NUCLEAR ISOLATION AND FLOW CYTOMETRY

1) Dissect ovaries (from 50-80 females) in Drosophila Ringers (EBR: 130mM NaCl, 5mM KCl, 2mM $CaCl_2$, and 10mM Hepes, pH 6.9) or Schneider's medium.
2) Digest in EBR 5mg/ml collagenase for 15 min. After incubation is complete, rinse ovaries with EBR to remove residual collagenase.
3) Homogenize ovaries in nuclear isolation buffer (15mM Tis-HCl, pH 7.4, 60 mM KCl, 15mM NaCl, 250mM sucrose, 1mM EDTA, 0.1mM EGTA, 0.15 mM spermine, 0.5mM spermidine) and 1.5% NP40, using a 2ml Kontes dounce with the B pestle.
4) Filter the homogenate 2X through a 100 □ Nitex mesh.
5) Place the homogenate on a sucrose step gradient of 2.5 M, 1.6M, 0.8M sucrose and centrifuge for 20 minutes at 20,000.
6) Remove the sucrose gradient and resuspend the pellet in nuclear isolation buffer.
7) Add DAPI to concentration of 0.5 □g/ml.
8) Keep nuclei on ice before sorting.

Treat all glass and tubes with a solution of BSA to prevent the nuclei from sticking.
Add a small amount of DAPI to the homogenate before placing it on the sucrose gradient allows one to follow the pelleted nuclei using a UV source.

This is a protocol I got from Mary Lilly. I follow it with a couple of exceptions. I use propidium iodide instead of DAPI where I treat the isolated nuclei after step 6 with 40□g/ml of RNase A for 30-40 minutes at 37^{O}C, then incubate in 30□g/ml Propidium iodide for 20 minutes at room temperature on the nutator. Once I am done, I try to keep the nuclei cold (~4^{O}C) until I analyze the ploidy. Usually it is better to analyze it on the same day that you prepared the nuclei, but doing the facs on the next day also works fine.

Tips for seeing good follicle cell peaks:
I found it better to use 2.0-2.2 M sucrose in the bottom gradient because I always had problems with getting good profiles when I used 2.5M sucrose.

Also aging the flies so that they are fat also helps to get a better distribution of different peaks. But make sure that the females are not too old (it is better to dissect females that are 1-2 days old for wild type to get a good distribution of all stages of oogenesis).

CELL SORTING AND HOECHST STAINING ON ISOLATED FOLLICLE CELLS.

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.