Science Education Collection

In ovo Electroporation of Chicken Embryos

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Abstract

Electroporation is a technique used in biomedical research that allows for the manipulation of gene expression via the delivery of foreign genetic material into cells. More specifically, *in ovo* electroporation is performed on early developing chicks (*Gallus gallus domesticus*) contained within their eggshells. In this procedure, DNA or knockdown constructs are first injected into a target tissue. However, the genetic material is unable to penetrate the plasma membrane to carry out its function within the cell. To solve this problem, an electrical field is applied, causing temporary disruptions to membrane stability. This electric field also causes the negatively charged nucleic acids to migrate toward the positively charged electrode through the holes in the plasma membrane, thus effectively driving the DNA or knockdown construct into the cell. The major advantage of this technique is that the delivery of genetic material can be localized to isolated cell types at specific developmental time points. As a result, the genetic mechanisms that govern individual developmental events can be examined.

This video provides an overview of the principles behind *in ovo* electroporation and introduces the tools required for the technique, including capillary needles, electrodes, and an electroporator. A step-by-step protocol for carrying out the procedure is also presented prior to discussion of a few fascinating examples of how the technique is used to perform a variety of genetic manipulations in chicken embryos.

Transcript

Electroporation is a technique used to introduce foreign genetic material into cells. Electroporation of chick embryos inside the egg, or *in ovo* electroporation, is a valuable tool for developmental biologists because the delivery of genetic material can be localized to specific tissues and specific developmental time points. This video will introduce the basic principles of *in ovo* electroporation, describe essential steps of the procedure, and discuss how this technique can be used to study biological processes during development.

To start, let's review the basic principles of electroporation to figure out how it can be used to get DNA into a cell.

First, microinjection is used to deliver the DNA in close proximity to a cell of interest. However, the DNA can't penetrate the plasma membrane to get into the cell.

To solve this problem, an electrical current is applied to disrupt the stability of the membrane, creating pores. A second consequence of this electric field is the migration of the negatively charged DNA towards the positive electrode. As a result, only cells on the side of the injection site closer to the positive electrode become transfected with the DNA.

Now that you know the basic principles, let's talk about how to prepare embryos for electroporation. If you need to use embryos older than Hamburger Hamilton stage 20, it's best to culture your chicks outside of the shell, or *ex ovo*, for improved tissue access. This video will focus on electroporations performed on early embryos developing within the shell, or *in ovo*.

To begin, eggs should be incubated in a humidified incubator at 37 °C until they have reached the desired developmental stage. While the eggs are incubating, prepare the tools for injection and electroporation. First, make capillary needles by pulling a 0.5 mm glass pipet with a pipet puller. To open the pipet, place it under a microscope and break off a small piece of the tip. Then, prepare the injection solution, which should contain an appropriate dilution of your construct as well as a dye to help you visualize the injected solution.

To control the movement of fluid within the needle, attach it to a human-powered mouth pipet. Alternatively, the needle can be loaded onto a microinjector, which provides adjustable pressure pulses of air. Fill the needle by submerging the tip in the injection solution and applying negative pressure.

In addition to needles, you'll also need electrodes. These consist of two exposed wires fixed together by an adaptor. A pair of cables connects the electrodes to the electric pulse generator, or electroporator, which controls the pulse duration, frequency, and voltage. For hands free operation, the electroporator can be activated by a foot pedal.

Once the eggs are ready, cut a window in the shell, and add a few drops of physiological salt solution, such as Hanks, to prevent the embryo from drying out. Under a microscope, position the egg such that the tissue of interest is accessible to the needle. Then, pierce the embryo with the capillary needle and deliver the solution by applying gentle pressure.

Now that we've injected the construct, it's time to perform the electroporation. Position the electrodes so that they are submerged in the Hank's solution and the tissue of interest is centered between them. In order to apply the electrical current, press the foot pedal and look for bubbles forming around the electrodes.

Once the electroporation is complete, remove the electrodes from the embryo and wipe them down with 70% ethanol. Add a few drops of salt solution supplemented with antibiotics to prevent infection, and seal the window with tape. Finally, place the eggs back in the incubator for continued development prior to phenotypic analysis.

Now that we've learned all about *in ovo* electroporation, let's look at some examples of how scientists are applying this technique in developmental research.

To begin, electroporation can be used to block gene expression by delivery of knockdown constructs. In this example, cells of the developing neural tube were electroporated with gene silencing constructs. The embryos were allowed to develop, and then axon trajectories were compared between normal and knockdown samples to assess the genetic control of axon guidance.

On the flipside, *in ovo* electroporation can also be used to express a protein in a subset of cells by introducing a plasmid containing a protein-encoding gene and a promoter: a sequence which binds RNA polymerase to initiate transcription. Here, scientists delivered a single gene into cells of the embryonic brain. Tissue staining to detect both the protein product of this gene and markers of specific neural subtypes shows that the electroporated gene alters neural development.

DNA constructs encoding fluorescent proteins can also be introduced by electroporation to visualize cells and structures during development. This allows for live imaging of complex processes, such as spinal cord development, giving insight into the dynamics of cell movements over time.

You've just watched JoVE's introduction to *in ovo* electroporation, a useful technique for delivering genetic material into chicks. This video discussed the basic principles of electroporation, steps required to inject and electroporate chicks, and applications of this technique in current developmental research. Thanks for watching!