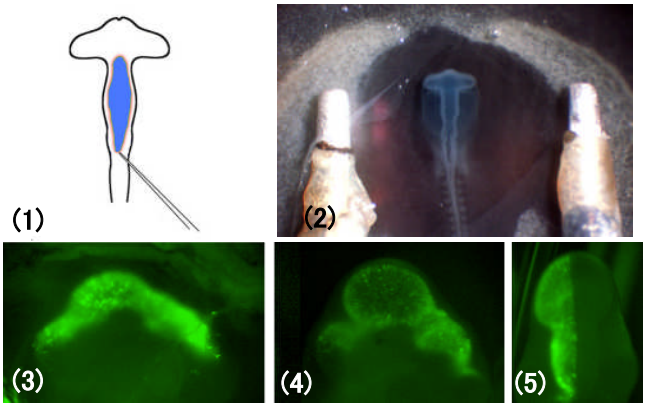
Applications (CUY21)

● Misexpression of the gene of interest by in ovo electroporation [Publication 1]

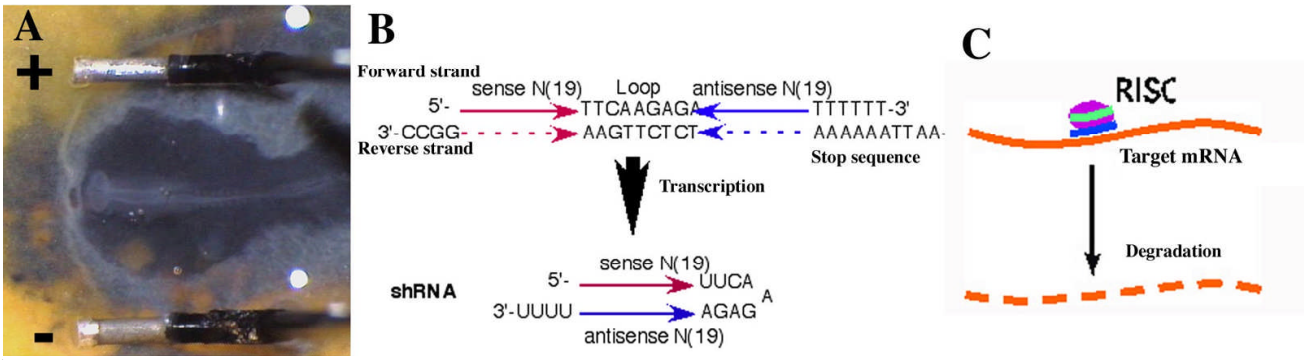


(1) Injection of the plasmid solution colored by Fast Green into the central canal
(2) A pair of electrodes are put on the vitelline membrane overlying the embryos, and a 25V、50 ms rectangular pulse was charged 4 times.
(3) We can monitor the transfection efficiency by electroporating GFP expression vector.
(4) Strong GFP fluorescence could be detected even 48 hours after electroporation.
(5) Right side of the embryo is transfected, hence the left side could be served as the control. (Dorsal view)

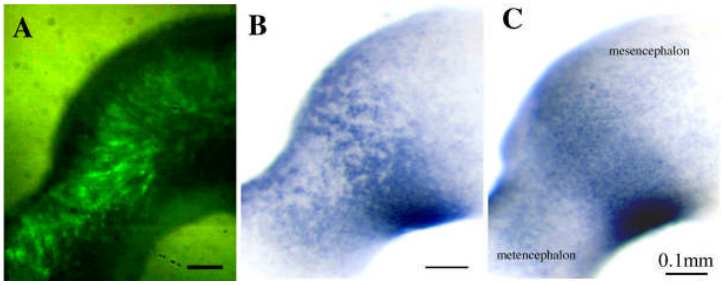
Mechanisms of brain regionalization and neural circuit formation have been studied by misexpression of transcription factors (En1/2, Pax2/5/6, Otx2, Gbx2), secreted factors (Fgf, Shh, semaphoring), signal transduction molecule (Ras, Sprouty2) and receptors (neuropilin)

Graduate School of Life Sciences, and Institute of Development, Aging and Cancer, Tohoku University
Harukazu Nakamura and Yuji Watanabe
*Development Growth & Differentiation, Volume 42, Issue 3, Page 199–201, June 2000

● Knock-down by transfection of shRNA expression vector by electroporation [Publication 2]



(A) shRNA expression vector is electroporated as shown above.
(B) Select target DNA sequence of 19 to 21 mer. Sense and antisense sequence were linked to a nucleotide spacer as a loop and put into expression vector that is driven by U6 or H1 promoter. Commercially available expression vector that dreives expression by mouse U6 promoter is effective in chick embryos.
(C) After transcription, hairpin is digested to form siRNA, and siRNA forms RISK (RNA-induced silencing complex) to dgrade the target mRNA.

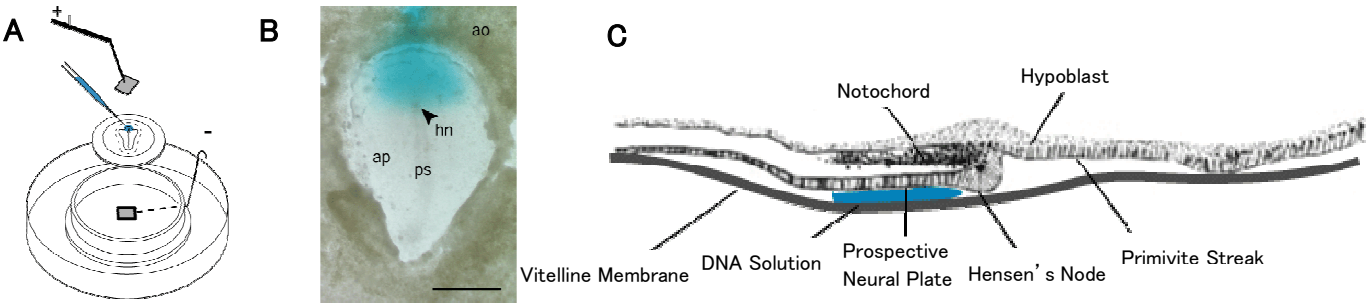


24 hours after electroporation.
(A) Transfection is monitored by co-electroporated GFP fluorescence.
(B), (C) Application of siRNA against En2 by electroporation shows degradation of En2 mRNA (B).
(C) Control side

Graduate School of Life Sciences, and Institute of Development, Aging and Cancer, Tohoku University
Harukazu Nakamura and Tatsuya Katahira
*Mechanisms of Development, Volume 121, Issue 9, Pages 1137–1143, September 2004
*Development Growth & Differentiation, Volume 45, Issue 4, Pages 361–367, August 2003

Applications (CUY21)

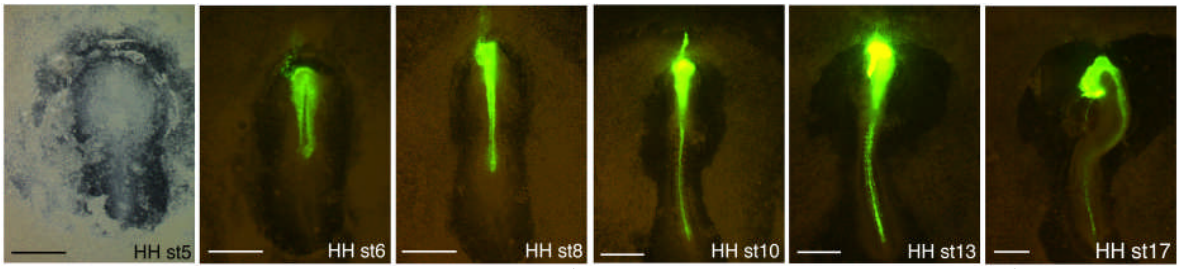
● Electroporation for early chick embryos using New's culture (gastrula) [Publication 3]



A: Schema illustrating the system.
B: Hamburger and Hamilton (HH) stage 4 chick embryo with DNA solution injected to the prospective neural plate region. DNA solution was colored with a FastGreen dye.
hn: Hensen's node, ps: primitive streak, ao: area opaca, ap: area pellucida
C: Schema illustrating the location of DNA solution between the vitelline membrane and the ectoderm.

◆Experimental Procedure

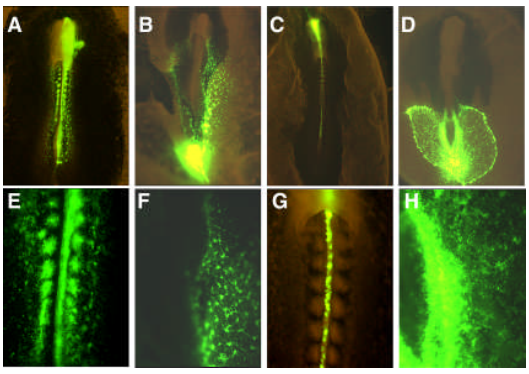
1. Dissect an embryo using a paper ring (dried filter paper) out of the egg.
2. Rinse gently to remove excess yolk, and place the embryo onto the platform of the cathode chamber.
3. Inject DNA solution into the space between the ectoderm and vitelline membrane using a glass pipette (for introduction to the ectoderm derivatives).
4. Adjust the target site right onto the cathode, and set the anode above it. Set the electrode distance at 5mm.
5. Deliver electric pulses (10V, pulse on 50ms, pulse of 100ms, 5 pulses) using a CUY21 electroporator.
6. Place the specimen on an agarose-albumin plate, and incubate at 39°C.



GFP expression becomes detectable by HH6 (about 5 hours after electroporation) in the neural plate, which spreads throughout the central nervous system at HH17. Scale bar: 1mm.

Transgene expression after the electroporation

GFP gene was introduced to the prospective neural plate at HH4, and the embryo was cultured for about 34 hours (HH17 equivalent). GFP expression was occasionally monitored under a fluorescent dissecting microscope. GFP fluorescence was detectable as early as 3 hours after electroporation at the central nervous system and head ectoderm. More than 80% of cells within the transfected area express GFP.



Gene transfection for tissues other than the CNS

Based on the fate map of the chick epiblast, transgenes can be introduced to the somites (A, E), hematopoietic system (B, F), notochord (C, G), or lateral plate mesoderm (D, H).

E-H are the high power views of A-D, respectively.

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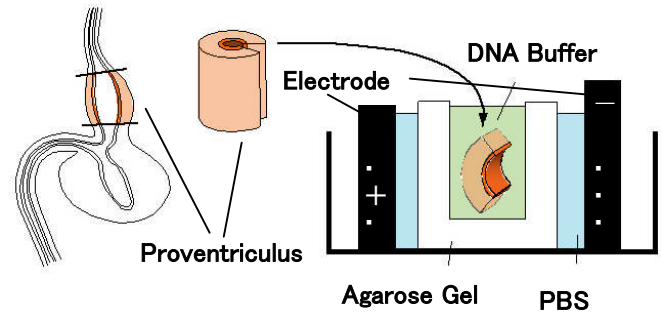
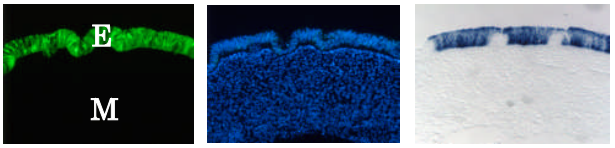
Applications (CUY21)

Method to introduce genes into epithelial cells of the chicken embryonic stomach (proventriculus) [Publication 4]

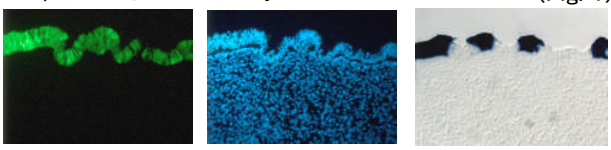


→ Organ culture for 2 days → Detection of GFP

1. Cut open the proventriculus with small scissors and transfer the tissue to PBS(-) solution.
2. Cut the agarose gel with a small well (we use minigel for the separation of DNA) into a piece of appropriate size and put it into the electrode well.
3. Fill the well of electrode with PBS(-) and the well of agarose gel with DNA solution (12-15μl). Put proventriculus tissue into the well with its epithelial side towards the cathode. Two or three samples can be put together.
4. Apply pulses of 30V for 15 times with pulse length of 50 ms, and interval time of 75 ms. Remove agarose gel immediately and wash it in Tyrode's solution. Then take out tissues from the gel and wash them well in Tyrode's solution. The tissues can now be cultivated organotypically.



E: Epithelium, M: Mesenchyme (Fig. 1)



(Fig. 2)

(Fig. 1) Two days after the introduction of the vectors. Expression of GFP is observed only in the epithelium (E, left), not in the mesenchyme (M). cSP gene, a luminal epithelium marker, is expressed normally indicating that the electroporation is not harmful for the normal development of the stomach (right). Central figure shows DAPI staining of the explant.
(Fig. 2) Three days after the introduction of the vectors. Small glands are already formed where cSP is not expressed. GFP protein can be found almost all epithelial cells of glandular and luminal epithelia, indicating that the efficiency of electroporation is very high.

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