

[Find Protocols](#)[Find a Kit](#)[Access Personal Page](#)[Submit Protocols](#)[Help](#)[Subscribe](#)[About CSH Protocols](#)**Institution:** WASHINGTON UNIV SCHL MEDICINE | [Sign In via User Name/Password](#)

Please cite as: CSH Protocols; 2007; doi:10.1101/pdb.prot4839



Protocol

Generation of Transgenic *Xenopus laevis*: II. Sperm Nuclei Preparation

Shoko Ishibashi¹, Kristin L. Kroll², and Enrique Amaya^{1,3}¹ The Healing Foundation Centre, Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, United Kingdom² Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110, USA³Corresponding author (enrique.amaya@manchester.ac.uk)

INTRODUCTION

Manipulating genes specifically during later stages of amphibian embryonic development requires fine control over the time and place of expression. These protocols describe an efficient nuclear-transplantation-based method of transgenesis developed for *Xenopus laevis*. The approach enables stable expression of cloned gene products in *Xenopus* embryos. Because the transgene integrates into the genome prior to fertilization, the resulting embryos are not chimeric, eliminating the need to breed to the next generation to obtain nonmosaic transgenic animals. The procedure is based on restriction-enzyme-mediated integration (REMI) and can be divided into three parts: (I) high-speed preparation of egg extracts, (II) sperm nuclei preparation, and (III) nuclear transplantation. This protocol describes a method for the preparation of sperm nuclei from *Xenopus laevis*. Sperm suspensions are prepared by filtration and centrifugation, and then treated with lyssolecithin to disrupt the plasma membrane of the cells. Sperm nuclei can be stored frozen in small aliquots at -80°C.

RELATED INFORMATION

For additional protocols essential to the generation of transgenic *Xenopus laevis*, please refer to [Generation of Transgenic *Xenopus laevis*: I. High-Speed Preparation of Egg Extracts](#) and [Generation of Transgenic *Xenopus laevis*: III. Sperm Nuclear Transplantation](#).

MATERIALS

Reagents

10% bovine serum albumin (BSA; Sigma, A7906), freshly prepared and stored on ice

10 mg/mL Hoechst No. 33342, diluted 1:100 prior to use

1000 U/mL human chorionic gonadotropin (HCG)

Liquid nitrogen

10 mg/mL L- α -lyssolecithin, egg yolk (Calbiochem, 440154)

Store the solid stock at -20°C. Discard the stock powder if it becomes sticky. Prepare the 10 mg/mL solution immediately before use at room temperature.

This Protocol

- ▶ **Abstract** **FREE**
- ▶ [Update/discuss this protocol](#)
- ▶ [Alert me when this protocol is cited](#)
- ▶ [Alert me when comments are published](#)
- ▶ [Alert me if a correction is posted](#)

Services

- ▶ [Similar protocols in this database](#)
- ▶ [Alert me to new releases of protocols](#)
- ▶ [Save to Personal Folders](#)
- ▶ [Download to citation manager](#)
- ▶ [Printer-friendly version](#)

Google Scholar

- ▶ [Articles by Ishibashi, S.](#)
- ▶ [Articles by Amaya, E.](#)

PubMed

- ▶ [Articles by Ishibashi, S.](#)
- ▶ [Articles by Amaya, E.](#)

Related Collections

- ▶ [Xenopus](#)
- ▶ [Molecular Biology, general](#)
- ▶ [Transgenic Technology, general](#)
- ▶ [Xenopus Transgenics](#)
- ▶ [Developmental Biology](#)
- ▶ [DNA Delivery/Gene Transfer](#)
- ▶ [DNA Delivery/Gene Transfer, general](#)
- ▶ [Non-Viral Methods](#)
- ▶ [Cell Biology, general](#)
- ▶ [Preparation of Macromolecules and Introduction into Cells](#)
- ▶ [Subcellular Fractionation](#)
- ▶ [Laboratory Organisms, general](#)
- ▶ [Related Protocols](#)

Social Bookmarking



What's this?

Icon Legend



- 10X Marc's modified Ringer's (MMR), diluted to 1X prior to use and stored on ice
- 2X nuclear preparation buffer (NPB), freshly prepared (to a concentration of 1X) and stored on ice
- 100 U/mL pregnant mare serum gonadotropin (PMSG)
- Sperm dilution buffer (SDB)
- Sperm storage buffer (SSB), freshly prepared and stored on ice
- 0.1% tricaine methanesulfonate, prepared in 0.1% sodium bicarbonate

Xenopus laevis, adult males

For details on the proper handling of frogs, please refer to [Handling *Xenopus laevis* Adults](#).

Equipment

Centrifuge and rotor (e.g., Sorvall HB-4 swinging bucket rotor)

Cheesecloth

Dissection tools: forceps and scissors

Fluorescence microscope

Funnel

Gloves, latex

Hemocytometer

Needles (26 gauge)

Paper towels

Petri dishes (60 mm)

Pipettes, plastic (5 and 10 mL)

Pipetman tips (1 mL and 200 μ L)

Razor blade

Syringes (1 mL)

Tubes (14 mL; Falcon, 2059)

Tubes, microcentrifuge (1.5 mL)

METHOD

1. About 3-5 d prior to HCG injection, prime one or two male *X. laevis* by injection with 50 U of PMSG using a 26-gauge needle fitted to a 1-mL syringe.

For details on the proper procedure for handling and injection of the frogs, see [Inducing Ovulation in *Xenopus laevis*](#).

2. Twelve to fifteen hours before nuclei preparation, inject the males with 500 U of HCG.

3. Anesthetize the male by immersion in tricaine for at least 20 min. Pith it.

4. Isolate the testes with dissecting scissors. Roll them on a paper towel to remove blood, blood vessels, and adhering fat.

For details on this procedure, please consult [Isolating *Xenopus laevis* Testes](#).

5. Wash the testes briefly in a 60-mm Petri dish containing cold 1X MMR. Remove any attached pieces of fat or debris with forceps.

6. Transfer the cleaned testes to a dry 60-mm Petri dish. Macerate the testes with a pair of clean forceps until clumps are no longer visible to the naked eye.

7. Add 2 mL of cold 1X NPB. Mix well by pipetting the solution up and down with a 10-mL plastic pipette.

8. Filter the sperm suspension through four thicknesses of cheesecloth placed into a funnel. Collect the filtrate in a 14-mL tube.

9. Rinse the Petri dish with an additional 3 mL of cold 1X NPB. Force this through the cheesecloth into the 14-mL tube.

10. Add 5 mL of cold 1X NPB to the funnel. Wearing gloves, squeeze the cheesecloth by hand to force any remaining liquid through the funnel into the 14-mL tube.
11. Pellet the sperm by centrifugation at 3000 rpm for 10 min at 4°C using a swinging bucket rotor with the appropriate adapters.
12. During the centrifugation, allow 1 mL of 1X NPB to equilibrate to room temperature.
13. Decant the supernatant obtained from Step 11. Resuspend the sperm in 9 mL of 1X NPB using a 10-mL plastic pipette.
14. Recentrifuge the sperm at 3000 rpm for 10 min at 4°C. Decant the supernatant.
15. Resuspend the pellet with a 1-mL pipette tip in the 1 mL of room-temperature 1X NPB (from Step 12).
16. Add 50 µL of freshly made 10 mg/mL lysolecithin. Mix gently. Incubate for 5 min at room temperature.
17. Add 10 mL of cold 1X NPB supplemented with 3% BSA to the suspension to stop the reaction.
18. Centrifuge the samples at 3000 rpm for 10 min at 4°C. Decant the supernatant.
19. Resuspend the pellet in 5 mL of cold 1X NPB supplemented with 0.3% BSA. Mix well by pipetting with a 5-mL plastic pipette.
20. Centrifuge at 3000 rpm for 10 min at 4°C. Decant the supernatant carefully.
21. Resuspend the pellet in 500 µL of SSB. Transfer the suspension into a 1.5-mL tube.
22. Count the number of sperm nuclei using a hemocytometer.
For details on this procedure, please consult [Estimation of Cell Number by Hemocytometry Counting](#).
 - i. Cut off the end of a 200-µL pipette tip with a razor blade.
 - ii. Mix the sperm nuclei well by pipetting.
 - iii. Dilute 1 µL of the sperm nuclei with 100 µL of SDB.
 - iv. Add 1 µL of the diluted Hoechst reagent.
 - v. Visualize the sperm nuclei under a fluorescence microscope.
For a 1:100 dilution, we typically obtain counts of 100×10^4 to 200×10^4 nuclei/mL in a $1 \times 1 \times 0.1$ -mm square of an improved Neubauer hemocytometer. At this concentration, the undiluted stock contains 1×10^5 to 2×10^5 nuclei/µL. See [Troubleshooting](#).
23. For best cryopreservation of the sperm, leave fresh nuclei overnight at 4°C to allow the glycerol to penetrate.
24. The next day, divide the sperm into 20-µL aliquots. Fast-freeze the samples in liquid nitrogen. Store the frozen aliquots at -80°C.

TROUBLESHOOTING

Problem: Sperm stock is substantially less concentrated (i.e., a count of <50 for a 1:100 dilution).


[Step 22]

Solution: Let the sperm settle for a few hours or overnight and remove some of the supernatant.

Problem: Sperm stock is substantially contaminated with debris.

[Step 22]

Solution: Make sure not to treat sperm nuclei longer than 5 min with lysolecithin. After the treatment with lysolecithin, the precipitate of sperm nuclei is loose. Be careful when you decant the supernatant.

 At Step 16, digitonin (Sigma) at a final concentration of 100 µg/mL can be used instead of lysolecithin. Digitonin is more specific for the plasma membrane, leaving the nuclear membranes intact.

 [CiteULike](#)  [Connotea](#)  [Del.icio.us](#)  [Digg](#)  [Reddit](#)  [Technorati](#) [What's this?](#)

Related Protocols

Estimation of Cell Number by Hemocytometry Counting

Joseph Sambrook and David W. Russell
CSH Protocols 2006: 4454. [\[Extract\]](#) [\[Full Text\]](#)

Handling *Xenopus laevis* Adults

Hazel L. Sive, Robert M. Grainger, and Richard M. Harland
CSH Protocols 2007: 4733. [[Abstract](#)] [[Full Text](#)]

Inducing Ovulation in *Xenopus laevis*

Hazel L. Sive, Robert M. Grainger, and Richard M. Harland
CSH Protocols 2007: 4734. [[Abstract](#)] [[Full Text](#)]

Isolating *Xenopus laevis* Testes

Hazel L. Sive, Robert M. Grainger, and Richard M. Harland
CSH Protocols 2007: 4735. [[Abstract](#)] [[Full Text](#)]

Generation of Transgenic *Xenopus laevis*: I. High-Speed Preparation of Egg Extracts

Shoko Ishibashi, Kristin L. Kroll, and Enrique Amaya
CSH Protocols 2007: 4838. [[Abstract](#)] [[Full Text](#)]

Generation of Transgenic *Xenopus laevis*: III. Sperm Nuclear Transplantation

Shoko Ishibashi, Kristin L. Kroll, and Enrique Amaya
CSH Protocols 2007: 4840. [[Abstract](#)] [[Full Text](#)]

[Find Lab Supplies](#)

[Find Lab Manuals](#)

[Find a Course](#)

[Advertise On This Site](#)

Copyright © 2007 by Cold Spring Harbor Laboratory Press. Online ISSN: 1559-6095 [Terms of Service](#)