Chicken embryonic spinal cord electroporation

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Organisation

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Specific Research Group or Service Animal Molecular and Cellular Biology (AMCB)

Country Belgium

SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research, Education and training
Type of method	In vivo
Used species	Chicken (Gallus gallus domesticus)
Targeted organ system or type of research	Central and peripheral nervous system

DESCRIPTION

Method keywords

Embryonic spinal cord

Electroporation

In ovo experiment

Expression vector

RNA interference

Gene overexpression

Gene downregulation

chicken

chicken embryo

Scientific area keywords

Development

neurobiology

Developmental neurobiology

Spinal cord

CNS

Neurogenesis

Neuronal differentiation

Neuronal migration

Gene expression

Embryo

Method description

The goal of chicken embryonic spinal cord electroporation is to increase or to reduce expression levels of genes of interest in the developing spinal cord, and to assess the phenotypic consequences of these alterations on neuronal differentiation or migration. Fertilized eggs stored at 14°C are incubated for ~60 hours at 38°C to obtain embryos at the expected developmental stage. Plasmid DNA or siRNA is injected in the lumen of the neural tube at Hamburger-Hamilton stages ~10 to ~18. Later stages cannot be injected due to the rotation of the embryo. Nucleic acids are internalized in neural progenitors and their progeny on one side of the neural tube using whole-embryo electroporation. The contra-lateral side can be used as a perfect matching control. Instead of the spinal cord, hindbrain, midbrain, or forebrain can also be targeted by adapting the position of the electroporation electrodes. Specific cell populations can be targeted by using a cell-specific promoter. Signaling pathway reporter constructs or labelling systems targeting neurites or synapses can also be (co-)electroporated. Development can be continued for 1 to 5 days depending on the developmental stage to be analyzed.

Lab equipment

- Egg storage cabinet (wine cooler) Haier
- Eppendorf FemtoJet injector
- Harvard Apparatus BTX ECM830 power source + electrodes
- FIEM egg incubators

Method status

History of use

Internally validated

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- cheap
- fast
- versatile regarding gene alterations, labelings, or reporter activity that can be obtained

- adapted for screening a reasonable number of candidate genes for a particular process

Challenges

- requires some initial training and skill
- limited time-window for the injection/electroporation (~HH10 to ~HH18)
- transient activity of the injected constructs
- limited duration of the post-electroporation period of time
- variability from one embryo to another (can be reduced with practice)

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Roy A., Francius C. (equal contribution), Rousso D.L., Seuntjes E., Debruyn J., Luxenhofer G., Huber A.B., Huylebroeck D., Novitch B.G. and Clotman F. (2012) Onecut transcription factors act upstream of Isl1 to regulate spinal motoneuron diversification. Development, 139 (17) pp. 3109-19

Harris A., Masgutova M., Collin A., Toch M., Hidalgo-Figueroa M., Jacob B., Corcoran L.M., Francius C. and Clotman F. (2019) Onecut factors and Pou2f2 regulate the distribution of V2 interneurons in the mouse developing spinal cord. Frontiers in Cellular Neuroscience, 13: 184

Toch M. and Clotman F. (2019) CBP and p300 coactivators contribute to the maintenance of Isl1 expression by the Onecut transcription factors in embryonic spinal motor neurons. Molecular and Cellular Neuroscience, 101:103411

Debrulle S., Baudouin C., Hidalgo-Figueroa M., Pelosi B., Francius C., Rucchin V., Ronellenfitch K., Chow R.L., Tissir F., Lee S.-K. and Clotman F. (2020) Vsx1 and Chx10 paralogs sequentially secure V2 interneuron identity during spinal cord development.

Cellular and Molecular Life Science, 77(20):4117-4131

Associated documents

Chicken embryo electroporation - NEDI lab (UCLouvain).pdf

Fixation procedure for chicken embryos.pdf

Links

Short movie of chicken embryonic spinal cord electroporation

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