Video Article

In Ovo Electroporations of HH Stage 10 Chicken Embryos

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Abstract

Large size and external development of the chicken embryo have long made it a valuable tool in the study of developmental biology. With the advent of molecular biological techniques, the chick has become a useful system in which to study gene regulation and function. By electroporating DNA or RNA constructs into the developing chicken embryo, genes can be expressed or knocked down in order to analyze in vivo gene function. Similarly, reporter constructs can be used for fate mapping or to examine putative gene regulatory elements. Compared to similar experiments in mouse, chick electroporation has the advantages of being quick, easy and inexpensive. This video demonstrates first how to make a window in the eggshell to manipulate the embryo. Next, the embryo is visualized with a dilute solution of India ink injected below the embryo. A glass needle and pipette are used to inject DNA and Fast Green dye into the developing neural tube, then platinum electrodes are placed parallel to the embryo and short electrical pulses are administered with a pulse generator. Finally, the egg is sealed with tape and placed back into an incubator for further development. Additionally, the video shows proper egg storage and handling and discusses possible causes of embryo loss following electroporation.

Video Link

The video component of this article can be found at https://www.jove.com/video/408/

Protocol

Egg handling

1. Eggs may be kept at 13°C for up to one week prior to incubation without significant loss of viability (a small wine cooler is ideal for this purpose).
2. Prior to incubation, allow eggs to warm to room temperature, then place in a humidified chicken incubator set to 37.8°C (100°F).
3. We usually electroporate embryos approximately 48 hours from the start of incubation, when the embryo has reached Hamburger and Hamilton stage 10.
4. Time of incubation may vary according to desired Hamburger and Hamilton stage. Temperature is critical and deviation from optimal incubation temperature will alter incubation time and decrease embryo viability.
5. During incubation, eggs should be kept on their sides so that the embryo will be properly positioned for electroporation. The embryo will float on top of the yolk and it is helpful to mark the top of the egg with a pencil before windowing so that you know where to cut.

Preparation

1. Warm Leibovitz L-15 media to 37°C. Pull glass capillaries into needles. Position electrodes and micromanipulator and connect electrodes to the pulse generator.

Windowing

1. Using a syringe with large bore needle, pierce the shell at the small end of the egg.
2. Pointing the needle carefully downward to prevent disruption of the yolk, remove about 5ml of albumin.
3. Seal the hole with a small piece of tape.
4. Cover the top of the egg with another piece of tape (about 4x4 cm).
5. Using curved scissors, and taking care not to disrupt the embryo, cut a hole just large enough to provide a window in which to work.

Optional: To visualize the neural tube, inject ink solution with 26 gauge needle underneath the embryo (insert needle under the embryo from outside the blood ring). India ink should be diluted 1:5 with Hanks or comparable sterile buffered solution.

Injection and Electroporation

1. Break the capillary tip to desired diameter using tweezers

2. Using the mouth pipette, load the capillary with plasmid/Fast Green dye.
3. Position the embryo with the head toward you and insert the needle into the neural tube at a shallow angle.
4. Inject the plasmid solution into the lumen of the neural tube until dye fills the entire space.

Note: Be careful not to have tip larger then neural tube diameter. In general, you will be able to tell if the optimal tip size has been achieved by how easy or hard it is to pull liquid into the capillary. If there is extreme resistance, the opening is probably too small and the tip should be broken again higher up. If there is little or no resistance, the opening is too large and a new needle should be used.

1. Place one to two drops of sterile L-15 media on the embryo.
2. Immediately place the electrodes on either side of the embryo, parallel to the neural tube, and pulse 5 times at 10-24 volts for 50 mseconds at 1 second intervals. You will see bubbles near the electrodes if it worked properly.
3. Carefully remove electrodes, put 5 drops of L-15 on top of the embryos and seal the egg with tape. Make sure that the egg is well sealed, as drying is a major contributor to embryo loss following electroporation.
4. Place eggs back into the incubator, window side up, and incubate until they reach desired H&H stage.

Discussion

This protocol provides a step by step guide to neural tube electroporation of HH10 chick embryos. In addition to showing the technique, guidelines for storage and handling of eggs is also provided. This technique has a broad range of applications for genetic analysis and the ease and low cost of experiments makes them feasible for many labs.

Disclosures

The authors have nothing to disclose.

References

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