DENUDED OOCYTE COLLECTION

I. Medium Preparation

1. Prepare a dish of 1xPBS and incubate at 37°C

2. Prepare two dishes of MEM-HEPES: if collecting 12 day old and older GV oocytes, make sure to add dbcAMP to medium (10 µL of 10 mg/ml stock for every 1mL of medium).

   Note: a cover drop of mineral oil can be added on top of MEM-$NaHCO_3$ dish to optimize medium conditions when dish is taken out of incubator. This significantly increases survival rate of cells when doing microinjection for instance.

II. Collecting Ovaries

1. Excise ovaries from mice and put them in the MEM-HEPES media.

2. Clean ovaries by removing surrounding tissue.

   Note: Ovary should be inside a bursa between a coil of fallopian tube and a sheath of fat, to remove the ovary intact, gently puncture/tear bursa and pull on fat and coil gently.

3. Transfer ovaries to a new MEM-HEPES media dish

III. Collecting Oocytes

1. Use needles to puncture the ovary repeatedly to pop follicles.

2. Allow a couple of minutes after each ovary to allow oocytes to ooze out of follicles. Use P1000 to pipette media with ovaries up and down – this helps with disrupting follicles and denuding the oocytes.

3. Use a mouth pipette to isolate and collect oocytes.

   Note: the right size for a pipette-tip is determined by what you are collecting. In this instance, you want 'nude' oocytes so you want your tip's diameter to be slightly bigger than that of the oocytes you are collecting; big enough to avoid squeezing and damaging them, but small enough to be accurate and selective during collection.

   Note: When collecting smaller oocytes from 15 day old (or younger) mice adding 20 µL Trypsin and 20µL collagenase to the media, mixing it with the pipette, and incubating for 5 min will help with denuding the smaller, more compact follicles.

4. Transfer oocytes into a third MEM-HEPES dish to clean them and get rid of excess granulosa cells.

5. **(optional)** To get MII cells, transfer fully grown GV oocytes into MEM-$NaHCO_3$ dish and incubate in incubator (37°C) overnight. MII oocytes can be collected the next morning.

IV. Storing oocytes

1. For Western blots transfer oocytes into a 0.6 mL tube while minimizing amount of solution transferred. Add Laemmli buffer and β-Mercaepthanol mix solution (1:1 ratio). Denature by heating to 95-100^{o}C for 5 minutes (the MJ Cycler). Freeze at -20^{o}C until use. Samples may also be frozen prior to denaturation in -80^{o}C.

2. For RNA extraction and RT-PCR transfer oocytes into a 0.6 mL tube while minimizing amount of solution transferred. Oocytes can be stored at -80^{o}C until ready to extract RNA and proceed to RT and PCR (avoid storing for long periods of time).

3. For whole-cell immunofluorescence transfer oocytes into 500 µL of fresh 2% formaldehyde in a concave 9-well glass plate and incubate at RT for at least 15 min (30 min max). Transfer cells into 500 µL of 3% BSA (in 1x PBS 0.1% Triton-X) blocking in a well of a 4-well plate for storage at 4^{o}C until ready to be used for staining. Staining can be done in 72-well plates with 13.5 µL of solution in each well used.

4. For micro-injections transfer oocytes from the MEM-HEPES dish to a MEM-$NaHCO_3$ dish with dbcAMP that has been incubating for at least 1 hour in the 37^{o}C incubator. Incubate for no more than 2 hours (the zona will be too loose and the oocyte harder to inject). Proceed to microinjection.