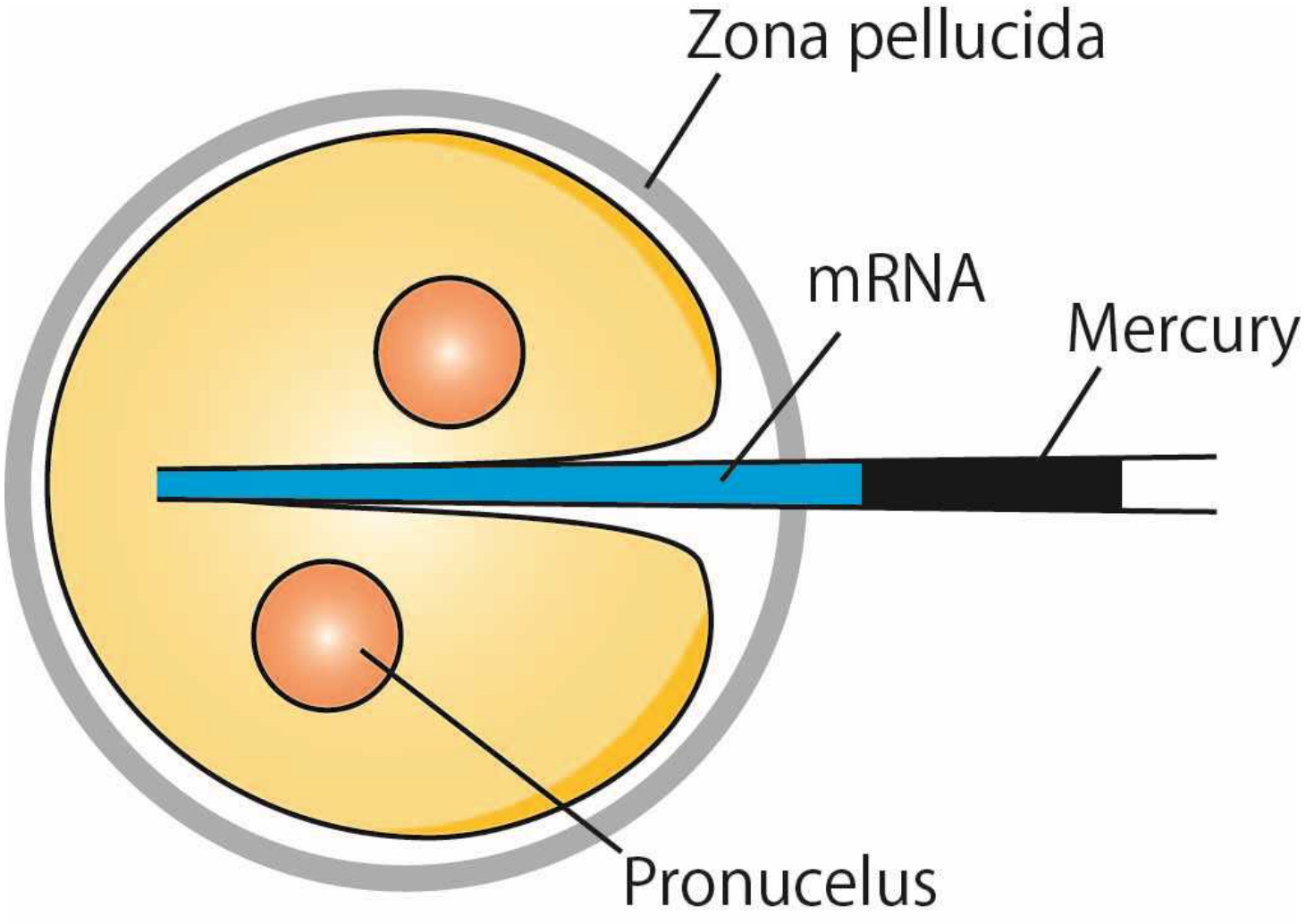
Microinjection of mRNA into fertilized or cloned zygotes

Bioresource Research Center
Bioresource Engineering Division
protocol #1

2020/05/20

Graphical Abstract



Contents

Introduction

Purpose

Overexpress a gene of interest by microinjection of mRNA into fertilized or cloned zygotes.

Introduction

The mRNA of interest is injected into a fertilized or cloned embryo at one cell stage using a micromanipulator.

Reagents and Equipment

Glass pipette	Sutter Instrument Drummond	B100-75-10 1-000-0500
Puller	Sutter Instrument	P-97/IVF
Microforge	NARISHIGE	MF-900
Micromanipulator with PIEZO	Nikon, Prime tech, etc.	
Dishes for micromanipulation	Depends on the microscope system	
Dishes for embryo culture	IWAKI	1010-060 1000-035
Embryo culture medium (KSOM, CZB, etc.)	Homemade	Homemade
Medium for micromanipulation (Hepes-KSOM, Hepes-CZB, M2 medium, etc.)	Homemade or MERCK	Homemade or MR-015-D
10%PVP	Homemade or Irvine Scientific	Homemade or 90123

Methods

1. Preparation of culture medium and mRNA

- **Culture medium**

  For long-term culture: KSOM or CZB, etc.

  For micromanipulation: Hepes-KSOM, Hepes-CZB, M2 medium, etc.

  For pipette washing: 10% PVP

- **mRNA**

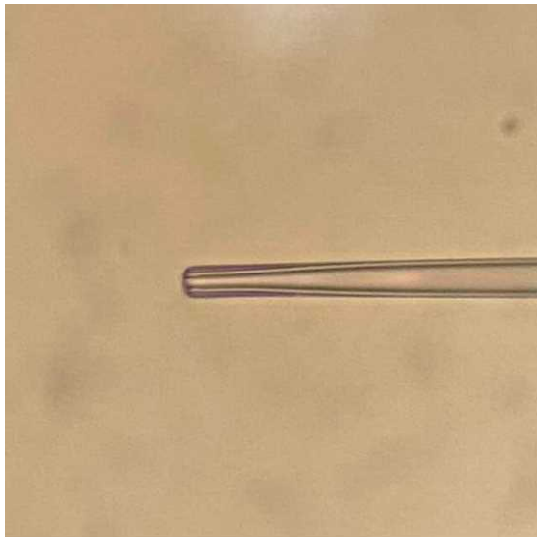
  For mRNA synthesis, see protocol #X. mRNA is dissolved in nuclease free water and aliquots were stored at -80℃. Thaw just before use.

  For the purification of mRNA for microinjection, ethanol-precipitation with LiCl is usually OK. However, if the embryo is highly sensitive to the purity of mRNA (e.g., marmoset), it is better to purify it with a column such as the MEGA clear kit.

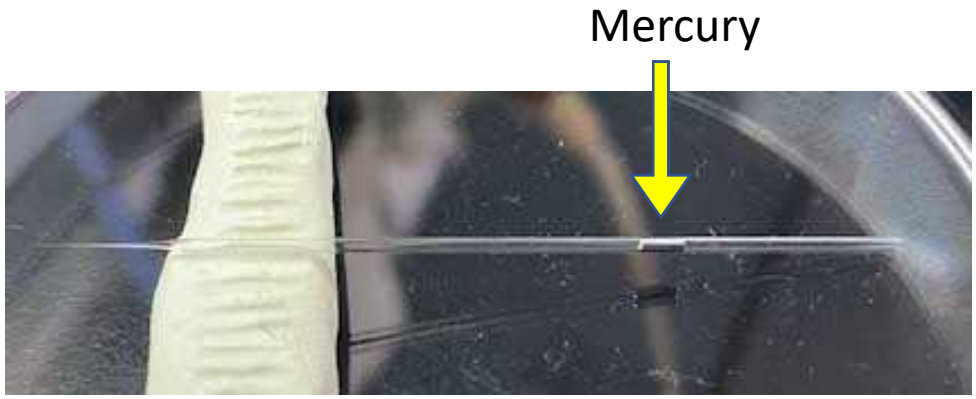
Methods

2. Preparation of microinjection needles

Pull a glass pipette with a puller and cut the tip so that the outer diameter of the tip is 4-5um. Then, heat the tip with a forge (close to the glass ball) to make it as round and smooth as possible. Inject mercury or its substitute (such as Fluorinert) into the pipette (1-2 mm in length).



Rounded glass pipette tip



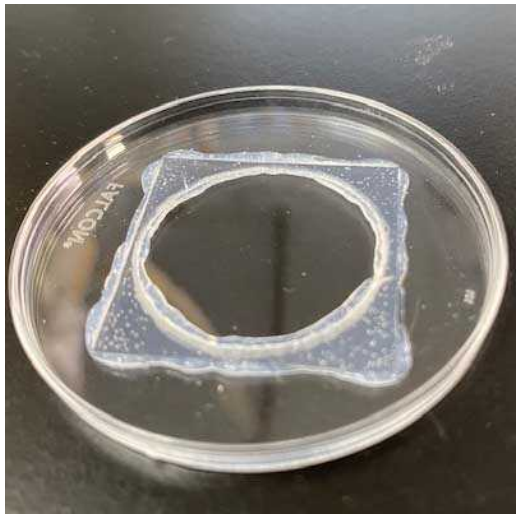
Glass pipette filled with mercury

Methods

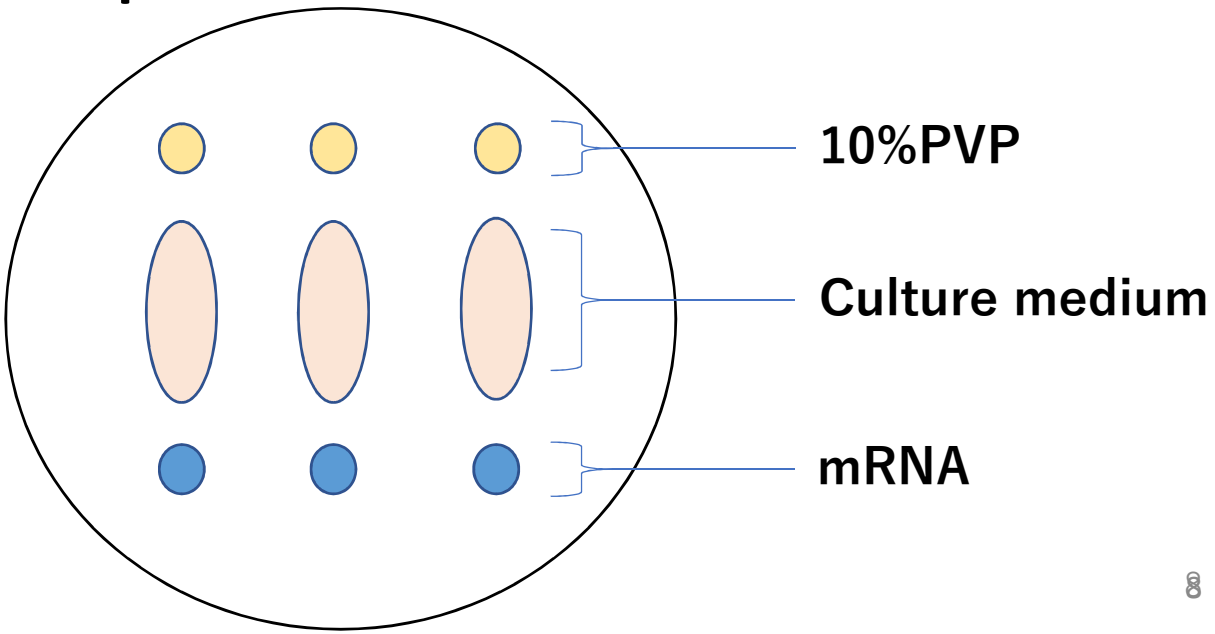
3. Preparation of a dish for microinjection

The dish should be prepared according to the microscope system used. (We make homemade glass bottom dishes.)

Three types of drops: 10% PVP for pipette washing, culture medium for embryo manipulation, and mRNA are arranged as shown in the figure. Finally, cover the drops with mineral oil.



Homemade glass bottom dish



Methods

4. Preparation of fertilized or cloned zygotes

Fertilized embryos or cloned embryos are prepared as pronuclear stage embryos 5-6 hours after fertilization or activation, respectively. This timing is arbitrary and unfertilized eggs and even later embryos (e.g., at the two-cell stage) can be injected, too. However, the survival rate after injection is higher in post-activated embryos than unfertilized eggs because they are more resistant to piezoelectric damage.

Methods

5. Microinjection of mRNA into zygotes

First, the embryos are transferred to the culture medium for embryo manipulation. Wash glass pipettes attached to micromanipulators with 10% PVP and mercury. Fill the pipette to the tip with mercury, move to the mRNA drop and suck a suitable amount of mRNA into the pipette (usually the equivalent of 5-10 injections). Return to the culture medium for embryo manipulation and hold the embryos with a holding pipette. Make a hole in the zona pellucida with a strong piezo. Then, the cytoplasmic membrane is pushed in with a pipette while avoiding the pronuclei. When the tip of the pipette reaches near the other side of membrane, break the membrane with a weak piezo. mRNA is injected into the cytoplasm in the right amount (about 10 pl for an amount of one anterior nucleus). After 5-10 injections, suck the mRNA again and repeat the injections.

Methods

6. Culture of injected zygotes

After injection, the embryos are allowed to rest in the culture medium for embryo manipulation for 10-15 min (returning to the incubator immediately after injection will reduce the survival rate). The embryos are then transferred to the normal culture medium for long-term incubation.

References

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Appendix

Contact

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- **Question**

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