In vitro transcription and micro-injection of *Kdm4a* mRNA into mouse oocytes

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Method Article

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Abstract

This experiment describes the *in vitro* transcription of *Kdm4a* wildtype and H188A catalytic dead mRNA. This details also its subsequent injection into mouse oocytes followed my IVF to track the impact on embryo development. The procedure is technically challenging and performed by the Transgenic Core Facility at the University of Copenhagen. Oocytes have a poorer survival rate following mRNA inject as against zygotes. However the objective was to demonstrate the earliest stage of intervention to rescue developmental failure of KDM4A maternal zygotic mutant embryos

Introduction

The objective and rational of the experiment is as follows.

- generate high quality mRNA suitable for microinjection.

- inject MII oocytes followed soon after by in vitro fertilization using KDM4A null sperm.

- track development rate of morulae and blastocyst.

Note: A pilot experiment to track efficacy of H3K9me3 demethylation by injected *Kdm4a* mRNA in wildtype zygotes is conducted through immunofluorescence against H3K9me3. Once a good batch of IVT mRNA is identified, aliquots of the same batch mRNA are used in actual experiment.

Reagents

Addgene plasmid RRID#101051: pcDNA-flag-hKDM4A-polyA

Mutagenesis primers:

1. hKdm4aH188A_Fw: 5’-AAGACATCCTTTGCTTGGGCAACTGAAGACATGGACCTC-3’

2. hKdm4aH188A_Rv: 5’-AAGACATCCTTTGCTTGGGCAACTGAAGACATGGACCTC-3’

Kits and enzymes:

1. Quikchange II XL Site-directed mutagenesis kit (Agilent 200521)

2. SfoI restriction enzyme(NEB R0606)

3. mMESSAGE mMACHINE T7 Ultra Kit (Life Technologies AM1345)

Equipment

FemtoJet® (Eppendorf),

CellTram® Air (Eppendorf)

Narishige micromanipulators

Procedure

1. The H188A mutation was achieved on the *in vitro* transcription ready pcDNA-flag-hKDM4A-polyA plasmid using the Quikchange II XL Site-directed mutagenesis kit (Agilent 200521).

2. The mutant plasmid was sequence verified and then both wild-type and mutant mRNA were linearized using *SfoI* (NEB R0606)

3. this was followed by *in vitro* transcription, poly(A) tailing and precipitation according to manufacturers instructions of the mMESSAGE mMACHINE T7 Ultra Kit (Life Technologies AM1345).

4. Multiple 10 microliter sized single thaw-and-use aliquots of 1000ng/uL wild-type and H188A mutant *Kdm4a* polyA-mRNA were prepared and frozen at -80 C.

5. Microinjection of MII oocytes take place 14 hours after hCG injection in M2 medium on a station equipped with FemtoJet® (Eppendorf), CellTram® Air (Eppendorf) and Narishige micromanipulators.

6. Approximately 10 pL of a stock concentration of 1000 ng/uL of either wild-type or H188A *Kdm4a* poly(A) mRNA was injected into cytoplasm of MII oocytes.

7. Injected oocytes are then transferred to a HTF medium drop and undergo IVF as described in an independent protocol listed here, soon (0.5-1 hr) hour after mRNA injection.

8. The resulting embryos are incubated at 37 °C, 5% CO_2 in KSOM-Embryomax medium (Merk Millipore) that is refreshed daily up to E5,5 stage, while observing the progress of development.

Troubleshooting

Time Taken

1. in vitro transcription and aliquoting (1day)

2. Oocyte isolation, microinjection and IVF (2-3 hours).

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).

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