

Submission ID #: 70602

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Project Page Link: https://review.jove.com/files_upload.php?src=21533658

Title: Physical Manipulation to Generate *Xenopus* Mini Embryos

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

If **Yes**, can you record movies/images using your own microscope camera? **Yes**

SCOPE shot: 3.2.1, 3.2.2, 3.2.3, 3.3.1, 3.3.2, 3.3.3, 3.4.1, 3.4.2

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 8

Number of Shots: 22 (8 SCOPE)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

Answers are slightly edited in accordance with the journal guidelines

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Hui Chen:** Our research investigates how cell size regulates early embryonic genome activation by physically generating miniature embryos at the beginning of development

- 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Hui Chen:** Existing methods lack direct assessment of size effect on genome activation; this method addresses this by directly changing the size of 1-cell embryo.

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Chuan Qin:** The most important challenge when performing this protocol is to ligature the 1-cell embryos to make the mini embryos.

- 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Chuan Qin:** The mini embryos obtained using this protocol can be used in imaging and sequencing for mechanistic studies.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Lily Acker:** Future studies could explore how embryo and cell size regulate early development and underlying mechanisms.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at University of South Carolina

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Preparation of Hair Knot for Embryo Constriction

Demonstrator: Lily Acker

- 2.1. ~~To begin, obtain previously prepared density gradient medium and an agarose-coated Petri dish [1].~~ To begin, prepare hair knots for constricting embryos by cutting thin hair into approximately 5-centimeter pieces [2]. Using a tweezer, insert one end of the hair into a P10 (*P-ten*) tip at a depth of about 1 centimeter [3].
 - 2.1.1. ~~WIDE: Talent walking into the room with prepared density gradient medium and agarose-coated petri dish.~~ Author's NOTE: Not filmed
 - 2.1.2. Talent cutting hair into 5 cm pieces
 - 2.1.3. Talent inserting one end of hair into p10 tip at 1 cm depth
- 2.2. Then, take wax on a spoon and heat it to melt the wax [1]. Quickly dip the hair-attached P10 tip in the wax and let it to dry for one minute [2]. Ensure the hair is fixed at the end of the P10 tip [3].
 - 2.2.1. Talent heating the wax in a spoon to melt it.
 - 2.2.2. Talent dipping the P10 tip, attached with hair into the wax and putting it aside for drying.
 - 2.2.3. CU: Shot of the hair is fixed at the end of the P10 tip.
- 2.3. Use a tweezer to carefully make an overhand knot of the hair [1]. Adjust the size of the loop to be approximately 1.5-millimeter [2]. Trim the overhanging hair [3].
 - 2.3.1. Talent using tweezer to make an overhand knot of the hair.
 - 2.3.2. Talent adjusting the size of the loop to be ~1.5 mm.
 - 2.3.3. Talent trimming the overhanging hair.

3. Generation of *Xenopus* Mini Embryos

Demonstrator: Chuan Qin

- 3.1 Using in vitro fertilization or IVF (*I-V-F*), obtain 1-cell embryos microinjected with 5-ethynyl-uridine to label nascent transcripts [1]. Add 15 milliliters of 3% density gradient medium containing 0.5 times MMR into previously prepared agarose covered 60-

millimeter Petri dish [2]. Once embryos reach at 1 hour post fertilization stage, transfer them into the Petri dish [3].

- 3.1.1 WIDE: Talent bringing a glass dish with microinjected embryos.
- 3.1.2 Talent adding 15 mL 3% density gradient medium/0.5× MMR into 60 mm agarose coated dish
- 3.1.3 Talent transferring embryos into 60 mm dish with 15 mL density gradient solution.

3.2 Under a stereomicroscope, use tweezers to rotate the embryo approximately 45 to 60 degrees to access the vegetal pole [1]. Hold the embryo with tweezer and use a 30 Gauge needle in the other hand to carefully puncture the vegetal pole [2].

3.2.1 LABMEDIA: 3.2.1.mp4: 00:00 – 00:13

3.2.2 LABMEDIA: 3.2.2.mp4: 00:14 – 00:22

3.2.3 ~~SCOPE: The embryo is being pressed and cytoplasm is released.~~ NOTE: This was moved after 3.4.1.

3.3 Now, place the hair knot attached to a P10 tip over the embryo [1] in such a way that the animal pole is enclosed within the hair knot [2]. Adjust the position of the knot according to the size of the mini embryo [3].

3.3.1 LABMEDIA: 3.3.1.mp4: 00:38 – 00:41

3.3.2 LABMEDIA: 3.3.2.mp4: 00:42 – 00:45

3.3.3 LABMEDIA: 3.3.3.mp4: 00:46 – 00:48

3.4 Using a P10 pipette and tweezers, pull the knot ends in opposite directions to tighten it around the embryo between the animal and vegetal poles [1]. Gently press the embryo and release some cytoplasm [2]. Cut the hair knot at the point where it attaches to the P10 tip [3].

3.4.1 LABMEDIA: 3.4.1.mp4: 00:48 – 00:50

3.2.3 LABMEDIA: 3.2.3.mp4: 00:51 – 00:58 NOTE: 3.2.3 is moved here.

3.4.2 LABMEDIA: 3.4.2.mp4: 00:59 – 1:06

3.5 After generating the required number of mini embryos, let them stay in 3% density gradient medium for approximately one to two hours [1]. Then, using a P1000 (*P-one-thousand*) pipette remove the medium without disturbing the embryos [2] and add 15 milliliters of 0.1 times MMR [3-TXT].

- 3.5.1 CU: Shot of embryos in 3% density gradient medium.
- 3.5.2 Talent removing the medium with P1000 pipette without disturbing the embryos.
- 3.5.3 Talent adding 15 mL of 0.1x MMR. **TXT: Collect the developed embryos**

NOTE: On-screen text was added since the shot was not filmed and does not fit the VO.

~~3.6 Once the embryos develop into the desired stages [1], using tweezers remove the knots from the mini embryos [2]. Then, collect the embryos for desired analysis [3].~~

~~3.6.1 Shot of the developed embryos~~

~~3.6.2 Talent using the tweezers to remove the knot from the mini embryos~~

~~3.6.3 Talent collecting the embryos~~

Author's NOTE: 3.6 was not filmed. VO is added in concise format in the previous step.

Results

4 Results

4.1 The microscopic image of the embryos obtained at 8.5 hours post-fertilization [1] indicated that the volume of some mini embryos is approximately one-third [2] of that of the control embryos suggests that the early *Xenopus* embryos can be manipulated to generate mini embryos [3].

4.1.1 LAB MEDIA: Figure 2

4.1.2 LAB MEDIA: Figure 2 *Video editor: Please emphasize mini embryos on the right side of the image under "Mini" heading encircled with white dashed line.*

4.1.3 LAB MEDIA: Figure 2 *Video editor: Please emphasize control embryos on the left side of the image under "Control" heading encircled with white dashed line.*

Pronunciation guides:

1. Constricting

Pronunciation link: <https://www.merriam-webster.com/dictionary/constricting>

IPA: /kən'strɪk.tɪŋ/

Phonetic Spelling: kuhn·strik·ting

2. Embryos

Pronunciation link: <https://www.merriam-webster.com/dictionary/embryo>

IPA: /'ɛm.bri.əʊ/

Phonetic Spelling: em·bree·oh

3. Fertilization

Pronunciation link: <https://www.merriam-webster.com/dictionary/fertilization>

IPA: /fɜː.tə.lə'zeɪ.ʃən/

Phonetic Spelling: fur·tuh·luh·zay·shuhn

4. Microinjected

Pronunciation link: <https://www.merriam-webster.com/dictionary/microinject>

IPA: /,maɪ.kroʊ.ɪn'dʒɛkt/

Phonetic Spelling: my·kroh·in·jekt

5. Ethynyl-uridine

Pronunciation link: No confirmed link found

IPA: /'ɛθɪnɪl 'jʊrɪdiːn/

Phonetic Spelling: eth·uh·nil yur·uh·deen

6. Nascent
Pronunciation link: <https://www.merriam-webster.com/dictionary/nascent>
IPA: /'næs.ənt/
Phonetic Spelling: nas·uhnt
7. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /'æg.ə.roʊs/
Phonetic Spelling: ag·uh·rohs
8. Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereomicroscope>
IPA: /,stɛr.i.əʊ'maɪ.krə'skoʊp/
Phonetic Spelling: stair·ee·oh·my·kruh·skohp
9. Vegetal
Pronunciation link: <https://www.merriam-webster.com/dictionary/vegetal>
IPA: /'vedʒ.ə.təl/
Phonetic Spelling: vej·uh·tuhl
10. Cytoplasm
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytoplasm>
IPA: /'saɪ.tə.plæz.əm/
Phonetic Spelling: sy·tuh·plaz·uhm
11. Xenopus
Pronunciation link: <https://www.merriam-webster.com/dictionary/Xenopus>
IPA: /'zɛn.ə.pəs/
Phonetic Spelling: zen·uh·pus
12. Microscopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopic>
IPA: /,maɪ.krə'skɑː.pɪk/
Phonetic Spelling: my·kruh·skop·ik
13. Post-fertilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/postfertilization>
IPA: /,poʊst,fɜː.tə.lə'zeɪ.ʃən/
Phonetic Spelling: pohst·fur·tuh·luh·zay·shuhn
14. Manipulated
Pronunciation link: <https://www.merriam-webster.com/dictionary/manipulate>

IPA: /mə'nɪp.jə.leɪ.tɪd/

Phonetic Spelling: muh·nip·yuh·lay·tid