FACs protocol (revised from Edgar Lab protocol)
Laura Johnston Lab; Columbia University

Dissociation of wing disc cells and FACS analysis

We have used a variety of Gal4 lines that have UAS-GFP recombined onto the Gal4-containing chromosome, including Engrailed-Gal4, C96-Gal4, Act>CD2>Gal4, Dll-Gal4, etc. These flies are crossed with the UAS line of choice. Eggs are collected for one-two hours on grape plates. Fifty newly hatched larvae are transferred to a 30ml vial of fly food, and raised at 25°C. The larvae are then analyzed at different ages during L3.

At room temperature, in a 100mm Petri dish with three drops of 1x PBS, the anterior halves of larvae are inverted in the first drop. The inverted anterior halves are stripped of fat body and gut and then transferred using forceps to the second drop of 1x PBS. The wing discs are dissected carefully, excluding other tissues e.g., trachea, and transferred to the third drop of PBS. This cleans the discs of other tissue. The discs are transferred to the dissociation solution using a 20ul micropipettor, to avoid damaging the discs. To prevent the discs sticking to the tip it is first coated by protein and fat from the dissected larvae by pipetting up and down in the first drop. In a 10ul volume the discs are transferred from the third drop to a 6 ml polystyrene tube that contained 500ul of 450ul 10x Trypsin-EDTA (Sigma), 50ul 10x PBS, and 0.5ul 500ug/ml Hoechst 33342 (note that the Trypsin solution is used as 10X, and not diluted to 1X). This solution can be made in advance and stored at –20°C, in foil to protect the Hoechst).

We dissociate cells for 2-4 hours with gentle agitation on a Nutator, taking care not to allow the liquid to spread up the sides of the tube. If clumps of cells are still visible, the tubes are shaken gently by hand. Using microscopy, cells are observed to be round in size instead of their original columnar shape, as well as having their nuclei stained. The GFP signal is also detectable for a portion of the cells.

We have used both a Becton Dickinson FACS Vantage and a Beckman Coulter Ultra Hypersort with equivalent results. We measure side and forward scatter of visible light, GFP signal, and Hoechst signal for each event. The data are analyzed using Cell Quest (Becton Dickinson). By excluding the debris from dead cells and the clusters of live cells, the differences in relative cell size and cell cycle profile between live, individual expressing (GFP+) cells and non-expressing (GFP-) cells can be observed.

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Developmental timing must be the same (eg, you can't compare delayed L2 larval discs with wildtype L3 larval discs). Also, optimize dissociation conditions so that cells fall apart into individual cells, but don't digest beyond about 4 hours, as it will be toxic. Finally, collect at least 10,000 events/sample, and run the same experiments at least 3 times.

ModFit (Verity) is used to integrate peak areas, to determine relative numbers of cells in each cell cycle phase (this cannot be done accurately with Cell Quest).

For analyzing GFP-containing FRT chromosomes, follow the same procedure. However, the GFP on our FRT chromosomes is much weaker than the UAS-GFP, and therefore to detect 0X vs. 1X vs. 2X copies of the GFP, linear scales for GFP height are probably necessary.