Immunofluorescence of mouse zygotes and preimplantation embryos

ADITYA SANKAR (adisankara2000@gmail.com)
University of Copenhagen https://orcid.org/0000-0002-1840-3356

Method Article

Keywords: Epigenetics, developmental biology, transposons, histone modification

Posted Date: January 27th, 2020

DOI: https://doi.org/10.21203/rs.2.21543/v1

Abstract

This protocol is based on previously published work and described the procedure of immunofluorescence of mouse zygotes and preimplantation embryos. The procedure takes 2 days from recovery of gametes/embryos from the mouse to imaging of samples in a confocal microscope. The procedure uses bent capillaries and a mouth pipette with a filter, allowing for movement of the samples between solutions following necessary incubation times. This simple procedure works quite efficiently and is an easy skill to master with the availability of the necessary tools.

Introduction

Reagents

Material:

- M2 medium supplemented with 4 mg/ml BSA;

- M2/BSA medium supplemented with hyaluronidase from 6 mg/ml stock diluted 1:20 (300ug/ml final concentration);

- PBS supplemented with 1% (w/v) BSA;

- PBS supplemented with 1% (w/v) BSA and 0.1% (v/v) Triton X-100;

- PBS supplemented with 4% (w/v) PFA (resolubilized by heating at 37C and cooled down before using).

- Oocytes/preimplantation embryos from mouse

Equipment

-Standard Leica bright field dissection microscope

- 96 well round U-bottom plate (Nunclon)

- 45 degree bent glass capillaries

- Mouth pipette for handling and denudation of oocytes/embryos

Procedure

Collection and storage of oocytes/pre-implantation embryos:

- Clean the oocytes/embryos from surrounding cumulus cells/sperm by incubating them in a 50 uL drop of hylarunidase diluted 1:3 with M2 media.

- Keep the drop at 37C for 3-4min on a heated stage.

- Collect and wash oocytes/embryos in M2 media.

- Remove zona pellucida (this also clears out any remaining cumulus cells) by washing in a 50 uL drop of Acid Tyrode's solution while moving the zygotes continuously. Once you see the zona dissolving and egg is naked, transfer into a drop of M2 media.

- Now wash through 2 such drops and finally collect all eggs in an organ well dish with M2 supplemented with 4mg/ml BSA medium. Purpose of BSA is allow easy movement of eggs and promoting non-stickiness

- Wash once in PBS-1%BSA.

- Fix in 4% PFA (pre-warmed to 42 C) for exactly 20 min.

- Wash 3 x 10 min in PBS-1%BSA.

- Now oocytes can be stored at 4C for 1-2 weeks.

Immunofluorescence

- Permeabilization in PBS-1%BSA-0.5%Triton for 30 min (triton 0.1% or 0.5% depending on the staining).

- Incubation with 1st antibody O/N at 4C in PBS-1%BSA-0.1%Triton.

- Wash 3 x 10 min in PBS-1%BSA-0.1%Triton.

- Incubation with Alexa coupled 2nd antibody diluted 1:300 in PBS-1%BSA-0.1%Triton for 1h at RT in the dark.

- Wash 1 x 10 min in PBS-1%BSA-0.1%Triton.

- Wash 2 x 10 min in PBS-1%BSA.

- Stain for 5 min with DAPI from 50 ug/ul stock diluted 1:50 in PBS-1%BSA (or) skip if using mounting media with DAPI in-built.

- Wash 1 x 10 min in PBS-1%BSA (only if you have manually stained with DAPI).

- Wash briefly in PBS.

- Mount with Prolong Gold or Vectashield medium with DAPI, seal the cover slips on glass slides with nail polish (use colorless nail varnish to limit potential autofluorescence), let the mounting medium polymerize O/N in dark and store at -20C until microscopic imaging.

Soluble protein pre-extraction protocol (this is advised if you are staining for chromatin bound proteins for high specificity in signal)- the protocol is different at the initial and final mounting stages.

Material:

- Ice-cold permeabilization solution with Triton X-100: NaCl 50 mM, $MgCl_2$ 3 mM, Triton X-100 0.5%, sucrose 300 mM in HEPES 25 mM pH 7.4.

- Ice-cold permeabilization solution without Triton X-100: NaCl 50 mM, $MgCl_2$ 3 mM, sucrose 300 mM in HEPES 25 mM pH 7.4.

Procedure:

Start from washed zygotes in PBS-1%BSA with intact zona pellucida.

Extract soluble protein for 10 min on ice using ice-cold permeabilization solution with Triton.

Wash 3 x 10 min in ice-cold permeabilization solution without Triton.

Fix and stain as described above.

Sequential washing steps with increasing concentrations of glycerol (10%, 20%, 40%, 80%, 100%) in PBS-1%BSA before mounting.

Troubleshooting

Time Taken

2 days

Anticipated Results

References

Nashun, B. *et al.* Continuous Histone Replacement by Hira Is Essential for Normal Transcriptional Regulation and De Novo DNA Methylation during Mouse Oogenesis. *Mol Cell* **60**, 611-625 (2015).

Acknowledgements

Buhe Nashun (post-doc), Petra Hajkova Laboratory