions

PBST

1X phosphate buffered saline (PBS)
0.2% Triton X-100

PEM

100 mM PIPES Disodium Salt
2.0 mM EGTA
1.0 mM $MgSO_4$
pH to 6.9 using KOH
Filter sterilize

PAXD

20% goat serum
5% BSA
Make in PBST