Protocol for fly ovary FLP-out clones. Edwards Lab Oct 2013

1. Virgin the "*HACG*" stock (see below).

2. Cross ~4 *HACG* virgin females to ~4 males of the *UAS* responder line (*Hto* insert, *UAS* construct, etc). This is Brood 1.

3. Place in Brood 1 in 25°C incubator.

4. After a few days, when you see larval activity, transfer the adults only to a new vial, "Brood 2". Place Brood 2 in 18°C as a backup.

5. In ~10 days, look for eclosing flies in Brood 1. They need not be virgins, but they should not be left on the old food more than a day or two at 25°C.

6. Pull out ~14 females of the correct genotype (*HACG/+*; *UAS/+*), and ~10 males of any type*. Put them in a "cross" vial with plenty of fresh yeast. (*males promote oogenesis, but we don't care what genotype the progeny would be since we only want the pre-fertilized egg chambers.)

7. This cross should be allowed to go for a day or two at room temp, and then the clones should be induced by heat shocking the vial.

8. To heat shock (HS), place the vial in the 37° water bath (don't let the plug get wet) for ~2 min to equilibrate the food to 37°, then place the vial **on its side** in the 37° air incubator for 55 min. Remove it and place at 25°C for 2.5 days.

> Whenever the flies eat all the yeast, before or after HS, brood them to a new vial. Especially make sure they have yeast in the last day before dissection. The ovaries will be POOR QUALITY if the flies run out of yeast.

9. At 2.5 days after HS, remove the flies and dissect out the ovaries. For example, if you HS Tues night, then you must dissect Fri morning. Or if you HS Tues morning, then you must dissect Thu night. Dissect ~ 5 females at a time, and do 1-2 batches of 5. [Go to the separate protocol for fixing and staining ovaries.]

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The ***HACG*** stock carries to two constructs on a single X chromosome:

[*Hsp70-FLP*], [*Act5C>CD2>GAL4*]

where ">" is an FRT recombination signal.

Upon HS, the FLP protein is produced from [*Hsp70-FLP*], in all cells. Then in random cells, the FLP protein will cause recombination between the two *FRT* sites, looping out and excising the *CD2* "stuffer fragment".

If this happens, the strong, constitutive *Act5C* promoter will be placed next to the GAL4 coding region, and GAL4 will be produced at high levels (just in that cell and its progeny).

After a few days, the cell will divide and grow into a clone of 2, 4, 8, or more cells, all expressing GAL4. Oogenesis is an assembly line that takes ~3 days, so if you wait too long after HS, the clones will be present only in mature eggs, or the eggs with clones will all be oviposited. At 2.5 days you should see an optimal mix of large and small clones across the stages of interest (Stages 9-11).

Time line:

DAY

1	Set up *HACG* x *UAS* Brood 1 at 25°C
2	
3	
4	
5	brood *HACG* x *UAS* to 18°C, keep larvae at 25°C
6	
7	
8	
9	
10	
11	Brood 1 ecloses, move vial to room temp
12	Collect *HACG/+*; *UAS/+* females, keep them with some males, on fresh yeast, at room temp. You will NOT need progeny from this vial; these conditions are only to encourage oogenesis.
13	
14	HS the vial at 37°C for ~1 hr (USE A TIMER; they will die after 90 min at this temp). Do the HS in the evening, if you want to dissect in the morning (in 3 days). NOTE THE TIME on the vial. Brood to a new vial with plenty of yeast, if needed. Return to 25°C.
15	24 hrs after HS. Brood to a new vial with plenty of yeast, if needed
16	48 hrs after HS. Brood to a new vial with plenty of yeast, if needed
17	2.5 days after HS, dissect and fix the ovaries (takes ~ 1-1.5 hr). Typically, stain with SYBR Green (unless using a GFP line) and WGA or phalloidin overnight.
18	Wash the dyes out overnight.
19	Mount the ovaries on slides.
20	The slides can be viewed for up to ~ a week