ZONA PELLUCIDA REMOVAL USING ACID TYRODE'S SOLUTION AND SNAP FREEZING

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MATERIALS

- Gloves
- Wiretrol or other embryo handling tool
- RNAse wipes
- Grid plate (Fisher Scientific) with lid or similar plastic dish suitable for cell culture
  - Use new plates for washing and treating embryos with Acid Tyrode's solution – left-over lids and plates are good for fixing embryos in paraformaldehyde, but they are not adequate for when collecting RNA because of possibility of RNase contamination.
- 1 aliquot autoclaved DEPC-treated DPBS/PVP 0.2%
  - Use aliquots from the plastic boxes inside the IVF fridge – Molecular biology use only. These aliquots are for single use.
- 1 aliquot of Acid Tyrode's Solution
  - Use aliquots frozen in the IVF freezer. These aliquots are for single use.
  - Purchase ready-made from Sigma: T1788
  - *Note:* to make from scratch (Cold Spring Harbor Protocols), dissolve 0.8 g NaCl, 0.02 g KCl, 0.024 g $CaCl_2 \cdot 2H_2O$, 0.01 g $MgCl_2 \cdot 6H_2O$, 0.1 g glucose and 0.4 g polyvinylpyrrolidone in 100 ml water. Prepare at room temperature and adjust to pH 2.5 with 37% HCl. Filter-sterilize and store in aliquots at -20°C.

SETUP

- Clean microscope, area around it, warmer plate and pipettes with alcohol followed by a RNAse wipe
- Pre warm media: make two 50 µl-Acid Tyrode's drops and six 25 µl drops of DPBS/PVP, close the lid of the plate and let the drops warm for 5 minutes
- Label Eppendorf tubes from the molecular biology area to identify your samples
- Fill small Styrofoam box partially with liquid nitrogen

PROTOCOL

- Wear gloves
- Take embryos from culture drop and wash through three drops of DPBS/PVP 0.2%
- Place embryos in first pre-warmed drop of Acid Tyrode's; quickly move to second drop, carrying over as little extra media possible
- Place dish back on the warmer plate
- Check embryos for ZP dissolution after 30 sec
- Remove embryos from Acid Tyrode's AS SOON as the ZP is dissolved (30 sec-2min)
- Wash embryos through three drops of DPBS/PVP 0.2% (embryos will be STICKY)
- From the last drop, pick embryos together with 5 µl of DPBS/PVP and place in the labeled Eppendorf tubes
- Immerse tube in liquid nitrogen to snap freeze
- Immediately place samples in box in the -80ºC freezer