

Planarian FACS sorting and neoblast isolation

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(When the FACS machine and staining are working well, 1 worm will yield about 1500 X1s, and 1500 X2s. For cell *in situs*, about 10,000 cells/spot/slide are needed.)

1. Collect planaria into 1.5ml eppendorf tubes at a density of ~30 worms/tube. Use as many tubes as cells you need.
2. Wash 2x with cold CMF in the eppendorf tubes.
3. GENTLY dounce worms in eppendorf tubes with a sterile plastic pestle until big fragments are all broken but don't over dounce.
4. Assemble the filter setup with a 20 μ m filter.
5. Pour the cells through the filter into a new 15ml conical tube. Cells may need a little coaxing to get through the filters, but treat them very gently to get them through.
6. Dilute cells to 12ml with CMF and nutate for 10-30min and use a transfer pipet to break up tissue clumps gently with no bubbles every 10min.
7. Centrifuge cells at 300g for 5min, low brake. Resuspend cells in CMF to 10worms/ml.
8. **Optional:** Get 3 additional 2054 Falcon tubes to calibrate the FACS machine with single dye labels (either Hoechst OR calcein OR PI). This is done by adding 0.5ml of WT cells to each tube, and 0.5ml of CMF.
9. Add Hoechst 342 (10mg/ml stock, and use at 25 μ g/ml (**2.5 μ l/ml**)) and incubate for 20' in the dark.
10. Add calcein (dilute 50 μ g powder in 250 μ l DMSO to get a stock of 0.2 μ g/ μ l. Use at 0.2 μ g/ml (**1 μ l/ml**)) and incubate for 10' in the dark.
11. Spin down cells (300g for 5', low brake). Resuspend cells in CMF at the density of 10 original worms/ml (4mls CMF for 40 worms). Control tubes of single dyes are not spun down.
12. Add PI (1mg/ml stock, use at 4 μ g/ml (**4 μ l/ml**)) and take straight to FACS machine. **Note:** on the new FACSaria, we are no longer using PI for neoblast isolation, only as a control in WT to ensure that viability is good.
13. Sort.
14. To spin down sorted cells in eppendorf tubes: spin at 500g for 8min.
15. Add 100 μ l of Trizol, vortex, store at -80°C.

CMF (Ca/Mg-Free media) 10x Glucose

NaH ₂ PO ₄	400mg/L
NaCl	800mg/L
KCl	1200 mg/L
NaHCO ₃	800 mg/L
glucose	2.4 g/L
BSA	to 1%
HEPES	to 15mM
pH7.3	