Isolated FDB muscle fibers for damage-repair assay

-The Han Lab

1. Dissect the FDB muscles tendon-to-tendon from the mouse feet.
2. Digest the muscle with 0.2% collagenase type I or type IV in PBS+1mM Ca2+ @ 37 C for 1 h with slow rotation.
3. Let the muscle sit down and decant the digestion solution, wash the muscle with fresh PBS+1mM Ca2+ twice.
4. Add 1ml PBS+1mM Ca2+ and gently triturate the muscles with a large open-mouth plastic pipette till you can see the muscle bundles are dissociated into single fibres.
5. Resuspend the fibres in fluorescein dextran (FDx, 10kDa, lysine fixable) solution.
6. Flush the fibres once using a 18G needle.
7. IMMEDIATELY put the fibre solution at 37 Â°C incubator for 2 min. (Timely)
8. Add equal amount of 4% PFA, at room temperature for 15 min.
9. Wash once with PBS

[The steps 10-11 are only needed certain antibodies to retrieve antigen]

10. Incubate with 100 mM Glycine in PBS for 10 min at room temperature.
11. Incuate in 0.05% SDS in PBS for 30 min at 50 Â°C.
12. Wash once with PBS
13. Incuate with primary antibody overnight @ room temperature.
14. Wash three times 5 min each with PBS
15. Incuate with secondary antibody at room temperature for 30 min.
16. Wash three times 5 min each with PBS.
17. Confocal microscopic examination.

(The end)

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