

Blastocyst harvest and growth in vitro

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“Manipulating the Mouse Embryo” has a nice protocol with pictures – Chapter 4, Protocol 11 on pp201-203 of 3rd Edition. This is just my optimization.

Blastocyst culture is necessary for studying early embryonic lethality of mice and also for the establishment of ES cells. If you are trying to get ES cells, see Chapter 8, protocol 5 of “Manipulating the Mouse Embryo”.

You will need M2 media (Sigma M7167) – order this when you set up your mating pairs.

Set up natural matings, checking plugs as soon as possible each morning (see Guide to Mouse Rooms for more info). The day a plug is discovered is Day 0.5 (Assume the mice mate at midnight, so noon is ½ a day after mating)

Three days later, harvest blasts from the mother.

I find it is essential to do harvest between 2pm and 4pm as the blasts are often already breaking out of the zona pelucida by then. Do not attempt after 6pm because the embryos will be implanting into the uterus and you’ll miss some. If this is your first harvest, plan on doing no more than 2 in an afternoon.

Materials:

0.1% or 0.2% gelatin in water or in PBS

U-bottomed 96-well plates (if you are genotyping cells) or, chambered slides if you are doing IF on the blasts later

M2 media

60mm TC plates (3 per female harvested)

Pulled capillary tubes (Kimax #34502, 0.8-1.10 x 100mm) mouth aspiration assembly (Sigma A5177)

DMEM with 15% FCS and non-essential amino acids (NEAAs)

Needle (20Gauge, 1 ½ inch length) that has tip filed off so it is blunt

1mL syringe dissection tools (forceps and scissors, 70% ethanol, paper towel)

Preparation (noon)

Begin by placing gelatin in 12 wells of the 96-well plate

Make DMEM with 15% FCS and NEAAs (if you don’t have it already)

Take one of the 60mm plates and draw stripes on the bottom, approximately every 5mm with a fine-tipped pen (blue sharpie is perfect).

Place 1mL of M2 media in the dish with the stripes, and 3mL in both non-striped dish, place these in a 37° C incubator to warm up.

Wear down the sharp tip of the needle using a metal file

Pull some capillary tubes in flame, aiming for medium-sized openings

Prepare dissection area by cleaning the area with 70% EtOH

Remove gelatin from wells, add DMEM (80µL per well), place in incubator

Blastocyst Harvest

Euthanize the pregnant mouse.

Dissect out the uterus, keeping the two uterine horns connected at the bottom, snipping the tops just below the skinny fallopian tubes. Dip the uterus in one non-stripped dish to remove some of the blood cells. Place uterus into the stripped dish.

From the other, non-stripped dish, suck 1mL M2 into syringe. Flush 0.5mL M2 down each uterine horn. Suck up 1mL M2 from non-stripped dish, flushing each horn from the connected, bottom holes.

Remove the uterus from the dish, clean up dissection area quickly (to get the mouse carcass out of sight and spare your labmates) and gently walk the stripped dish to a TC microscope with 10x objective. Get mouth aspirator, pulled capillary tubes, lab pen and 96-well plate.

Starting in center, search up and down along the stripes (10x magnification) for blastocysts. To find the correct focal plane, focus on the red blood cells settling on the dish or use capillary tube to make scratches on the dish, then focus on the scratches.

When a blast is located, transfer by mouth aspiration to well in the 96-well plate, and number the well. From a BL/6 mouse, 6 blasts is common, though 8-12 blasts occur occasionally. I believe that about 1/8 mice that have plugged will not have any blasts.

Allow the blasts to grow for 48 hours, change the media. (this is Day 5.5) on Day 7.5, change media again. Take pictures as needed.

The next morning (day 8), trypsinize the blast and genotype.