NEW FACS Protocol for Live Striatal EGFP Neurons 2/19/09

Striatal Dissociation Protocol for FACS

Dissection

Set up for Dissection

1mm Matrix
12 and 14 guage needles (for dStr and NAc)
petri dish
Hibernate A (MUST USE Low Fluorescence)
Accutase enzyme (~2ml per sample)
DNase (Sigma 1mg/ml, ~2000Units/mg)
Dissecting tools: scissors, tweezers, etc
Timer
Control mouse (wildtype no EGFP)
Experimental Mice
Dissection is carried out in the mouse facility
Foil

1. Dissect brain and place in Hibernate A LF in a petri dish
2. Slice 1mm sections in matrix
3. Punch NAc and dStr in Hibernat A LF (do control mouse first and for each pooled sample can punch pooled brains together-usually 2-3 mice)
4. Place punches in accutase enzyme + add 50ul DNase (pipet up and down to mix)
5. Invert tube a few times then place on ice for 30min (keep samples under foil- EGFP bleaches faster in dissociated adult neurons)
6. Transfer samples back to lab (try to periodically invert tube to mix punches in enzyme)

Dissociation

1. Triturate with 3-4 glass pipettes of decreasing tip diameter
2. Centrifuge @ 1250rpm @ 4°C for 6min
3. Prepare Hibernate A LF/AOI solution: For every 1 ml: 900µl Hibernate A LF, 100 reconstituted AOI (this is reconstituted in Hibernate A LF), 25ml DNase – make ~2.5ml per sample, can make less for control
4. Remove supernatant and resuspend cell pellets (resuspend with glass pipet or 1ml pipet tip) in Hibernate A LF/AOI solution
5. Prepare discontinuous density gradient: Add 2-3ml AOI to a 15 ml tube and carefully layer cell suspension atop
6. Centrifuge @ 900rpm @ 4°C for 6min
7. Discard supernatant and resuspend pellet in 2-3ml FACS media
8. Pass cell suspension through 70µm mesh and collect in a 50 ml tube

9. Rinse first tube with ~1ml FACS buffer to obtain any remaining cells and pass through mesh
10. Pour ~1ml clean FACS media over mesh to clear extra cells from the mesh
11. Centrifuge at 1200rpm for 5 min
12. Remove supernatant and resuspend in 1-2 ml FACS media
13. Transfer to sterile FACS tube and add 2µl PI (propodium iodide) (1mg/ml) to stain dead cells red

*__keep cells on ice in between centrifugation and use a 4°C centrifuge__

__note: addition of phenol red can interfere with the lasers' ability to properly detect fluorescence__

FACS/RNA extraction

1. FACS cells immediately- prolonging FACS has resulted in decreased EGFP+ cell recovery
2. At Mount Sinai cells are sorted on an Influx machine low pressure (12psi). At UCLA cells were sorted on a FACSvantage also at low pressure.
3. Use wt (non-EGFP) cells to set parameters
4. Keep cells at 4°C during FACS
5. Collect cells in 1.5ml tube (FACS media) at 4°C
6. Spin down cells immediately following FACS and proceed with RNeasy Plus Micro Kit
7. Check RNA integrity on Bioanalyzer picochip

FACS Media recipe (50ml)
-50ml Hibernate A Low Fluorescence
-50mg BSA (0.1%)
-1ml DNase (2,020 units/mg)