EC-tagging Fly Delivery Methods

ADULTS

Reagents:
Sucrose Solution (100 mM Tris pH 7.5, 10% sucrose, make fresh)

5-ethynylcytosine (EC) and 5-ethynyluridine (EUd)
- Make 200 mM stock solutions in DMSO
- Light Sensitive (keep covered when possible)
- Aliquot into small tubes that can be individually thawed just before use

Trizol

25 gauge needles and 3cc syringes

Protocol
1. starve flies over night (16 hours) in clean empty food bottle (don't forget to make air holes)

2. before the next morning prepare cotton-stuffed bottles
    - unfold cotton ball, and tear into 3 strips
    - place the strips one on top of the other, use a spatula to force cotton tightly into bottom
    - make holes in the top of the bottle to allow air flow

3. Add sucrose solution with EC or EUd just prior to feeding
a. Dilute sucrose solution 1:10 (our food bottles require 15 ml)
b. add EC or EUd at concentration of 1.0 mM
c. pipet entire 15 mls containing EC or EUd onto cotton
d.. using spatula, press wet cotton against sides, make sure there are no air pockets

4. flip adults from "starving" bottle into bottle containing soaked cotton. Feed at 25C or 30C.

5. after ~ 4 hrs, the cotton will require more sucrose media. Typically feed for 8 hours.

6. flip adults into empty food bottle and homogenize in Trizol
a. 15 adults per tube works best
b. add 100 ul of Trizol and homogenize with tissue grinder
c. add 900 ul of Trizol to bring volume up to 1 ml
d. pass the solution 3x through a 25 gauge needle attached to a 3cc syringe. CAREFUL! Wear eye protection / work in a hood.
e. Centrifuge at max speed in a microcentrifuge for 2 minutes to pellet insoluble material. Transfer the soluble Trizol solution to a new 1.5 ml tube.

f. store at –80C until ready to proceed with RNA extraction

LARVAE

Reagents:

Yeast (-) media "mocha caps"
- 20 ml dH20, 0.4 g sucrose, 0.18 g agar, 1 g dextrose, and 0.5 g brewer's yeast
- Mix and heat to briefly boil in microwave
- Allow to cool to 55 C before pouring caps and/or when adding EC or EUd

5-ethynylcytosine (EC) and 5-ethynyluridine (EUd)
- Make 200 mM stock solutions in DMSO
- Light Sensitive (keep covered when possible)
- Aliquot into small tubes that can be individually thawed just before use

Trizol

Protocol
1. perform standard egg collection, rinse eggs onto a filter

2. dechorionate in 50% bleach for 2 min, rinse with dH20 and blot dry

3. using a paintbrush, gently transfer eggs onto mocha caps, wait for larvae to hatch onto cap, transfer larvae to fresh cap each day until desired stage is reached

4. on the day of EC tagging, make fresh mocha cap containing 0.5 mM EC, pour media into top (lid) & base. **Fold tape over the edge of the base before placing lid so that a small opening forms between the lid and base (otherwise the lid may seal and suffocate the larvae)

5. allow larvae to feed for desired time at 25C or 30C

6. Transfer larvae to 1.5 ml tube for tissue homogenization, add larvae to about the 50 ul mark
a. add 50 ul of Trizol and homogenize
b. add 950 ul of Trizol to bring volume up to 1 ml
c. pass the solution 3x through a 25 gauge needle attached to a 3cc syringe. CAREFUL! Wear eye protection / work in a hood.
d. Centrifuge at max speed in a microcentrifuge for 2 minutes to pellet insoluble material. Transfer the soluble Trizol solution to a new 1.5 ml tube.

e. store at –80C until ready to proceed with RNA extraction.

EMBRYOS

Reagents:

Schneiders media - Sigma S0146

5-ethynylcytosine (EC) and 5-ethynyluridine (EUd)
- Make 200 mM stock solutions in DMSO
- Light Sensitive (keep covered when possible)
- Aliquot into small tubes that can be individually thawed just before use

PBS (phosphate-buffered saline, 1X)

Trizol

Protocol
1. transfer eggs onto a mesh filter secured in a "mesh basket" (flystuff.com)

2. dechorionate in 50% bleach for 3 min, rinse with dH20 and blot dry / air dry so no water remains

3. move basket into small weighing boat and/or clean small petri dish

4. pour FRESH octane (don't use old bottle, minimize octane exposure to air) over embryos, leave for 3 min.

5. remove basket from the octane treatment; unscrew the cap to remove the mesh and blot dry on Kimwipe then leave on paper towel to air dry for 3 min.

6. carefully secure mesh filter back into basket (careful not to squish embryos) and place into fresh weighing boat and/or clean small petri dish

7. pour Schneiders media with 1 mM EC or EUd over embryos, enough to fill weigh boat / dish and bottom of basket. Ideally embryos will float at top of the media.

8. label for desired time (typically 2 – 4 hours) at 25C or 30C

9. rinse embryos with dH20, remove mesh from basket and blot dry briefly

10. using a paintbrush, transfer embryos from the mesh into a 1.5 ml tube containing 1.0 ml of PBS (fold and place mesh into the PBS then use brush to release embryos from the mesh)
a. Centrifuge at max speed for 1 minute in a microcentrifuge to pellet the embryos. Remove all of the PBS by pipetting.
b. add 50 ul of Trizol, homogenize with tissue grinder
b. add 950 ul of Trizol to bring volume up to 1 ml
    - no need to pass embryo samples through needle or centrifuge out insoluble material

c. store at –80C until ready to proceed with RNA extraction