**Protocol**

# Calibration of the Injection Volume for Microinjection of *Xenopus* Oocytes and Embryos

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Adapted from *Early Development of* Xenopus laevis, by Hazel L. Sive, Robert M. Grainger, and Richard M. Harland. CSHL Press, Cold Spring Harbor, NY, USA, 2000.

## INTRODUCTION

Microinjection of *Xenopus* oocytes or embryos with messenger RNA (mRNA) or DNA is a powerful technique for studying development. Before microinjection can be performed, the injection volume must be calibrated carefully. This protocol describes the calibration procedure for a pressure injector.

## RELATED INFORMATION

A protocol is available for **Microinjection of *Xenopus* Oocytes** (Sive et al. 2010a). Information is also available on **Microinjection of *Xenopus* Embryos** (Sive et al. 2010b).

## MATERIALS

### Reagents

Paraffin oil

Solution for backfilling the injection needle

### Equipment

Injection needles (drawn-out micropipettes)

Microinjector

Microscope, dissecting, with eyepiece micrometer

Microscope slides

Pipettor, automatic, with a long, narrow tip (e.g. the type of tip used to load sequencing gels)

## METHOD

1. Backfill the needle using the automatic pipettor and mount on the microinjector.

2. Break the injection needle tip to produce an orifice of ~10 µm.

3. Place a small drop of paraffin oil on a microscope slide. Mount the slide on the stage of a dissecting microscope.

4. Calibrate the eyepiece micrometer for the appropriate magnification. Perform a trial injection into the drop of oil.
*The injected liquid forms a sphere within the oil droplet.*

5. Measure the diameter of the sphere using the eyepiece micrometer. Calculate the injected volume by using the equation $v = 4/3\,(\pi r^3)$, where $v$ is the volume and $r$ is the radius of the sphere.

6. Alternative methods for calibrating the injection volume include the following:
i. Deposit the drop directly onto the stage micrometer.
*The micrometer must be siliconized for the drop to be near spherical.*

ii. Allow the drop to hang at the end of the needle, where its diameter can be measured using the eyepiece micrometer.
*Measure the drop quickly, because evaporation causes it to shrink rapidly.*

iii. Inject the drop into a dish of paraffin oil. Approximate the volume of the drop using the equation in Step 5.
*Alternatively, obtain the volume using the figures in Table 1.*
7. Once the appropriate pressure has been established, use the duration of the pressure burst to control the volume precisely.

## REFERENCES


1. Sive HL, Grainger RM, Harland RM. 2010a. Microinjection of *Xenopus* oocytes. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot5536.[Abstract/Free Full Text]
2. Sive HL, Grainger RM, Harland RM. 2010b. Microinjection of *Xenopus* embryos. *Cold Spring Harb Protoc* doi: 10.1101/pdb.ip81.[Abstract/Free Full Text]