Tech-Trends

Volume 1, Series 7

Gene Transfer Through Ex-Plant Electroporation

Gene Transfer into Mouse Prepancreatic Endoderm by Whole Embryo Electroporation

Context Understanding gene function in the developing pancreas is a major issue for pancreatic cell therapy. The in vivo analysis of gene function has essentially been performed by modulating gene expression in transgenesis. A faster and easier method is electroporation of mouse embryos. This technique, coupled with whole embryo culture, enables one to deliver genes and analyze their effects in a spatially and temporally regulated manner.

Objective We wanted to adapt the electroporation technique for gene transfer of whole e8.5 mouse embryos into the endoderm to allow expression of transgenes in the pancreas or liver.

Results Using two platinum plate electrodes, low voltage and a precise positioning of the embryo in the electroporation cuvette we could target and express DNA constructs in the prepancreatic or prehepatic territories, identified with cell markers. We also demonstrated that this technique is a valuable tool in the study of transcriptional regulation in the developing endoderm.

Conclusions Targeted electroporation of whole embryos is a useful method of characterizing the gene network which controls pancreatic development.

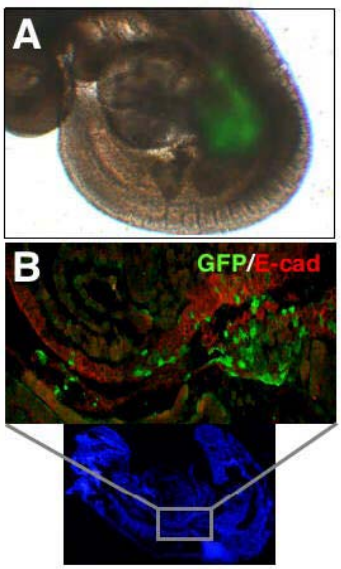


Figure 2. Gene targeting of a restricted region of the mouse endoderm by whole embryo electroporation. **A.** Overlay of bright field and fluorescence images of an embryo electroporated at the 8-somite stage with a GFP expression vector and cultured for 24 h, showing GFP activity (green) only in the midgut region. **B.** Immunolocalization of GFP (green) in the E-cadherin (red)-positive epithelium of the primitive gut. The GFP positive region is boxed in the lower panel, which shows Hoechst staining of a section of the whole embryo.

ECM® 830* Ex- Plant Electroporation Protocol

Tissue preparation:

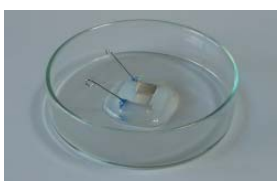
Inclubate embryos with the plasmid solution so the DNA can be absorbed into the surface of the endoderm Transfer embryos with a 30 µL drop of plasmid solution (concentration from 0.5 to 1.5 µg/µL) into a bacterial culture dish and incubate for 10 min at room temperature. Embryos were transferred with the DNA solution in the electroporation cuvette between the two platinum plated tweezertrodes 5 mm apart. To target the prepancreatic endoderm, embryos (6- to 8-somite) were oriented in the cuvette so that the ectoplacental cone was tilted at an angle of approximately 45 degrees to the anode. To target the prehepatic endoderm, younger (4- to 6-somite) embryos were used and oriented in a similar way.

Electroporation Settings:

Voltage:	9V
Pulse length:	50 msec
Number of pulses:	3
Interval:	1s
Field Strength:	18 V/cm

Reference;

(1) Pierreux, C., Poll, A., Jacquemin, P., Lemaigre, F., Rousseau, G., 2005. Gene Transfer into mouse Prepancreatic Endoderm by Whole Embryo Electroporation. JOP. J Pancreas (online) 2005; 6(2): 128-135



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