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BEAD INJECTION PROTOCOL V.1

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Designing protein-base...



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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes workflow of the bead injection in mouse oocytes.

Materials

Rat anti-GFP antibody (D153-3, MBL), PBS, MonoMag Protein G-conjugated beads (1 μm diameter, MPG1000, or 3 μm diameter, MPG3000, Ocean NanoTech), mRNA-injected GV-stage oocytes, piezo pulse device.

Protocol

- 1 Prepare Rat anti-GFP antibody (D153-3, MBL) at 1:30 dilution in PBS.
- 2 Add MonoMag Protein G-conjugated beads (1 μm diameter, MPG1000, or 3 μm diameter, MPG3000, Ocean NanoTech) at 1:20 dilution to the antibody solution.
- 3 Incubate the mixture for 2.5 hours at 4 °C with rotation to immobilize the antibody to the beads.
- 4 Wash the antibody-bound beads with PBS.
- 5 Store the antibody-bound beads in PBS until use.
- 6 Introduce the anti-GFP-bound beads into mRNA-injected GV-stage oocytes by microinjection using a piezo pulse.
- 7 Inject either 4 beads (3 μm diameter) or 4–11 beads (1 μm diameter) per oocyte.
- 8 Culture the microinjected oocytes at 37 °C for 1 hour before meiotic resumption.