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Construction of protein-based artificial kinetochore in mouse oocytes

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Designing protein-base...

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We use this protocol and it's working

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Abstract

This protocol describes the workflow of construction of protein-based artificial kinetochore in mouse oocytes.

Materials

Pregnant mare serum gonadotropin (PMSG); mice (8–12 weeks old); M2 medium; IBMX (Sigma); mMESSAGE mMACHINE T7 kit (Thermo Fisher Scientific); mRNAs for NDC80-FKBP, NUF2, H2B-mCherry, hAG-Pfv-Sapphire-GS-FRB, FKBP-GS-FKBP; rapamycin (Funakoshi).

Troubleshooting

1 Inject mice (8–12 weeks old) with 0.2 ml (10 IU) pregnant mare serum gonadotropin (PMSG).

2 Collect fully grown GV-stage oocytes 48 hours after PMSG injection.

3 Incubate oocytes in M2 medium containing 200 μM IBMX (Sigma) at 37 °C.

4 Transcribe and purify mRNAs using the mMESSAGE mMACHINE T7 kit (Thermo Fisher Scientific).

5 Prepare the mRNA mixture containing 0.3 pg NDC80-FKBP, 0.3 pg NUF2, 0.45 pg H2B-mCherry, 3.5 pg hAG-Pfv-Sapphire-GS-FRB, and 0.15 pg FKBP-GS-FKBP.

6 Inject the mRNA mixture into GV-arrested oocytes, followed by 2 hours incubation at 37 °C.

7 Induce meiotic resumption by washing out IBMX.

8 Culture oocytes in M2 medium containing 500 nM rapamycin (Funakoshi) to induce formation of the protein-based artificial kinetochore (NDC80-GEM-Linker).