Primary myoblast culture

-The Han Lab

1. Dissect out limb muscle from 1-3 days old pups, leave the muscle in DMEM/2% pen/strep
2. Mince the muscle into pieces in tissue culture hood.
3. Transfer the minced muscle into 15 ml tube containing 7ml DMEM+3ml 0.25% trypsin.
4. Use 10ml pipette to triturate the tissue for at least 3 min (no bubbles), leave the tube at 37 C for 2 more min.
5. Spin down the undigested tissue at 500 rpm for 5min. transfer the sup into 50ml tube containing 20ml F10 culture media
6. Add 7ml DMEM + 3ml 0.25% trypsin to the undigested tissue and triturate for 3 min and then leave at 37 C for 2 more min.
7. Repeat this digestion cycle for at least 4 times.
8. Spin the four 50ml tubes at 1000 RPM for 10min
9. Discard the sup and re-suspend the cells with 10ml culture media, Flow the cells through a 70-um nylon mesh filter.
10. pre-plate the cells in a 100mm culture dish for 10min
11. Collect the culture media from pre-plating plate. Count the cell number and plate at a density of ~10^6 cells/ml into gelatin or collagen-coated culture dishes.

Coat the culture dish or chamber with 0.1% gelatin or (rat tail collagen I) for at least 20min.

F10 culture medium (500ml):

F10 395ml
FBS 100ml
Pen/strep 5ml
Insulin 0.01mg/ml final
FGF 25ng/ml final
EGF 10ng/ml final

Differentiation medium (200ml)

DMEM 194ml
HS 4ml

(The end)

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