Myocyte isolation protocol (JCI 112:608 Norris 2003)

This protocol obtains genomic DNA from skeletal muscle preparations crudely enriched for myocytes. As reported in ***Norris AW Chen L…Kahn CR. J Clin Invest. 2003 Aug;112(4):608-18. PMID: 12925701***. Protocol developed from J Pharm Pharmacol 38:288 (1986).

Isolation Buffer (IB):

solute	g/L
NaCl	6.8
KCl	0.4
Glu	0.9
Na2HP04	0.21
NaH2PO4	0.06

Collagenase Buffer (CB):

IB +
2.5 mM CaCl2
2 mg/mL collagenase (Sigma C-9891 – Type 1A)
2 mg/mL dispase (Gibco 17105-041)
1 mg/mL hyaluronidase (H-3506)

Procedure:

1) Sacrifice mice
2) Rapidly dissect hindlimb muscle – trimming away obvious fat. Make several longitudinal slices in the muscles to speed digestion.
3) Add muscle to 5 mL CB
4) Incubate 37 C, triturate every 15 minutes with P-1000 pipette having cut pipette tip back to widen orifice. The mix should be a homogenous slurry in 1 – 2 hours.
5) Filter slurry through 250 micron nylon mesh. Gristle / sinews & undigested muscle will be retained on the filter. Collect filtrate in tube on ice.
6) Centrifuge filtrate 3g for 7 minutes, at 4 C. Sediment will be enriched for myocytes. Supernatant will contain many cell types, and adipocytes will float in lipid layer.
7) Layer pellet ontop of 12 mL 4% BSA (Sigma A-3059 – Fraction V) in IB in tube on ice.
8) Allow myocytes to sediment through the BSA layer on ice (30 – 90 minutes).
9) The sediment at the bottom of the BSA layer will be highly enriched for striated myocytes. Remove these from the bottom of the tube carefully with a long-necked Pasteur pipette, placing in a new tube on ice.
10) Collect cells in the various fractions by centrifuging at 800 g, 5 minutes at 4C. Lighter cells, especially adipocytes, are likely not pelleted.
11) Begin genomic DNA isolation by resuspending cellular pellet in equal volume cellular digestion buffer and following standard genomic DNA isolation protocols.

NOTES:

1) The myocyte enrichment of the cells can be followed easily by light microscopy looking for striated cells. The fractions often contain single myocytes as well as myofascicles (that may be expected to contain endothelial cells?).

2) Often the 800g pelleted cells will separate into tan and brick colored fractions. I have found both be highly enriched in striated cells. Perhaps the brick-colored layer are the heavier Type I myocytes (?).