BLASTOCYST OUTGROWTHS

Required materials for dissection/flushing:

KSOM medium

MEM-HEPES

Mineral oil

(4) 35 mm Petri dishes

(2) 35 mm tissue culture dish

65 mm Petri dishes

3 ml syringe

30-gauge needle, blunted

Micropipettes

Forceps and dissection scissors

Before beginning:

1. The day before collection, superovulate 7-week-old female mice according to protocol (see: *Superovulation* protocol).

2. The night before collection, prepare KSOM medium:

   Under sterile conditions (i.e. in laminar flow hood), place three (3) 50 µl drops of KSOM for embryo collection in a 35 mm culture dish. Cover drops completely with mineral oil.

   Place five (5) 40 µl drops of KSOM for washing in a second 35 mm culture dish. Cover drops completely with mineral oil.

   Place dishes in 37°C/5% CO_2 incubator to equilibrate overnight.

3. At least 30 minutes before collection, add 2 ml MEM-HEPES to each of four (4) 35 mm Petri dishes for collection and preheat medium to 37^{o}C for at least 30 minutes.

Dissection and flushing:

1. Sacrifice superovulated females on the afternoon of day 1.5 after mating.

2. Remove oviducts together with a small part of the uterus and place in the first collection dish. Under a microscope, remove as much fatty tissue from oviducts as possible.

3. Fill 3 ml syringe with ~1 ml MEM-HEPES for flushing.

4. Wash one set of oviducts by transferring to the second collection dish. Remove any remaining fatty tissue using forceps and dissection scissors.

5. Transfer the set of oviducts to a 65 mm dish. Cover the oviducts with some prewarmed MEM-HEPES to prevent them from drying out.

6. Insert the end of the blunted 30-gauge needle into the opening of one oviduct. Hold it in place with forceps.

7. Gently flush embryos out of the oviduct using the MEM-HEPES in the syringe. Try to use no more than 0.5 ml of medium.

8. Repeat for the second oviduct.

9. Under the microscope, find embryos that were flushed out of the oviducts into the dish. Transfer embryos as you find them to one of the 50 µl drops of KSOM.

10. Once all embryos have been found, wash by transferring them to a 40 µl drop of KSOM. Place the dish back in the incubator.

11. Repeat for all remaining sets of oviducts, using a new 65 mm dish for each uterine horn.

12. Once embryos have been collected from all oviducts, wash groups of 15-20 embryos a second time in another of the 40 µl KSOM washing drops.

13. Transfer all embryos to the 50 µl drops of KSOM culture media.

14. Place the culture dish with the embryos in the 37°C/5% CO_2 incubator.

15. Incubate the embryos for 3 days until they reach the late blastocyst stage.

Important notes:

Place embryos into the incubator AS SOON AS POSSIBLE after collection.

Embryos should not remain in MEM-HEPES collection medium for more than 30 minutes. Embryos in MEM-HEPES should be kept on a heated 37°C microscope stage.

Do not keep embryos out of the incubator for more than 10 minutes.

Use a new micropipette each time you collect embryos after culturing.

Required materials for blastocyst outgrowth:

0.1% gelatin

2 ml fetal bovine serum (FBS)

D-MEM (or equivalent culture medium)

2 ml Tyrode's solution

MEM-HEPES

1x PBS

24-well plate

(2) 35 mm Petri dishes

Blastocyst outgrowth:

1. Prepare blastocyst outgrowth culture plate:

   Pipette 500 µl of 0.1% gelatin into well(s) of 24-well plate. Incubate 10 minutes at room temperature to allow gelatin to coat bottom of wells. Remove all 500 µl of solution and allow gelatin to dry at room temperature for 10 minutes.

   Prepare 20% FBS-supplemented culture medium by adding 2 ml FBS to 8 ml D-MEM. Pipette 1 ml of culture medium into each well.

   Incubate plate in incubator at 37°C in 5% CO_2 in air for at least 30 minutes to allow medium to equilibrate.

2. Add 2 ml Tyrode's solution to one 35 mm Petri dish and 2 ml MEM-HEPES to a second 35 mm Petri dish. Heat media to 37°C for at least 30 minutes.

3. Remove the zona pellucidae of the blastocysts by incubating them for 10-30 seconds in 2 ml prewarmed Tyrode's solution until you see the zona dissolve under a microscope. (Note: easiest to do this in groups of 5-10 blastocysts at a time.)

4. Immediately transfer blastocysts to a wash dish containing MEM-HEPES.

5. Transfer 1 blastocyst to each well of the prepared blastocyst outgrowth culture plate.

6. Culture at 37°C/5% CO_2 for 96 hours. Blastocysts will adhere to the plate and grow outwards.

7. After 96 hours, examine the blastocysts under a microscope. Record their appearance, and take pictures of them for future reference.

8. Remove the culture media from each well and wash each well 3 times with 1x PBS.