Somatic gene transfer for FDB Muscle.v1

This protocol is based on DiFranco, Quinonez, Capote & Vergara (2009) J Vis Exp Oct 19(32) to have efficient gene transfer for this small skeletal muscle and allowing for sensitive detection of Mito-Timer signal for assessment of mitochondrial stress and damage in adult skeletal muscle.

1. Prepare plasmids at 2 μg/μl in sterile saline and need 20 μl for one FDB muscle. If co-injecting an over-expression vector with a reporter gene (i.e. pMitoTimer or pGFP3), make a ratio of 3:1 (over-expression vector:reference reporter gene).
2. Prepared a solution containing 0.36 mg/ml hyaluronidase in sterile saline. For the extra hyaluronidase, aliquot and store at -20 °C.
3. Anesthetize the mouse using 3% isoflurane in O_2 and monitor anesthetic depth by toe pinch reflex.
4. Inject 10 μl of the hyaluronidase solution under the footpad using a 27G or smaller syringe. Insert the needle at a point close to the heel of the foot and advance the needle subcutaneously towards the base of the toes for ~¼ inch. Repeat with other foot if desired.
5. Place the mouse in cage and allow it to recover from anesthesia.
6. Anesthetize the mouse for a second time 1 hour later. Using the same injection procedure, inject 20 ul of the plasmid DNA. A small plasmid construct will generally result in better transfection efficiency.
7. Allow the mouse to recover from anesthesia for 10 min.
8. Anesthetize the mouse for a third time.
9. Insert a gold-plated acupuncture needle under the skin at the heel and a second at the base of the toes so they are parallel to each other and perpendicular to the long axis of the foot.
10. Connect the needles to the electrical stimulator using micro-clip connectors.
11. Apply 10 pulses, 20 ms in duration at 1 Hz. Depending on the spacing of the electrodes, adjust the voltage amplitude to ~75 V/cm. No contractions in response to the stimuli may be observed. Repeat on the other foot if required.
12. Allow the animal to recover and monitor as outlined in animal ethics procedure. It may also be necessary to administer an analgesic (Buprenorpine HCL) if required prior to the procedure and for up to 5 days after the procedure.

Whole Mount

1. Harvest FDB. If both confocal microscopy and protein analysis is required, carefully slice the muscle in half longitudinally. Immediately homogenize half of the muscle in sample buffer and incubate the other half with 1 mL 4% PFA for 20 minutes (at room temperature) and then transfer to 1 mL PBS and incubate for 5 min. Try not to expose to tissue to bright light in order to preserve the fluorescence of the reporter if you use pMitoTimer.
2. Carefully remove the tendon running the length of the muscle. Place the tissue on a gelatin-coated slide. Use small paint brushes to orientate the tissue and flatten it on the slide. Make sure there are no air bubbles under the tissue.
3. Allow the tissue to adhere to the slide. This typically takes 5-10 min, but it can vary on the thickness and type of tissue.
4. On one side of the slide pipette 50:50 PBS:Glycerol and place a coverslip over the slide. You want the coverslip to be as close to the slide as possible. This is easy for thin tissues, and you can push the coverslip and slide together if the tissue is deformable (press and wiggle).
5. Remove any excess 50:50 that has come out from under the coverslip with blotting paper.

6. Seal the coverslip by painting the edges with nail polish. This will prevent the 50:50 from evaporating over time.
7. Image immediately.

Parameters for imaging Mito-Timer and ER-Timer

Use FITC (green) and TRITC (red) channels.
Aspect ratio: 800x800
Green HV: 565
Red HV: 685
Gain: 1
Offset: 10%
Lazer intensity: 15%
2 pixels/sec
0.5 um slice
check sequential imaging (line)
Take at least 10 images at 100X magnification