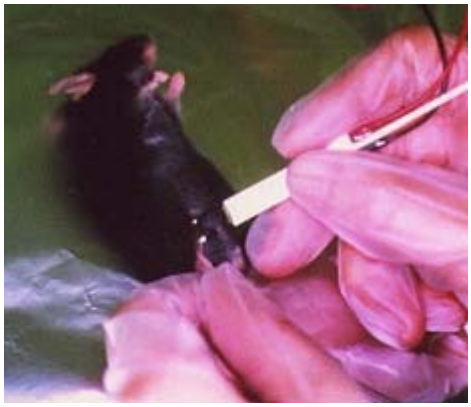
[Muscle] Gene transfer into muscle by electroporation in vivo

Injection of plasmid DNA into muscle



In Vivo electroporation

Electric pulses were delivered using an electric pulse generator (Square Wave Electroporator CUY21EDIT; Nepa Gene Co.,Ltd.). Electrodes consisted of a pair of stainless steel needles of 5 mm in length and 0.4 mm in diameter, fixed with a distance (gap) between them of 3 mm or 5 mm (Nepa Gene Co.,Ltd.)

Protocol

1) Intramuscular DNA Injection

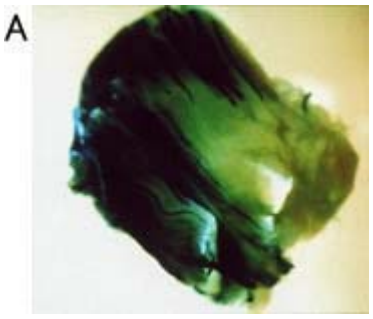
Anesthetize mice by intraperitoneal injection of 0.01 ml/g body weight of 6 mg/ml pentobarbital sodium solution. Inject the tibialis anterior muscles with 50 μg of purified closed circular DNA of pCAGGS-lacZ plasmid at 1.5 μg/μl in PBS using an insulin syringe with a 27-gauge needle.

2) Electroporation in Vivo

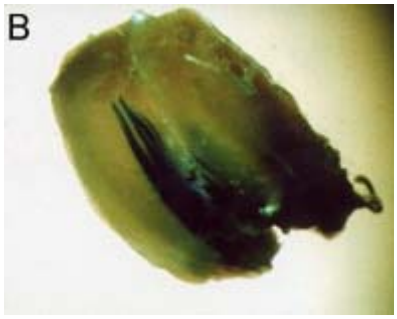
Insert a pair of electrode needles into the muscle to a depth of 5 mm to encompass the DNA injection sites. Deliver electric pulses using an electric pulse generator. Three 50-msec-long pulses of the indicated voltage (50-100 V) followed by three more pulses of the opposite polarity are administered to each injection site at a rate of one pulse per sec.

3) β-galactosidase Expression

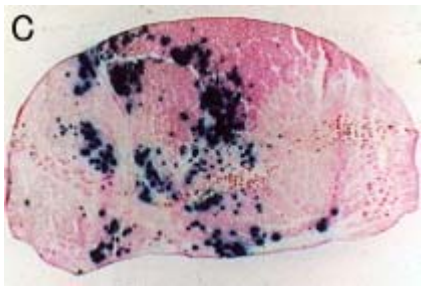
Five days after DNA transfer, the expression of the lacZ gene is visualized by X-gal staining for β-galactosidase activity



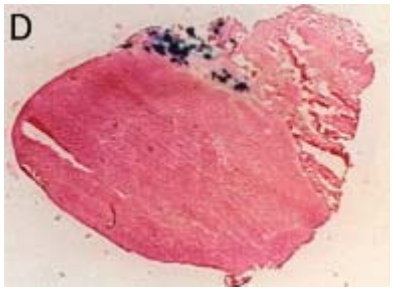
Whole muscle with electroporation



Whole muscle without electroporation



Transverse section with electroporation



Transverse section without electroporation

◆X-gal Staining

The tibialis anterior muscles were fixed in cold 4% paraformaldehyde in PBS for 3 h, then washed in PBS for 1 h, and stained at 37°C for 18 h in the presence of 1mM X-gal to detect E. coli β-galactosidase activity. For transverse sections, muscles were embedded in O.C.T. compound and frozen in dry ice-acetone. Serial sections (15μm thick) were sliced with a cryostat and placed on slide glasses coated with 3-amino-propyltriethoxysilane. The slices were fixed in 1.5% glutaraldehyde for 10 min at room temperature and then washed three times in PBS. Samples were then incubated at 37°C for 3 h in the presence of 1 mM X-gal. The muscle sections were counterstained with eosin. The control muscle (without electropulsation) showed only a small number of stained muscle fibers. Electroporation increased both the number of positively stained muscle fibers and the density of staining.

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