Protocol

Microinjection of *Xenopus* Oocytes

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Microinjection of *Xenopus* oocytes has proven to be a valuable tool in a broad array of studies that require expression of DNA or RNA into functional protein. These studies are diverse and range from expression cloning to receptor–ligand interaction to nuclear programming. Oocytes offer a number of advantages for such studies, including their large size (~1.2 mm in diameter), capacity for translation, and enormous nucleus (0.3–0.4 mm). They are cost effective, easily manipulated, and can be injected in large numbers in a short time period. Oocytes have a large maternal stockpile of all the essential components for transcription and translation. Consequently, the investigator needs only to introduce by microinjection the specific DNA or RNA of interest for synthesis. Oocytes translate virtually any exogenous RNA regardless of source, and the translated proteins are folded, modified, and transported to the correct cellular locations. Here we present procedures for the efficient microinjection of oocytes and their subsequent care.

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.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at <http://cshprotocols.cshlp.org/site/recipes>.

Reagents

Antibiotics

Gentamycin (1000× stock solution [10 mg/mL in H₂O])

Streptomycin (1000× stock solution [100 mg/mL in H₂O])

Ficoll 400 (Sigma-Aldrich) (optional; see Step 4)

Medium for oocyte culture

Modified Barth's Saline (MBS) for oocytes <R>

Oocyte culture medium (OCM) <R>

A number of formulations exist for OCM (Wallace and Misulovin 1978; Opresko et al. 1980; Wylie et al. 1996). The formulation cited here is used for host transfer studies (Schneider et al. 2010a).

Oocyte reagent 2 (O-R2) <R> (Wallace et al. 1973)

The choice of medium depends upon what the oocytes will be used for experimentally.

Xenopus laevis oocytes (defolliculated)

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From the *Xenopus* collection, edited by Hazel L. Sive.

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Oocytes should be obtained surgically from anesthetized adult females or sourced from commercial vendors (Nasco, EcoCyte Bioscience, Xenocyte) and defolliculated. See Protocol: **Isolation of *Xenopus* Oocytes** (Newman et al. 2018) for oocyte isolation and defolliculation procedures.

Equipment

Capillary tubes (Drummond Scientific 3-000-203G/X [borosilicate glass, 1.14-mm outer diameter, 0.53-mm inner diameter, 90 mm long] or Sutter Instrument BF100-50-10 [borosilicate glass with filament, 1.0-mm outer diameter, 0.5-mm inner diameter, 100 mm long])
Dissecting microscope and dual gooseneck LED illuminators (low heat)
Forceps (Dumont #4 or #5)
Hair loop
Incubator at 18°C–20°C
Mesh (nylon or polypropylene; 0.5–0.8 mm) (as needed; see Step 4)
Microinjector (e.g., Drummond Nanoject II Auto-Nanoliter Injector or Narishige IM-300)
An automated injection system (roboinject) is optional; see <http://www.multichannelsystems.com/products/xenopus-oocyte-research>.
Micromanipulator (e.g., Warner Instruments MM-33 or Singer Mk1)
Petri dishes (35-mm) and/or six-well microplates (35-mm/well) (plastic)
Pipette puller (e.g., Narishige PC-10 or Sutter Instrument P-97)

METHOD

Preparing for Microinjection

1. Prepare pipettes for microinjection from glass capillary tubes using a micropipette puller. Using forceps and with the aid of a dissecting microscope, gently break the pipette tip to produce a clean open tip. Measure the diameter.

Capillary tube dimensions may vary according to the injector to be used; check the manufacturer's specifications. The length of the pipette should be 5–8 cm. A final tip diameter of <20 μ m is optimal.

Pipette quality is critical for postinjection oocyte viability. A good quality pipette has a fine, beveled tip, and will slide in and out of the oocyte leaving a small injection mark without leakage of sample or cellular material (Fig. 1B,C). A poor quality pipette has a wide and/or blunt ended tip which is difficult to insert, and will leave a large hole in the oocyte that will leak ooplasm (Fig. 1D; Maldifassi et al. 2016).

2. To perform sample loading, refer to the manufacturer's instructions for specific details for your injection apparatus.

Loading of the sample commonly involves backfilling the pipette with mineral oil after breaking the tip as described in Step 1, and then front-filling with sample. Injection volumes vary according to the sample, the oocyte stage and the microinjector. In general, maximum injection volumes are: stage V/VI, up to 50 nL; stage IV, 20 nL; stage III, 10 nL; stage II, 4.6 nL; stage I, 1–2.3 nL.

3. Secure the injection apparatus to a micromanipulator which moves the pipette for injection. Position the pipette at a 35–55 degree angle.

FIGURE 1. (A) Stage VI oocytes aligned in various orientations over a 0.5-mm nylon mesh for microinjection. (B) Example of nuclear injection using a beveled needle (arrow). (C) An enlargement of the area surrounding the injection (boxed area in B) shows how the injection hole constricts and eventually heals. (D) Injection holes produced by a too broad or blunt-tip pipette (arrows); compare with (B).

Performing Microinjection

Steps 4.i and 4.ii describe the two predominant techniques used to microinject defolliculated oocytes. Either technique can be used in Step 4.iii, depending on the preference and skill of the user.

Microinjectors generally have two options for injections: pressing a button or using a foot pedal. Use of a foot pedal frees the hands and is more efficient.

A general injection procedure can be viewed at <https://www.youtube.com/watch?v=jv-BgKTvqfU> (Oocyte 2009). A microinjection procedure for electrophysiological experiments is described and shown by Maldifassi et al. (2016).

4. Microinject defolliculated oocytes using the desired technique.

- i. Immobilize oocytes by positioning them into the openings of a nylon or polypropylene mesh (0.5–0.8 mm) at the bottom of a Petri dish containing culture medium (Fig. 1A). Carefully align oocytes for microinjection with the desired pole facing upward using a hair loop or blunt-end forceps. Prepare 50–100 oocytes at a time for microinjection. After each injection is complete (Step iii), gently withdraw the pipette and move to the next oocyte by moving the microinjection dish.
- ii. Alternatively, place 20–50 oocytes in the center of a Petri dish filled with culture medium. Carefully orient one oocyte at a time with the desired pole facing up using forceps, and hold it in place while performing each injection (Step iii). Gently withdraw the pipette, move the injected oocyte to another position in the dish (e.g., from center to top), and bring the next oocyte to the pipette.
- iii. Using either of the above methods, position the selected oocyte under the loaded injection pipette and move the pipette down using the micromanipulator. (The oocyte surface will dent under pressure from the pipette until penetration; further movement is unnecessary.) Inject the sample, then wait a few seconds before withdrawing the pipette to minimize sample leakage. Periodically release one injection shot into solution to ensure the pipette has not clogged.

The use of 4% Ficoll 400 in the culture medium is optional, but may prevent leakage of sample from the injection site.

For nuclear injections, the germinal vesicle (GV) is found just beneath the surface of the animal pole (darkly pigmented half). We recommend practicing nuclear injections into the animal pole using a dye solution (bromophenol blue 0.1% or FITC 0.1%) to ensure injections are targeting the GV. Another method for targeting the nucleus is described by Bataillé et al. (1990) and Dworetzky and Feldherr (1988). This method involves centrifugation of defolliculated oocytes at 650g for 8–10 min causing migration of the GV to a position just underneath the plasma membrane at the animal pole, which is easily visualized as a targetlike structure due to the displacement of pigment granules in the cortex. Nuclear injections can then be accomplished, preferably within 15 min after centrifugation. The maximum volume suggested for nuclear injections is 20 nL.

See Troubleshooting.

5. When microinjection is complete, carefully separate the oocytes into individual dishes or microplate wells (up to 50 cells per 35-mm dish or well) containing the same medium used during injection, including Ficoll (if used). Return the oocytes to the 18°C incubator. If Ficoll was used, replace the medium with Ficoll-free medium after >2 h.

Oocytes can remain in medium containing Ficoll 400 overnight.

6. Twice a day, monitor the oocytes for viability, removing damaged or dying oocytes to prevent deleterious effects on healthy oocytes. Replace medium with fresh medium each time. Include antibiotics, such as gentamycin or streptomycin, in the culture medium to prevent bacterial infection (see Protocol: **Isolation of Xenopus Oocytes** [Newman et al. 2018]).

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 4): The pipette clogs during injection.

Solution: Remove the pipette from the medium and empty it, using pressure to force out the particulate material. As a last resort, use forceps to carefully break off slightly more of the tip.

Problem (Step 4): There is leakage of injected material.

Solution: Change the pipette, as the quality may be poor. Include 4% Ficoll 400 in the medium used for injections.

Problem (Step 4): Oocytes are soft and difficult to penetrate.

Solution: Only inject relatively firm oocytes. If too many are soft, use oocytes from another female. Note that using Ficoll 400 in the medium may initially make the oocytes seem soft.

Problem (Step 6): The viability of injected oocytes is poor.

Solution: Sample toxicity, old solutions with low pH, or damage to the oocytes due to a poor quality pipette are the most common reasons for poor viability. Use freshly made buffers and inject a range of sample volumes and concentrations to control for sample toxicity.

Problem (Step 6): There is low protein expression after injection.

Solution: Poor quality oocytes, oocytes insufficiently recovered from defolliculation processes, a degraded sample (particularly if working with RNA), or an insufficient amount of injected sample can contribute to low expression. Use oocytes that have been allowed to recover overnight from defolliculation and from several females to control for oocyte variability. Inject a range of sample concentrations to control for expression variability.

DISCUSSION

Examples of studies using oocyte injection are listed in Table 1. Based on PubMed searches, the major uses of *Xenopus* oocyte microinjection are heterologous transporter and channel expression followed

TABLE 1. Example studies using oocyte injection

Application	Reference
Cell cycle regulation	Daldello et al. 2015
Cellular polarization	Peter et al. 1991
Cryopreservation	Guenther et al. 2006; Yamaji et al. 2006
Dominant negative constructs	Reed et al. 2016
Electrophysiology	Miledi et al. 1982; Fujita et al. 2007; Hansen and Brauner-Osborne 2009; Papke and Smith-Maxwell 2009; Wang et al. 2014
Enzymatic activity	Sato et al. 1996; Hehl et al. 2001; Sallacz and Jantsch 2005; Ota et al. 2011
Genome editing	Miyamoto et al. 2015; Nakajima and Yaoita 2015; Ratzan et al. 2017
Germline specification	Aguero et al. 2016
Lipid metabolism	Stifani et al. 1990
mRNA knockdown	Hulstrand et al. 2010
Nuclear import/export pathways	Lane et al. 1980; Terns et al. 1993; Smillie et al. 2004; Loop and Pieler 2005
Nuclear reprogramming	Jullien 2015
Oocyte host transfer	Schneider et al. 2010a; Mei et al. 2013
Protein expression (homologous/heterologous)	Bartnik and de Robertis 1983; Duchesne et al. 2003; Bernareggi et al. 2011; Clemencon et al. 2015; Crespin et al. 2016; Miller and Zhou 2000; Aoshima et al. 2005; Maciver et al. 2008; Reed et al. 2016; Plautz et al. 2016.
Protein–protein interaction	Schneider et al. 2010b; Nijjar and Woodland 2013
RNA–protein interaction	Mabuchi et al. 2008; Matsumoto et al. 2000
Signal transduction	Lin-Moshier and Marchant 2013; Sato 2015
Transcript/protein localization	Chang et al. 2004; Nijjar and Woodland 2013

by electrophysiological or tracer flux analysis. Oocytes can be injected with up to 10 ng of mRNA, 3 ng of cDNA or 80 ng of Morpholino antisense oligonucleotides (Hulstrand et al. 2010). In general, the efficiency rate of protein expression using mRNA is higher than using cDNA (Sigel 2001). Microinjection of dominant negative constructs, antisense Morpholinos or transcripts encoding mutant proteins are extensively used in loss-of-function and gain-of-function experiments. Genetic information can be introduced or depleted with high efficiency by microinjection into either the cytoplasmic or nuclear compartments, thus avoiding the complications of transfection. For some experiments, it may be desirable to culture oocytes. Oocytes can be cultured and remain in a healthy, stable condition for several days if not weeks (Wallace and Misulovin 1978). One cautionary note: oocytes exist within an ovarian follicle comprising different somatic cells (i.e., blood vessels and thousands of follicle cells). These somatic cells can be a source of molecular contamination, depending on the goal of the experiment. Defolliculation does not remove 100% of these somatic cells.

RECIPES

Modified Barth's Saline (MBS) for Oocytes

1. Prepare a 0.1 M CaCl_2 solution by dissolving 1.11 g of CaCl_2 in deionized H_2O for a final volume of 100 mL.
2. Prepare a 10× MBS salt solution by dissolving the following reagents in deionized H_2O for a final volume of 1 L. Adjust the pH to 7.8 with 10 M NaOH, and sterilize by autoclaving. Store indefinitely at room temperature.

HEPES	11.92 g
NaCl	51.43 g
KCl	0.75 g
NaHCO_3	2.10 g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	2.46 g

3. To prepare 1× MBS, combine 100 mL of 10× MBS salt solution and 7 mL of 0.1 M CaCl_2 with 893 mL of autoclaved deionized H_2O . Add antibiotics. Store for up to 1–2 mo at 16°C–18°C (the same temperature used for oocyte incubation). Check before use.

The final reagent concentrations in 1× MBS are 0.7 mM CaCl_2 , 5 mM HEPES, 88 mM NaCl, 1 mM KCl, 2.5 mM NaHCO_3 , and 1 mM MgSO_4 .

Oocyte Culture Medium (OCM)

Reagent	Amount to add	Final concentration
Leibovitz's L-15 medium with L-glutamine	0.35 L	70%
Bovine serum albumin (fraction V)	0.2 g	0.4 mg/mL
Penicillin-streptomycin (100×/100×, P4333 Sigma-Aldrich)	5 mL	100 U/mL penicillin 0.1 mg/mL streptomycin

Bring the volume to 0.5 L with deionized H_2O , and adjust the pH to 7.8 with NaOH. Filter to sterilize. Store at 4°C–8°C for up to 1–2 mo. Check before use.

Oocyte Reagent 2 (O-R2)

1. Prepare Stock Solution A by dissolving the following reagents in deionized H₂O for a final volume of 1 L. Store indefinitely at 4°C–8°C.

HEPES	11.915 g
NaCl	48.221 g
KCl	1.864 g
CaCl ₂ · 2H ₂ O	1.47 g
MgCl ₂ · 6H ₂ O	2.03 g
NaOH	1.520 g
2. Prepare Stock Solution B by dissolving 1.420 g of Na₂HPO₄ in deionized H₂O for a final volume of 1 L. Store indefinitely at 4°C–8°C.
3. Combine 100 mL of Stock Solution A, 100 mL of Stock Solution B, and 800 mL of deionized H₂O. Add antibiotics. Store for up to 1–2 mo at 16°C–18°C (the same temperature used for oocyte incubation). Check before use.

The pH should be 7.8. The final reagent concentrations are 5 mM HEPES, 82.5 mM NaCl, 2.5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 3.8 mM NaOH, and 1 mM Na₂HPO₄.

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