Video Article

Windowing Chicken Eggs for Developmental Studies

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Abstract

The study of development has been greatly aided by the use of the chick embryo as an experimental model. The ease of accessibility of the embryo has allowed for experiments to map cell fates using several approaches, including chick quail chimeras and focal dye labeling. In addition, it allows for molecular perturbations of several types, including placement of protein-coated beads and introduction of plasmid DNA using in ovo electroporation. These experiments have yielded important data on the development of the central and peripheral nervous systems. For many of these studies, it is necessary to open the eggshell and reclose it without perturbing the embryo's growth. The embryo can be examined at successive developmental stages by re-opening the eggshell. While there are several excellent methods for opening chicken eggs, in this article we demonstrate one method that has been optimized for long survival times. In this method, the egg rests on its side and a small window is cut in the shell. After the experimental procedure, the shell is used to cover the egg for the duration of its development. Clear plastic tape overlying the eggshell protects the embryo and helps retain hydration during the remainder of the incubation period. This method has been used beginning at two days of incubation and has allowed survival through mature embryonic ages.

Video Link

The video component of this article can be found at https://www.jove.com/video/306/

Protocol

1. Remove eggs from incubator

1. Maintain at 37°C with relative humidity set above 60%.
2. Remove the eggs; turn eggs 90° so that the large base lies horizontal.

2. Swab eggs to sterilize

1. Saturate a stack of non-sterile gauze with 70% ethanol.
2. Use two to three pieces to swab up to 5 eggs. Discard when the gauze is soiled.

3. Preparing albumen removal site

1. Cut and place a 1" x 1" piece of 3M plastic tape just left of the base to protect the area where the albumin will be drawn out.

4. Removal of albumen

1. Use the point of a pair of scissors to make a small hole in the middle of the tape.
2. Using a 10 cc syringe with an 18-gauge, 1-inch needle, slowly drill the needle through the hole made by the scissors.
3. Drive the needle down at a 45°C angle towards the bottom of the egg.
4. Tilt the needle towards the center and draw up 3 to 4 mL of albumen.

5. Windowing

1. Cut a 3" x 3" piece of plastic tape and stretch it to fit on the top of the egg. Extend the corners of the square around the rounded ends of the horizontal surface of the eggs, being careful not to pull too hard. Pull the tape so that it is tight against the surface of the eggs with no folds.
2. Using a pair of sharp-straight 4" dissection scissors, twist a hole into the bottom center of the area where the tape was placed. Slowly guide the lower blade of the scissors into the egg being sure to keep the tips up against the inside of the shell. Direct the blade towards the base and slowly begin to cut the shell. Proceed in a counter-clockwise fashion, stopping just before reaching the top center. Remove the scissors and repeat going in the opposite direction until only a small bit of the egg remains attached. Check to be sure the egg is fertilized. Shut the window.

6. Closing, reopening and sealing the egg.

1. Cut about a 2-3" long by 1/2" wide plastic tape and shut the window so it fits back into the hole that was cut. Take another 1 x 1" piece of tape and seal the hole from which the egg was drained. Use a pair of forceps to reopen the egg to do any manipulations. When you're ready to return the eggs to the incubator, cut a piece of tape that is large enough to seal the window and cover the entire horizontal surface of the egg.

Discussion

This method allows for long survival times following experimental manipulations. For example, dye-labeling studies allowed survival to E10 or later in a fate mapping study (1). In addition, it has been used for in ovo electroporation studies that provide alteration of gene expression levels and required development to proceed to later developmental stages (2-6). This windowing technique can be used in combination with any procedure that requires survival after manipulations to the embryo.

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