

Submission ID #: 70630

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21542198>

Title: Isolating Single Cells from *Xenopus* Early Embryos and Sorting Them by Size

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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**, all done
- 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: 09

Number of Shots: 22

Introduction

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

INTRODUCTION:

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

- 1.1. **Hui Chen:** Our research focuses on how cell size regulates genome and cell fate decisions in early embryo 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 overlook size heterogeneity in early *Xenopus* embryos; this protocol enables precise physical separation and sorting by cell size.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Hui Chen:** This protocol allows researchers to study functions and mechanisms mediated by cell size.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.4. **Chuan Qin:** The most important consideration for this protocol is to carefully handle the dissociated cells as they are fragile.

- 1.4.1. 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?~~

- 1.5. **Chuan Qin:** After this protocol, the sorted cells can be used for cell cycle analysis, single-cell sequencing, and confocal imaging.
 - 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 the University of South Carolina

Protocol

2. Isolation and Size-Based Sorting of Early Embryonic Blastomeres from *Xenopus laevis* Embryos

Demonstrator: Chuan Qin

- 2.1. To begin, add ~15 milliliters of CMFM to a new 60-millimeter Petri dish coated with 1.5 percent agarose gel [1-TXT]. Using a plastic transfer pipette, transfer 10 to 100 *Xenopus laevis* embryos into the dish [2]. Place the dish under a stereomicroscope [3].
 - 2.1.1. WIDE: Talent pouring CMFM into a 60-millimeter agarose-coated Petri dish on the bench. **TXT: CMFM: Calcium and Magnesium-Free Medium**
 - 2.1.2. Talent using a plastic transfer pipette to move embryos into the prepared dish.
 - 2.1.3. Talent positioning the Petri dish under a stereomicroscope and adjusting it into view.
- 2.2. Carefully catch the vitelline membrane on the surface of the embryos with a tweezer [1]. Peel off the membrane with another tweezer [2-TXT].
 - 2.2.1. SCOPE: [SCOPE-2.2.1-and-2.2.2.avi](#). 00:00-00:10
 - 2.2.2. SCOPE: [SCOPE-2.2.1-and-2.2.2.avi](#). 00:11-end
TXT: Avoid damage to the embryos
- 2.3. Incubate the devitellined embryos in CMFM for 1 hour at room temperature [1]. Gently shake the dish every 5 minutes to facilitate cell dissociation [2]. Check regularly until no piles of blastomeres are visible [3].
 - 2.3.1. Talent placing the Petri dish containing embryos on the bench for incubation at room temperature.
 - 2.3.2. Talent gently shaking the dish by hand.
 - 2.3.3. SCOPE: [SCOPE-2.3.3.avi](#). 00:26-end
- 2.4. During blastomere dissociation, submerge the 40-micrometer, 70-micrometer, and 100-micrometer cell strainers in individual Petri dishes filled with CMFM [1]. Arrange the dishes from left to right in the indicated order [2]. Place two additional CMFM-filled dishes between the groups for brief washing of the strainers during sorting [3].
 - 2.4.1. Talent placing three cell strainers labeled 40 micrometer, 70 micrometer, and 100 micrometer into separate CMFM-filled Petri dishes.
 - 2.4.2. Shot of the dishes arranged from left to right in increasing mesh size order.

- 2.4.3. Talent positioning two additional CMFM-filled dishes between the groups for washing.
- 2.5. Now gently swirl the dish containing the isolated single blastomeres to enrich them at the center [1]. Then carefully transfer the blastomeres into the 40-micrometer strainer submerged in CMFM [2].
 - 2.5.1. Talent gently swirling the dish to concentrate blastomeres in the center.
 - 2.5.2. Talent transferring the blastomere suspension into the 40-micrometer strainer.
- 2.6. Gently shake the strainer a few times to allow blastomeres smaller than 40 micrometers to pass through [1]. Quickly move the strainer to a CMFM-filled dish for brief washing [2]. Then carefully transfer the blastomeres retained in the strainer into the 70-micrometer strainer [3].
 - 2.6.1. Talent gently shaking the 40 micrometer strainer over the dish.
 - 2.6.2. Talent transferring the 40-micrometer strainer into a washing dish containing CMFM.
 - 2.6.3. Shot of the retained blastomeres being transferred into the 70 micrometer strainer.
- 2.7. Repeat the sorting procedure using progressively larger strainers until the blastomeres are collected in the 100 micrometer strainer [1].
 - 2.7.1. Talent sequentially transferring blastomeres through the 70 micrometer and then the 100 micrometer strainers.
- 2.8. Gently swirl each dish containing sorted blastomeres to enrich them at the center [1]. Then carefully transfer approximately 4 milliliters of blastomeres [2] onto the top of 0.5 milliliters of 15 percent density gradient medium in a fluorescence-activated cell sorting tube without mixing [3].
 - 2.8.1. Talent swirling a dish with sorted blastomeres.
 - 2.8.2. Talent pipetting out 4 mL of the blastomeres suspension.
 - 2.8.3. Talent slowly layering the blastomere suspension over the density gradient medium in a fluorescence-activated cell sorting tube.
- 2.9. After 10 minutes, the intact blastomeres will settle to the bottom by gravity [1], separating them from yolk globules derived from burst blastomeres [2].
 - 2.9.1. Shot of the tube showing settled intact blastomeres at the bottom.
 - 2.9.2. Talent pointing to the layer of separated yolk globules above the intact blastomeres.

Results

3. Results

3.1. The unsorted control and size-sorted single blastomeres were fixed and imaged under a bright-field microscope [1].

3.1.1. LAB MEDIA: Figure 4A.

3.2. The sorted blastomeres showed narrow size distributions within their respective expected ranges [1], relative to the control unsorted blastomeres [2].

3.2.1. LAB MEDIA: Figure 4C. *Video editor: Highlight each sorted group "< 40 micrometer," "40–70 micrometer," and "> 70 micrometer" and show their tighter clusters along the diameter axis.*

3.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the "Control" group on the y-axis*

3.3. The less than 40 micrometers group showed a mean diameter of approximately 33 micrometers[1]. The 40 to 70 micrometers group showed a mean diameter of approximately 55 micrometers [2]. while the larger than 70-micrometers group showed a mean diameter of approximately 91 micrometers [3].

3.3.1. LAB MEDIA: Figure 4A. *Video editor: Zoom in on the image labeled "< 40 micrometer" and display the text "33 ± 5.3 micrometer."*

3.3.2. LAB MEDIA: Figure 4A. *Video editor: Zoom in on the image labeled "40–70 micrometer" and display the text "55 ± 11 micrometer."*

3.3.3. LAB MEDIA: Figure 4A. *Video editor: Zoom in on the image labeled "> 70 micrometer" and display the text "91 ± 17 micrometer."*

Pronunciation Guide:

Xenopus

Pronunciation link:

<https://www.merriam-webster.com/medical/Xenopus>

IPA: /'zɛnəpəs/

Phonetic Spelling: zen-uh-puhs

Blastomere / Blastomeres

Pronunciation link:

<https://www.merriam-webster.com/dictionary/blastomere>

IPA: /'blæstəˌmɪr/

Phonetic Spelling: blas-tuh-meer

Stereomicroscope

Pronunciation link:

<https://www.howtopronounce.com/stereomicroscope>

IPA: /ˌstɛrɪoʊˈmaɪkrəˌskoʊp/

Phonetic Spelling: ste-ree-oh-my-kruh-skohp

Agarose

Pronunciation link:

<https://www.merriam-webster.com/dictionary/agarose>

IPA: /'ægəˌroʊs/

Phonetic Spelling: ag-uh-rohs

Vitelline

Pronunciation link:

<https://www.howtopronounce.com/vitelline>

IPA: /'vɪtəˌlaɪn/

Phonetic Spelling: vit-uh-line

Devitellined

Pronunciation link:

<https://www.howtopronounce.com/devitellined>

IPA: /diːˈvɪtəˌlaɪnd/

Phonetic Spelling: dee-vit-uh-lined

Micrometer

Pronunciation link:

<https://www.merriam-webster.com/dictionary/micrometer>

IPA: /maɪˈkrəˌmɛtər/

Phonetic Spelling: my-krah-muh-ter

Dissociation

Pronunciation link:

<https://www.merriam-webster.com/dictionary/dissociation>

IPA: /dɪˌsoʊsiˈeɪʃən/

Phonetic Spelling: dih-soh-see-ay-shun

Fluorescence

Pronunciation link:

<https://www.merriam-webster.com/dictionary/fluorescence>

IPA: /flʊ'resəns/

Phonetic Spelling: floo-res-uhns

❓ Confocal

Pronunciation link:

<https://www.merriam-webster.com/dictionary/confocal>

IPA: /kɑ:n'fəʊkəl/

Phonetic Spelling: kon-foh-kuhl

❓ Gradient

Pronunciation link:

<https://www.merriam-webster.com/dictionary/gradient>

IPA: /'greɪdiənt/

Phonetic Spelling: gray-dee-uhnt

❓ Embryos / Embryo

Pronunciation link:

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

IPA: /'ɛmbriəʊ/

Phonetic Spelling: em-bree-oh