Video Article

DNA Transfection of Mammalian Skeletal Muscles using *In Vivo* Electroporation

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Abstract

A growing interest in cell biology is to express transgenically modified forms of essential proteins (e.g. fluorescently tagged constructs and/or mutant variants) in order to investigate their endogenous distribution and functional relevance. An interesting approach that has been implemented to fulfill this objective in fully differentiated cells is the *in vivo* transfection of plasmids by various methods into specific tissues such as liver[1], skeletal muscle [2,3], and even the brain[4]. We present here a detailed description of the steps that must be followed in order to efficiently transfect genetic material into fibers of the *flexor digitorum brevis* (FDB) and *interosseus* (IO) muscles of adult mice using an *in vivo* electroporation approach. The experimental parameters have been optimized so as to maximize the number of muscle fibers transfected while minimizing tissue damages that may impair the quality and quantity of the proteins expressed in individual fibers. We have verified that the implementation of the methodology described in this paper results in a high yield of soluble proteins, i.e. EGFP and ECFP[3], calpain, FKBP12, β2a-DHPR, etc. ; structural proteins, i.e. minidystrophin and α-actinin; and membrane proteins, i.e. α1s-DHPR, RyR1, cardiac Na/Ca^{2+} exchanger , NaV1.4 Na channel, SERCA1, etc., when applied to FDB, IO and other muscles of mice and rats. The efficient expression of some of these proteins has been verified with biochemical[3] and functional evidence[5]. However, by far the most common confirmatory approach used by us are standard fluorescent microscopy and 2-photon laser scanning microscopy (TPLSM), which permit to identify not only the overall expression, but also the detailed intracellular localization, of fluorescently tagged protein constructs. The method could be equally used to transfect plasmids encoding for the expression of proteins of physiological relevance (as shown here), or for interference RNA (siRNA) aiming to suppress the expression of normally expressed proteins (not tested by us yet). It should be noted that the transfection of FDB and IO muscle fibers is particularly relevant for the investigation of mammalian muscle physiology since fibers enzymatically dissociated from these muscles are currently one of the most suitable models to investigate basic mechanisms of excitability and excitation-contraction coupling under current or voltage clamp conditions[2,6-8].

Protocol

Experimental Procedures for *in vivo* electroporation of FDB and IO muscles

1. Before starting the *in vivo* electroporation protocols, mammalian expression plasmids must be amplified to yield concentration in the range of to 2-5 µg plasmid/µl of TE. *Note: We routinely use commercial amplification kits and follow the manufacturer' procedures. Commercial expression plasmids carrying the CMV promoter work very well in skeletal muscle in vivo transfections.*
2. Aliquot the necessary volume (10-20 µl) of the plasmid solution and save it in two 0.5 ml Eppendorf tubes (one for each foot of the mouse).
3. Prepare a solution containing 2 mg/ml hyaluronidase in sterile Tyrode.
4. Using an anesthetizing box, deeply anesthetize a mouse using 4% isoflurane in O2 with an approved gas anesthetic machine. Place the animal on a heating pad (37 °C) and maintain the anesthesia using a rodent face mask. Monitor the anesthetic depth by toe pinch reflex.
5. Under observation with a dissection microscope, inject 10 µL of the hyaluronidase solution under the footpads of one foot of the mouse using a 1" long 33 gauge sterile needle. Penetrate the skin at a point close to the heel of the foot and advance the needle subcutaneously towards the base of the toes for ~1/4".
6. Repeat the procedure with the other foot if so desired.
7. Disconnect the anesthesia and place the mouse in a cage. Allow it to fully recover from anesthesia.
8. After one hour, anesthetize the animal for a second time and place it on the heating pad. Following the same procedure described for the hyaluronidase solution, inject a total of 20-50 µg of the plasmid DNA (depending on the size of the plasmid construct). The total injection volume should be less than 20 µL/foot. *Note: when 15-20 µL is necessary, it is advisable to close the skin at the needle entry point with tissue-glue.*
9. Disconnect the anesthesia and place the mouse in a cage. Allow it to fully recover from anesthesia and wait for 10-15 min.
10. Anesthetize the animal for the third time and place it on the heating pad.
11. Select one foot of the animal. Place one gold-plated acupuncture needle under the skin at heel, and a second one at the base of the toes. Electrodes are oriented parallel to each other and perpendicular to the long axis of the foot.
12. Connect the head of the needles (electrodes) to the electrical stimulator using micro-clip connectors. Electroporate the muscles by applying 20 pulses, 20 ms in duration/each, at 1Hz. Depending on the spacing of the electrodes, the pulses' voltage amplitude is adjusted (by monitoring with an oscilloscope) to yield an electric field of ~100 V/cm. *Note: No contractions in response to the stimuli should be observed if the level of anesthesia is adequate.*
13. If so desired, repeat the above procedures in the contralateral foot of the animal.
14. Return the animal to its cage and once fully recovered from anesthesia maintain it under observation. Note: if the procedure went normally, the animal should regain full mobility within 30 minutes and afterwards is ready to be sent back to the animal room at the vivarium. The injections of hyaluronidase and DNA in the footpads do not have noticeable adverse effects on the animals. Once recovered from anesthesia, mice are able to amble normally around the cage. As an additional precaution, add to the drinking water of the animal Carprofen at 0.0027 mg/ml for 2 days as an analgesic.
15. Protein expression can be assayed 2-8 days after transfection. However, sustained expression of many proteins has been observed for months.

