CELL SORTING AND HOECHST STAINING ON ISOLATED FOLLICLE CELLS. Pelin Cayirlioglu/Duronio lab
Based on Bryant et al PNAS 96:5559-5564 (1999).

1) Dissect out 80-150 females (fat) in Schneider's medium (you don't need to fine dissect them, just get the two ovaries out of the females).

2) Transfer them into an eppendorf tube in Schneider's medium.

3) Remove the Schneider's and wash the ovaries with 1XPBS once.

4) Incubate the ovaries in 0.5% trypsin (1:250 sigma) for 15 minutes, with intermittent vortexing.

5) Filter the supernatant (that has the dissociated follicle cells) through a 80µ mesh into 1ml of Schneider's solution.

6) Spin the supernatant at 4200rpm for 7 minutes to pellet the cells.

7) Resuspend the pellet in 200-300µl Schneider's and keep on ice.

8) Repeat steps 4-7 2-3 more times, until you stop getting any cells in the supernatant (you will understand this because the supernatant will no longer be blurry if there are no cells in there).

9) After you got all the cells, if you want to do some FACS on them, stain them with 5µg/ml Hoechst 33342 for 45 minutes on the nutator at room temperature. Store on ice until sorting.

10) If you want to get RNA/DNA from them, spin down all the cells, and you can start the extraction step immidiately or you can save the cell pellet at –80.