Collection and handling of mouse oocytes/embryos

CURRENT STATUS: POSTED

ADITYA SANKAR
University of Copenhagen

adisankara2000@gmail.com*Corresponding Author*
ORCiD: https://orcid.org/0000-0002-1840-3356

DOI:
10.21203/rs.2.21552/v1

SUBJECT AREAS
Developmental biology *Genetics*

KEYWORDS
Embryology

Abstract

This procedure describes how to retrieve mouse oocytes and/or embryos, clean and process them for ChIP or RNASeq experiments

Reagents

M2 medium (Merck Millipore MR-015-D)

KSOM Embryomax medium (Merck Millipore MR-106-D)

M2 medium with hylarunidase (MR-051-F)

Acid Tyrodes solution (Sigma T-1788)

Equipment

Leica brightfield dissection microscope

Basic mouse dissection tools with forceps

Pulled glass capillaries

Mouth pipette with filter for embryo handling

Petridish

Procedure

1. For collection of MII oocytes, four to eight-week-old donor female mice were injected with 5 units of pregnant mare serum gonadotropin (PMSG) at 18.00 (100 µl of 50 I.U/ml solution)

2. this is followed by 5 units of human chorionic gonadotropin (hCG) at 17.00 (100 µl of 50 I.U/ml solution) the following day.

3. Donor mice were sacrificed by cervical dislocation 14-15 hours post hCG injection.

4. Oviducts are transferred to a clean petridish dish with M2 (Sigma) medium.

5. Cumuli were released from the ampulla in M2 medium and disaggregated in M2 medium containing 0.3 mg/ml hyaluronidase

6. This is followed by four washes in M2 medium.

7. For zona removal, oocytes/ preimplantation embryos are transferred in a 50µl drop of Tyrode’s solution (Sigma) to remove the zona pellucida.

8. this is followed by three washes in M2 medium droplets before downstream processing for Chip or RNA Seq.

9a. For Chip Seq, oocytes were given a fourth final wash in M2 before crosslinking and fixation as described in a separate protocol.

9b. For RNASeq, the oocytes and embryos were washed three times in sterile PBS before a final wash in nuclease free water supplemented with RNAse inhibitor, prior to tubing single oocytes/embryos in single 0.25mL units of a sterile 8-tube PCR strip for lysis and downstream cDNA amplification.

Time Taken

1 day from time of harvest of oocytes/embryos

Acknowledgements

Javier Martin Gonzalez, Transgenic Core Facility, University of Copenhagen