Video Article

# Dissection of Organizer and Animal Pole Explants from Xenopus laevis Embryos and Assembly of a Cell Adhesion Assay

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## Abstract

## Video Link

The video component of this article can be found at http://www.jove.com/video/187/

## Protocol

## Preparing Adhesion Chambers (the day before the injection/dissection)

### Adhesion Assay Chambers

If you have a conventional compound microscope (optical locates above the stage), it is ncecessary to make a low-height adhesion chamber as follows. If using the inverted-optics microscope, then "NUNC Lab-Tek Chambered Coverglass" can just be used, and skip the steps #1 - 2.

1. Attach one of "press-to-seal silicon insulator" on a 40 x 24mm cover slip. Firmly press the insulator to the cover slip on clean benchtop and look from the other side to make sure that the insulator is completely attached without any air-bubbles.

2. *Optional:* Put the adhesion chambers in a glass Petri dish with Whatman filter paper and autoclave in dry-cycle to sterilize. Cool them down to RT.

### Coating the Chamber

1. Put a 200ml droplet of working solution of cadherin (10mg/ml) or fibronectin (20mg/ml) onto the center of an autoclaved adhesion chamber, using sigmacoated yellow tips. Incubate at RT for 4 hours.

2. Take out the cadherin or fibronectin solution and transfer to a 1.5ml tube. This solution can be reused for next two weeks.

3. Dispense 500ml of 4% BSA in 1x MBS-H to block the chamber. Incubate at RT for 1 hour.

4. Aspirate the BSA solution. Wash the chamber with 500ml of 1x MBS-H twice. After the second wash, aspirate most of MBS-H (not completely), and store in a sealed container at 4ºC. This adhesion chamber is good for the next 2 - 3 days.

### Dissection and Dissociation of Animal Cap Explants

1. Incubate the injected embryos in 0.1x MBS-H, diluted from the autoclaved 1x MBS-H, as non-autoclaved medium sometime carries little germs that can disturb the dissociated animal cap cells.

2. Make 1x Barth's agarose plates and CMF-MBS agarose plates with 60mm dishes, at least as many as the number of your samples. Pour autoclaved 1x MBS-H to 1x Barth's plates. For CMF-MBS plates, pour 10ml of CMF-MBS.

3. When the embryos become stage 8.5, start dissecting the animal caps in a 1x Barth's plate. 10 - 15 explants per samples are usually enough for an experiment.

4. Transfer the animal cap explants to a CMF-MBS plate. Incubate the explants for 45 - 60 minutes until the explants are completely dissociated. Gentle agitations in every 15 minutes will help the dissociation.

## Cell Adhesion Assay

1. Seeding the cells: Dispense 500ml of 1x MBS-H to the adhesion chamber prepared in the steps in the sections above, Adhesion Assay Chambers and Coating the Chamber. Transfer the dissociated animal cap cells from the CMF-MBS plate to the adhesion chamber using P200 with a sigmacoated and chopped-off yellow tips.

2. *Optional:* If possible, carefully suck up the medium up to approximately 2/3 to halfway, without exposing cells to the air. Add approximately the same amount of fresh MBS-H, to make sure that the medium contains sufficient $Ca^{2+}$ and $Mg^{2+}$.

3. Incubate at RT for 45 - 90 minutes. Check the cells occasionally to see if they are attaching to the bottom surface.

4. Now you can take the picture of the cells under a microscope.

5. Put the adhesion chamber on a grid slide glass. Align one of the corners of the adhesion chamber to a corner of the grid slide. Count and record the number of the attached cells in several different grids.

6. Pour 1L of MBS-H in a large plastic bucket (e.g., Tupperware, etc.). Gently put the adhesion slide chamber to the bottom of the bucket. Rotate it on the orbital shaker for five minutes at low speed (50 - 60rpm).

7. Put the adhesion chamber back on the grid slide, aligning the same corner of the chamber to the same corner of the grid slide, as was done in step #5 of this section, Cell Adhesion Assay. Count the number of cells which remain attached on the same grid that you have counted before the washing. Calculate the percentage of cells that remain attached after the wash.