

Protocol 14

Microinjection of RNA into Mouse Zygotes

This protocol describes the injection of RNA into zygotes. It was provided by Dr. Yojiro Yamanaka, Goodman Cancer Research Centre, McGill University, Montreal, QC, Canada.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

Reagents

Fertilized mouse embryos (0.5 dpc) (Chapter 3)
M2 and KSOM embryo culture medium (Chapter 4)
mRNA prepared in Protocol 9

Equipment

Bunsen burner
Injection needles (see Step 2)
MICROFIL needles (MF34G, WPI), autoclaved
Can be recycled. Rinse with RNase-free and DNase-free H₂O using a 1-mL syringe, air-dry, and autoclave.
Microinjection setup as described in Protocol 12
Powder-free gloves
Wear throughout the protocol to avoid RNase contamination.

METHOD

Loading the mRNA into Injection Needles

1. Place the tube of mRNA into a 1.5-mL microfuge tube and centrifuge for few minutes at room temperature just before injection.
2. Briefly sterilize the back side of a freshly pulled injection needle with a flame. Place the injection needle on the lid of a 35-mm dish.
3. Using a MICROFIL needle, load the mRNA into the tip of an injection needle under a dissection microscope.
4. After loading the injection needle, quickly insert it in the needle holder and place it in the medium. Letting it sit out in the air can cause RNA to dry at the very tip and clog the needle before you even start injecting.

Injecting RNA into Zygotes

5. Inject the RNA by applying the injection pressure in a two-step procedure (Meyer et al. 2010), first into the pronucleus and then into the cytoplasm upon withdrawal of the needle. Note the transient subtle changes in the cytoplasm.

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 5): There is a high rate of lysis using the two-step injection method.

Solution: Instead, inject only the pronucleus.

REFERENCE

Meyer M, de Angelis MH, Wurst W, Kühn R. 2010. Gene targeting by homologous recombination in mouse zygotes mediated by zinc-finger nucleases. *Proc Natl Acad Sci* 107: 15022–15026.