Video Article

Method for Culture of Early Chick Embryos ex vivo (New Culture)

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Abstract

The chick embryo is a valuable tool in the study of early embryonic development. Its transparency, accessibility and ease of manipulation, make it an ideal tool for studying the formation and patterning of brain, neural tube, somite and heart primordia. Applications of chick embryo culture include electroporation of DNA or RNA constructs in order to analyze gene function, grafts of growth factor coated beads such as FGFs and BMPs , as well as whole mount in situ hybridization and immunohistochemistry. This video demonstrates the different steps in chick embryo culture; First, the embryo is explanted in saline. Then, the embryo is centered on a glass ring. The membranes surrounding the embryo are lifted along the walls of the ring. The ring is then placed in a culture dish containing a pool of albumine. The culture dish is sealed and placed in a humid chamber, where the embryo is cultured for up to 24 hrs. Finally, the embryo is removed from the ring, fixed and processed for further applications. A troubleshooting guide is also presented.

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=903

Protocol

Part 1: Bench set up

1. A humid chamber is prepared by placing Kimwipe/ddH_2O in plastic chamber.
2. A Falcon tube to collect albumin, dishes for culture, rings, watchglass and waste disposal are placed on bench.
3. Pyrex dish is filled with 1.4 l saline (see notes [a]).

Part 2: Embryo is explanted in saline

1. Eggs are removed from the incubator after 16 hrs (stage 4). The egg is opene by tapping the shell with forceps. Shell pieces are removed.
2. The thin albumin is collected in Falcon tube. The thick albumin is removed with forceps.
3. The embryo is placed in a plastic dish inside the saline dish. The remaining albumin is removed with forceps.

Part 3: Embryo is centered on ring

1. The yolk sac is tilted with forceps so that embryo faces upwards.
2. The yolk sac is cut at the level of the equator or below.
3. Using fine forceps, the vitelline membrane is swiftly peeled. The vitelline membrane is oriented so that its granular side (non shinny) faces upwards.
4. Using fine forceps, the vitelline membrane is placed on the watch glass.
5. Using fine forceps, a glass ring is applied on top of vitelline membrane and the embryo is centered.
6. The vitelline membrane is lifted around edges of the glass ring. The assembly is removed from the saline dish.

Part 4: The culture is set up under microscope

1. The vitelline membrane is lifted over the glass ring using fine forceps under microscope.
2. Using Pasteur pipette, the saline is removed from the outer edge of the ring.
3. Using fine scissors, excess vitelline membrane is removed from the inner edge of the glass ring. Care is taken not to pierce the membrane with pipette or forceps.
4. The embryo is gently rinsed with saline to remove loose membranes and yolk cells.
5. 200 µl saline is added to outer edge of the ring (this will facilitate later transfer).
6. The assembly is covered with an inverted plastic dish.

Part 5: The culture is transferred to incubator

1. A Falcon culture dish is labelled.
2. 2.5ml of thin albumin is added to the bottom of the Falcon dish.
3. 200µl of thin albumin is added to the inner edge of the lid of the Falcon dish.

4. The inverted dish is removed from the assembly.
5. Using fine forceps, the glass ring is slided along the edge of the watch glass, and transfered to the Falcon dish.
6. All remaining saline is removed from inner surface of the ring.
7. The Falcon dish is covered with lid and sealed.
8. The assembly is placed in humid chamber and then in the incubator.
9. The embryos are cultured for up to 24 hrs at 38°C.

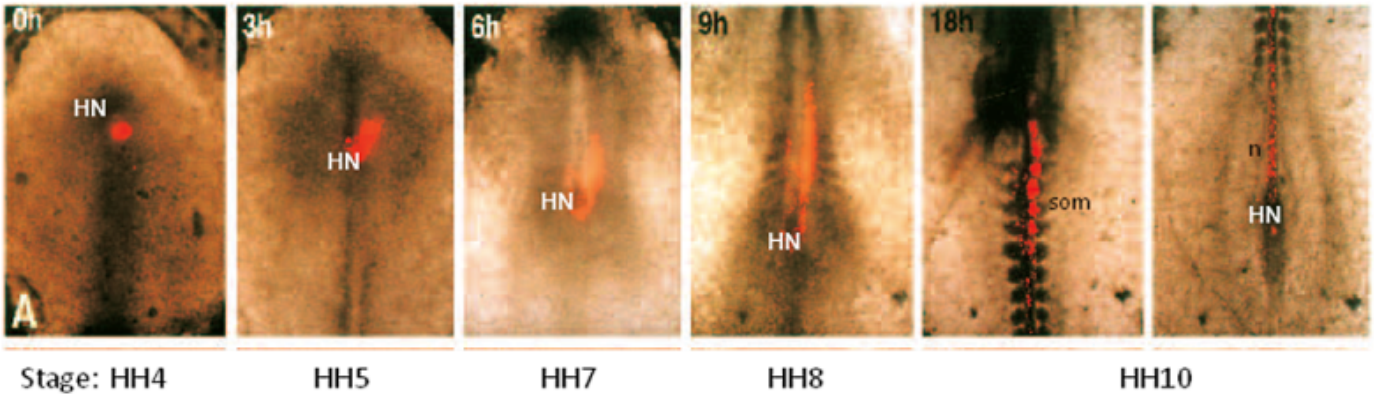
Part 6: Following culture, the embryo is fixed; culture is transferred to incubator

1. A fixation dish is filled with ice-cold PBS (or depc-PBS if embryos are to be processed for *in situ* hybridization).
2. The culture is removed from the incubator and immediately placed on ice. The culture dish is immediately filled with ice-cold PBS/depc-PBS.
3. The embryo is detached from the vitelline membrane. The embryo is transferred to the fixation dish, using the blunt end of a Pasteur pipette.
4. The embryo is pinned down using blunt-ended fine forceps. The PBS covering the fixation dish is removed. Fixative is added (see notes [b]).
5. Depending on application, the embryo is fixed for 6-8 hr at 4°C (cryostat), O/N at 4°C (in situs), or 1 hr at RT for whole mount immunohistochemistry.
6. The fixative is removed and replaced with ice-cold PBS/depc PBS.
7. For downstream applications such as *in situ* hybridization or immantibody staining: the nervous system and the heart are perforated using a blunt end microcapillary needle or a microdissection knife; this will prevent trapping of probe/antibody in later steps.

Notes: [a] Saline consists of: solution A (for 1 l): 121.0 g NaCl, 15.5 g KCl, 10.4 g $CaCl_2.2H_2O$, 12.7 g $MgCl_2.6H_2O$; solution B (for 1 l): 2.4 g $Na_2HPO_4.2H_2O$, 0.2 g $NaH_2PO_4.2H_2O$; Autoclave; Prior to use, mix 120 ml A with 2700 ml H_2O; Add 180 ml B. Mix [1]; [b] Fixative is prepared just prior to use (4% PFA in PBS or depc-PBS for in situs).

Part 7: Representative Results

Prior to culture, the embryo is at primitive streak stage (HH4). At the end of the culture period, the embryo has developed to HH10 (length 2-3 mm) and is visible in the center of the culture dish. It is possible to label a group of cells with carbocyanine fluorescent DiI just before culture (0h) and follow their movement throughout the culture period. In this case, cells below Hensen's node (HN) were labelled with DiI. These cells are shown to contribute to progressively developing somites (som) and notochord (n).



Part 8: Troubleshooting

Problem	Cause	Remedy
Embryo remains with yolk instead of coming off with membrane (step 2)	Poor egg quality	Request freshly produced eggs; Store eggs at 13°C upon receipt. Incubate eggs the same day as arrival date.
Vitelline membrane slide away from watchmaker's glass following placement of ring (step 3)	Albumin remnants	In step 2, make sure that all albumin is removed by pulling it away from membrane with tilted forceps
Embryo is inaccessible: lies underneath the membrane (step 4)	Wrong side of membrane is upwards	In step 3, make sure the side of the membrane containing yolk granules is facing upwards. The shiny, polished side should face downwards.
Embryo submerged in saline/albumin following culture(step 6)	Saline/albumin left inside ring prior to culture; hole in membrane	In step 5, make sure that all albumin/saline is removed from inside the ring; In step 4, make sure you do not pierce membrane with forceps (use blunt ended forceps)
Embryo disintegrated following culture	Bacterial infection	Sterilize all tools and glassware; Use antibiotic/antimycotic

Discussion

The New culture method [2] can be used for a wide variety of applications, ranging from grafts of growth factor containing beads [3], to whole mount *in situ* hybridization and whole mount immunohistochemistry [4]. Culture over a 24 hr period enables the continuous monitoring of embryonic development in applications such as time lapse cell movement analysis [5] or monitoring of GFP containing electroporated constructs [6].

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