

Submission ID #: 70591

Scriptwriter Name: Deepthi Das

Project Page Link: https://review.jove.com/files_upload.php?src=21530303

Title: Metabolic Labeling of the Nascent Transcriptome in *Xenopus* Early Embryogenesis

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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**

VWR® Laboratory LED Zoom Stereo Series (VWR Cat. No.: 10836-006)

ACCU-SCOPE® 3075 Zoom Stereomicroscopes (VWR Cat. No.: 470351-130)

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope.

2.3.4

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: 18

Number of Shots: 55

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:** In this study, we investigate how zygotic genome activation and transcriptional regulation occur during early *Xenopus* embryogenesis.
 - 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 struggle to detect newly activated zygotic transcripts amid abundant maternal RNAs; this protocol labels and enriches nascent transcripts.
 - 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:** Careful purification of metabolically labeled nascent RNAs is the most critical factor for ensuring sensitivity and specificity in this protocol.
 - 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:** EU-labeled nascent RNAs produced using this protocol can be further analyzed biochemically in-vitro.
 - 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. **Chuan Qin**: This approach enables future studies to investigate spatial genome activation patterns, tissue-specific gene expression, and regulatory mechanisms at single-cell resolution.

- 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. Microinjection of 5-Ethynyl Uridine (5-EU) into 1-Cell Embryos

Demonstrator: Chuan Qin

2.1. To begin, place the microinjection chamber on a clean surface [1] and add 1 milliliter of Ficoll solution into the chamber [2-TXT]. Obtain *Xenopus laevis* embryos stored in 0.1 times Marc's Modified Ringer's or MMR (M-M-R) solution [3]. Use a plastic transfer pipette to transfer 20 or more embryos to the center of the microinjection chamber [4].

2.1.1. WIDE: Show the experimental setup while the talent is arranging the microinjection chamber.

2.1.2. Talent adding 1 mL Ficoll solution into the microinjection chamber

TXT: Ficoll solution: 3% Ficoll in 0.5× MMR

2.1.3. Shot of the *Xenopus* embryos stored in 0.1 x MMR. *Videographer: Zoom in to show the label if possible.*

2.1.4. Talent transferring embryos to the center of the microinjection chamber using a plastic transfer pipette.

2.2. Prepare the injection needle by cutting the fine tip of a glass needle with tweezers [1]. Position the needle vertically with the tip facing down and load 5-ethynyl uridine or 5-EU (E-U) using a P2 pipette with a microloader tip [2-TXT]. Mount the needle onto a micromanipulator [3]. Using a P10 pipette, place 10 microliters of halocarbon oil on a stage micrometer and position it under a stereomicroscope [4].

2.2.1. Talent cutting the tip end of the glass needle with a fine tweezer.

2.2.2. Talent loading 0.5 µL of 5-EU into the vertically positioned glass needle **TXT: Add 0.5 µL of 50 mM 5-EU**

2.2.3. Talent mounting the needle onto the micromanipulator.

2.2.4. Talent adding 10 µL of halocarbon oil onto the stage micrometer and placing the stage micrometer under the stereomicroscope.

2.3. For volume calibration, press the microinjector foot pedal [1] and inject a droplet into the halocarbon oil on the micrometer [2]. Adjust the pressure and injection time on the microinjector until the droplet diameter is 0.27 millimeters [3]. Hold the 1-cell stage embryo with tweezers and control the needle with the micromanipulator to inject 5-EU into it [4-TXT].

- 2.3.1. Shot of the talent pressing the foot pedal
- 2.3.2. Talent injecting a droplet into the halocarbon oil on the micrometer. **NOTE: Authors provided a scope shot for better visuals (SCOPE-2.3.2.avi: 00:12-00:26)**
- 2.3.3. Talent adjusting the pressure and injection time on the microinjector while checking the droplet diameter on the micrometer.
- 2.3.4. LAB MEDIA: SCOPE-2.3.4.avi: 00:06-00:14 **TXT: Use 10 nL of 50 mM 5-EU**

2.4. Transfer the injected embryos into a new glass dish containing Ficoll solution and incubate for 1 hour [1]. Remove the Ficoll solution [2] and add 0.1 times MMR solution [3]. Visually inspect embryos during development and remove any abnormal embryos if necessary [4].

- 2.4.1. Talent transferring injected embryos into a new glass dish containing Ficoll solution and placing it aside.
- 2.4.2. Talent removing the Ficoll solution from the dish.
- 2.4.3. Talent adding 0.1x MMR solution to the embryos.
- 2.4.4. Talent checking the embryo development and removing abnormal embryos from the dish.

2.5. Transfer 10 embryos at the desired developmental stage into a 1.5-milliliter microcentrifuge tube [1]. Remove any residual medium from the tube, snap freeze the embryos in liquid nitrogen, and store in a minus 80 degrees Celsius freezer [2-TXT].

- 2.5.1. Talent transferring 10 embryos into a 1.5 mL microcentrifuge tube.
- 2.5.2. Shot of the talent snap freezing the embryos in liquid nitrogen. **TXT: Perform RNA extraction and quantification**

3. ~~Total RNA Extraction and Quantification~~ **NOTE: This section is not filmed**

~~3.1. Add 700 microliters of Buffer RLT (R-L-T) from the ribonucleic acid or RNA (R-N-A) extraction kit to the thawed embryo tube and mix by pipetting at least 15 times [1]. Incubate on ice for 20 min and then homogenize fully by pipetting 10 more times [2].~~

~~3.1.1. Shot of talent adding Buffer RLT to the thawed embryo tube and mixing multiple times.~~

~~3.1.2. Shot of the talent taking the samples from ice and pipetting the samples multiple times.~~

~~3.2. Now, add 700 microliters of 70 percent ethanol to the homogenized embryos and mix by pipetting [1]. Load 700 microliters of the mixture into a spin column [2]. Centrifuge~~

~~the column at 10,000 g for 1 minute [3] and discard the flow through [4-TXT].~~

~~3.2.1. Talent adding 700 µL of 70% ethanol to the homogenized embryos and mixing it.~~

~~3.2.2. Talent transferring 700 µL into a spin column.~~

~~3.2.3. Talent placing the spin column into the centrifuge.~~

~~3.2.4. Talent discarding the flow through.~~

TXT: Repeat centrifugation until entire mixture is processed

~~3.3. Add 350 microliters of Buffer RW1 (*R-W-1*) to the column, centrifuge at 10,000 g for 1 minute, and discard the flow through to wash the column [1]. Add 80 microliters of deoxyribonuclease I (*one*) to the center of the column membrane [2-TXT]. Wash the column once with 350 microliters RW1, twice with 500 microliters Buffer RPE (*R-P-E*) as shown earlier and dry the spin column by centrifuging at 10,000 g for 2 min [3].~~

~~3.3.1. Representative shot of talent adding RW1 buffer into the column.~~

~~3.3.2. Talent adding deoxyribonuclease I to the center of the column membrane and keeping it aside. **TXT: Incubate: 15 minutes, RT**~~

~~3.3.3. Talent placing the spin column into the centrifuge for the drying spin.~~

~~3.4. Transfer the column to a new ribonuclease or RNase (*R-nase*) free tube [1]. Add 35 microliters of RNase free water to the center of the column membrane [2]. Centrifuge the column at 10,000 g for 1 minute [3]. Collect the eluted RNA and place it on ice [4].~~

~~3.4.1. Talent transferring the spin column into a new RNase free tube.~~

~~3.4.2. Talent adding 35 µL of RNase free water to the center of the membrane.~~

~~3.4.3. Talent placing the spin column into the centrifuge.~~

~~3.4.4. Talent collecting the eluted RNA and keeping it on ice.~~

~~3.5. Prepare the working solution from the RNA quantification kit by diluting the RNA reagent dye 1 to 200 in RNA buffer [1-TXT]. Then, set up a 0.2 milliliter 8-tube strip [2]. Put the 8-tube strip in a fluorometer [3]. Select the RNA assay, calibrate with the standards [4]. Then read the concentrations of the samples [5].~~

~~3.5.1. Talent preparing the working solution by diluting the RNA reagent dye in the RNA buffer. **TXT: Total volume = 200 µL × (standards + samples)**~~

~~3.5.2. **TEXT ON PLAIN BACKGROUND:**~~

~~In a 0.2-mL 8-tube strip, prepare dilutions:~~

- ~~• Standards: 10 µL standard + 190 µL working solution~~

- ~~Samples: 1 μ L RNA + 199 μ L working solution~~

~~3.5.3. Talent placing the 8-tube strip into the fluorometer.~~

~~3.5.4. Show the fluorometer interface selecting the **RNA Assay** and calibrating with the standards~~

~~3.5.5. Talent finger points to the sample concentrations.~~

4. RNA Biotinylation and Cleaning

4.1. To make a 50-microliter click reaction, mix the components in a 1.5-milliliter microcentrifuge tube [1]

4.1.1. TEXT ON A PLAIN BACKGROUND

EU buffer

2 mM CuSO₄

0.25 mM biotin azide

10 mM additive 1

12 mM additive 2

4.2. Incubate the reaction mix on a nutator at room temperature for 30 min [1]. Now, add glycogen, ammonium acetate, and chilled ethanol to each reaction mix [2-TXT]. Mix well by pipetting 5 times [3]. Incubate overnight in a minus 80 degrees Celsius freezer [4].

4.2.1. Shot of the talent placing the reaction mix on a nutator.

4.2.2. Talent adding the reagents to the reaction tube.

TXT: 1 μ L glycogen; 50 μ L 7.5 M ammonium acetate; 700 μ L 100% ethanol

4.2.3. Talent pipetting the reaction mixture up and down to mix.

4.2.4. Talent placing the tube in a minus 80 °C freezer.

4.3. Centrifuge at 13,000 *g* for 20 minutes at 4 degrees Celsius, then remove the supernatant without disturbing the pellet [1]. Wash the pellet twice with 700 microliters of 75 percent ethanol [2]. Air-dry the pellet at room temperature for 5 to 10 minutes and resuspend the pellet in 10 microliters of RNase-free water [3].

4.3.1. Shot of the talent taking the tube out of the centrifuge and removing the supernatant. **NOTE: This is filmed in 2 separate shots**

4.3.2. Shot of talent adding 75% ethanol to the tube and mixing it.

4.3.3. Show the air-dried pellet and then show talent resuspending the pellet in 10 μ L RNase-free water. **NOTE: This is filmed in 2 separate shots**

5. Nascent EU-RNA Purification

5.1. Dispense 5 microliters of streptavidin-coated magnetic beads into a new 1.5-milliliter tube with the sample [1]. Add 9 microliters of Wash Buffer 2 to the beads and mix well [2]. Place the tube on a magnetic rack for 2 minutes [3]. Remove the supernatant [4-TXT]. Resuspend the beads in 5 microliters of Wash Buffer 2 [5].

- 5.1.1. Talent dispensing magnetic beads into a new tube with the sample
- 5.1.2. Talent adding Wash Buffer 2 to the beads and mixing the bead suspension.
- 5.1.3. Talent placing the tube on a magnetic rack.
- 5.1.4. Talent removing the supernatant from the tube on the magnetic rack. **TXT: Repeat the wash cycle twice**
- 5.1.5. Talent resuspending the beads in Wash Buffer 2.

5.2. Prepare a master mix in a 1.5-microliter microcentrifuge tube [1]. Add 15 microliters of the master mix to each tube containing the RNA sample [2]. Place the tube on a heat block and incubate it at 69 degrees Celsius for 5 minutes [3]. Afterward, put the tube on ice [4].

5.2.1. **TEXT ON A PLAIN BACKGROUND:**

Master mix:

- i. 12.5 μ L of RNA binding buffer (Component G)
- ii. 0.2 μ L of 1:10 diluted RNase OUT
- iii. 2.3 μ L of RNase-free H₂O

- 5.2.2. Talent adding 15 μ L master mix to the tubes containing the RNA sample.
- 5.2.3. Talent placing the tube on a heat block.
- 5.2.4. Talent placing the tube on ice.

5.3. Then, add 5 microliters of pre-washed beads to each RNA sample [1], and incubate on a nutator at room temperature for 30 min [2].

- 5.3.1. Talent adding 5 μ L pre-washed beads to the RNA sample.
- 5.3.2. Talent placing the tube on a nutator.

5.4. Wash the bead-RNA complexes 5 times with 50 microliters Wash Buffer 1, followed by five washes with 50 microliters Wash Buffer 2 [1]. Resuspend the beads in 5 microliters Wash Buffer 2 [2].

- 5.4.1. Representative shot of Talent adding Wash Buffer 1 to the tube.
- 5.4.2. Talent resuspending the beads in Wash Buffer 2.

5.5. Use the beads containing the nascent EU-RNAs directly for complementary deoxyribonucleic acid synthesis and library preparation [1].

- 5.5.1. Shot of the resuspended beads in the tube ready for direct complementary deoxyribonucleic acid synthesis and library preparation.

Results

6. Results

6.1. Increasing numbers of differentially activated genes were detected during zygotic genome activation when the nascent transcriptome at 6 hours post-fertilization was compared with that at 5 hours post-fertilization [1], with further increases observed at 7 hours post-fertilization [2], 8 hours post-fertilization [3], and 9 hours post-fertilization [4].

6.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the MA plot titled “6 hpf vs 5 hpf” and emphasize the red dots.*

6.1.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the MA plot titled “7 hpf vs 5 hpf” and emphasize the red dots.*

6.1.3. LAB MEDIA: Figure 2A. *Video editor: Highlight the MA plot titled “8 hpf vs 5 hpf” and emphasize the red dots.*

6.1.4. LAB MEDIA: Figure 2A. *Video editor: Highlight the MA plot titled “9 hpf vs 5 hpf” and emphasize the red dots.*

6.2. Zygotic genes were detected at higher levels and at earlier times in the nascent EU-RNA sequencing data [1] compared with the total RNA sequencing data [2].

6.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the red signal tracks labeled “Nascent EU-RNA-seq.”*

6.2.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the golden signal tracks labeled “Total RNA-seq.”*

1. Ficoll

Pronunciation link: <https://www.howtopronounce.com/ficoll>

IPA: /'fɪkəl/

Phonetic Spelling: fih·kawl

2. Xenopus laevis

Pronunciation link: <https://www.howtopronounce.com/xenopus-laevis>

IPA: /'zɛnəpəs 'li:vɪs/

Phonetic Spelling: zen·uh·puhs lee·vihs

3. Microinjection

Pronunciation link: <https://www.howtopronounce.com/microinjection>

IPA: /,maɪkroʊɪn'dʒɛkʃən/

Phonetic Spelling: mye·kroh·in·jek·shuhn

4. Ethynyl
Pronunciation link: <https://www.howtopronounce.com/ethynyl>
IPA: /'εθɪnəl/
Phonetic Spelling: eth-ih-nuhl
5. Uridine
Pronunciation link: <https://www.merriam-webster.com/dictionary/uridine>
IPA: /'jʊrəˌdiːn/
Phonetic Spelling: yoor-uh-deen
6. Micromanipulator
Pronunciation link: <https://www.howtopronounce.com/micromanipulator>
IPA: /ˌmaɪkrəʊməˈnɪpjəˌleɪtər/
Phonetic Spelling: mye-kroh-muh-nip-yuh-lay-ter
7. Halocarbon
Pronunciation link: <https://www.merriam-webster.com/dictionary/halocarbon>
IPA: /ˌheɪləʊˈkɑːbən/
Phonetic Spelling: hay-loh-kar-buhn
8. Stereomicroscope
Pronunciation link: <https://www.howtopronounce.com/stereomicroscope>
IPA: /ˌstɛrɪəʊˈmaɪkrəˌskoʊp/
Phonetic Spelling: ster-ee-oh-mye-kruh-skohp
9. Microinjector
Pronunciation link: No confirmed link found
IPA: /ˌmaɪkrəʊɪnˈdʒektər/
Phonetic Spelling: mye-kroh-in-jek-ter
10. Microcentrifuge
Pronunciation link: https://www.oed.com/dictionary/microcentrifuge_n
IPA: /ˌmaɪkrəʊˈsentrɪˌfjuːdʒ/
Phonetic Spelling: mye-kroh-sen-trih-fyooj
11. Ribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic%20acid>
IPA: /ˌraɪboʊnuːˈkliːɪk/
Phonetic Spelling: rye-boh-noo-kee-ik
12. Homogenize
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenize>
IPA: /həˈmɑːdʒəˌnaɪz/
Phonetic Spelling: huh-maj-uh-nyze
13. Deoxyribonuclease
Pronunciation link: <https://www.howtopronounce.com/deoxyribonuclease>
IPA: /diˌɒksiˌraɪboʊˈnuːkliəs/
Phonetic Spelling: dee-ok-see-rye-boh-noo-kee-ayce

14. Ribonuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonuclease>
IPA: /ˌraɪboʊˈnuːkliːɪs/
Phonetic Spelling: rye·boh·noo·klee·ayce
15. Fluorometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorometer>
IPA: /flɒˈrɒmətər/
Phonetic Spelling: floor·om·uh·ter
16. Biotinylation
Pronunciation link: <https://www.howtopronounce.com/biotinylation>
IPA: /ˌbaɪoʊˈtɪnəˈleɪʃən/
Phonetic Spelling: by·oh·tin·uh·lay·shuhn
17. Streptavidin
Pronunciation link: <https://www.howtopronounce.com/streptavidin>
IPA: /strɛpˈtævɪdɪn/
Phonetic Spelling: strep·tav·ih·din
18. Zygotic
Pronunciation link: <https://www.howtopronounce.com/zygotic>
IPA: /zaɪˈɡɒtɪk/
Phonetic Spelling: zye·got·ik
19. Transcriptome
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcriptome>
IPA: /trænˈskrɪpˌtoʊm/
Phonetic Spelling: tran·skrip·tohm
20. Fertilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/fertilization>
IPA: /ˌfɜːtələˈzeɪʃən/
Phonetic Spelling: fur·tuh·luh·zay·shuhn
21. Differentially
Pronunciation link: <https://www.merriam-webster.com/dictionary/differentially>
IPA: /ˌdɪfəˈrɛnʃəli/
Phonetic Spelling: dif·uh·ren·shuh·lee
22. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
23. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpərˈnɛtənt/
Phonetic Spelling: soo·per·nay·tuhnt

24. Ammonium

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

IPA: /ə'moʊniəm/

Phonetic Spelling: uh·moh·nee·uhm

25. Glycogen

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

IPA: /'glʌɪkədʒən/

Phonetic Spelling: glye·kuh·juhn