Primary myoblast culture from fresh human muscle biopsy

50 ml myoblast proliferation medium

F10	38ml
FBS	10ml
10 ug/ml bFGF (BD #354060)	0.125ml
1 mg/ml Insulin (sigma # I1882)	0.5ml
100x Glutamax (invitrogen #35050-061)	0.5ml
100x P/S	0.5ml

Conditioning medium

Medium 199	4.9 ml
FBS	4 ml
100X P/S	0.1 ml

Freezing medium

DMEM	7 ml
FBS	2 ml
DMSO	1 ml

Myoblast primary culture:

1. After obtaining the muscle tissue from operation, keep it in wet gauze in a sterile Petri dish.
2. Keep the dish on ice and send it to lab as soon as possible.
3. Muscle tissue can be divided for different purpose: primary culture and snap freeze with liquid nitrogen pre-cooled isopentane.
4. For myoblast primary culture, take a small piece (~3mm^3). Rinse it with myoblast proliferation medium; remove adipose tissue, connective tissue and blood vessels.
5. Mince the muscle tissue and spread the mince muscle tissue on a 60 mm sterile petri dish, leave the dish in culture hood for about 5 minutes to let the tissue adhere to the substrate, but pay attention not to let the tissue over dry.
6. Gently add 5 ml pre-warmed myoblast proliferation medium to the plate, pay attention not to detach the adhered tissue pieces.
7. Culture under 37C with 5% CO2.
8. Muscle cells start to grow from the tissue pieces in about one week. When there are sufficient cells, collect cells by trypsinization and plate them in another petri dish for proliferation. Re-feed the original plate with fresh medium, cells will grow from the adhered tissue continually.

Freezing of vital muscle biopsy for back up

At the same time, keep one or two small pieces of muscle tissue in a 60 mm petri dish with 5 ml condition medium, maintained at 37C 5% CO2 overnight. The next morning, transfer the tissue pieces to a cyptubes containing 1 ml freezing medium, freeze the cells in a cryo-freezing container.

Yaqun Zou, NINDS/NIH, January 2014