

Submission ID #: 70592

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21530608>

Title: Visualizing Zygotic Genome Activation in Single Cells of Early 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? **No**

- 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: 22

Number of Shots: 51

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**

INTRODUCTION:

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

- 1.1. **Hui Chen:** Our research focuses on how the genome becomes activated in individual cells during early embryonic 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 a direct visualization of large-scale genome activation at the single-cell resolution.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Hui Chen:** This protocol can be used in any embryonic system for studying single-cell genome activation.
 - 1.3.1. 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?~~

- 1.4. **Hui Chen:** This protocol allows researchers to directly visualize and quantify nascent transcripts during early embryonic genome activation.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 4.6.1*

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

- 1.5. **Chuan Qin:** The most important challenge for this protocol is to label the nascent RNAs in whole embryos.

- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.8.1*

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

- 1.6. **Chuan Qin:** Future studies include single-cell mechanisms and spatial coordination of genome regulation in early embryogenesis.

- 1.6.1. 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

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

2. Fixing the 5-Ethynyl Uridine (5-EU)-Microinjected Embryos

Demonstrator: Chuan Qin

- 2.1. To begin, add about 1.8 milliliters of 4 percent paraformaldehyde in MEM into a 2-milliliter scintillation glass vial [1]. Using a plastic transfer pipette, transfer approximately 20 5-ethynyl uridine microinjected embryos into the glass vial [2-TXT].
 - 2.1.1. WIDE: Talent adding about 1.8 milliliters of 4 percent paraformaldehyde in 1 times MEM into a 2 milliliter scintillation glass vial.
 - 2.1.2. Talent transferring approximately 20 5-ethynyl uridine microinjected embryos into the glass vial with a plastic transfer pipette. **TXT: Minimize the amount of culture medium added to the glass vial**
- 2.2. Fill the glass vial to the top with an additional 4 percent paraformaldehyde in MEM [1]. Close the vial with the cap, ensuring that no air bubbles remain inside.[2].
 - 2.2.1. Talent filling the glass vial to the top with additional 4 percent paraformaldehyde in 1 times MEM.
 - 2.2.2. Talent capping the glass vial with no air bubbles left inside.
- 2.3. Place the glass vial on a rotator and rotate the embryos at a low speed for at least 2 hours at room temperature [1].
 - 2.3.1. Talent placing the glass vial on a rotator for fixation at a low speed for at least 2 hours at room temperature.
- 2.4. Then, using a pipette, carefully remove the fixative [1]. Fill the vial with 100 percent methanol and rotate the embryos as demonstrated earlier for 5 minutes [2].
 - 2.4.1. Talent carefully removing the fixative from the glass vial with a pipette.
 - 2.4.2. Talent filling the glass vial with 100 percent methanol.
- 2.5. After that, carefully remove the methanol [1] and add fresh methanol [2]. Repeat the rotation and dehydration at least 2 more times so that the embryos are completely dehydrated [3-TXT].
 - 2.5.1. Talent carefully removing the methanol from the glass vial.
 - 2.5.2. Talent adding fresh methanol to the glass vial.
 - 2.5.3. Talent placing the glass vial on a rotator. **TXT: Store the embryos in methanol at -20 °C**

3. Conjugating Nascent RNAs with a Fluorophore via Click Reaction

- 3.1. To rehydrate the embryos, first remove the methanol from the glass vial [1]. Add 2 milliliters of 75 percent methanol in 0.5 times SSC, close the vial with the cap, and rotate the embryos as demonstrated earlier [2].
 - 3.1.1. Talent removing the methanol from the glass vial.
 - 3.1.2. Talent adding 2 milliliters of 75 percent methanol in 0.5 times SSC to the glass vial and capping it.
- 3.2. Sequentially replace the solution with 50% methanol in 0.5 times SSC, followed by 25% methanol in 0.5 times SSC, and finally 0.5 times SSC [1], rotating the embryos at low speed for 5 minutes during each step [2-TXT].
 - 3.2.1. Talent removing the liquid and adding 2 milliliters of 50 percent methanol in 0.5 times SSC to the glass vial.
 - 3.2.2. Talent placing the vial on a rotator. **TXT: Repeat the final 0.5× SSC wash at least 2x to fully rehydrate embryos**
- 3.3. Next, add 2 milliliters of freshly prepared bleaching solution containing 2 percent hydrogen peroxide and 5 percent formamide in 0.5 times SSC into the glass vial [1]. Place the glass vial on a piece of aluminum foil [2] and position it under a light-emitting diode light at a distance that does not overheat the samples [3-TXT].
 - 3.3.1. Talent adding 2 milliliters of freshly prepared bleaching solution containing hydrogen peroxide and formamide in SSC into the glass vial.
 - 3.3.2. Talent placing the glass vial on a sheet of aluminum foil.
 - 3.3.3. Talent positioning the glass vial under a light-emitting diode light source. **TXT: Bleach embryos under light until pigments turn white (≈5–6 h)**
- 3.4. Check the embryos periodically [1].
 - 3.4.1. Talent checks the embryos.
- 3.5. After that, carefully remove the bleaching buffer from the glass vial [1] and add 2 milliliters of 0.5 times SSC to quickly rinse the embryos [2-TXT].
 - 3.5.1. Talent carefully removing the bleaching buffer from the glass vial with a pipette.
 - 3.5.2. Talent adding 2 milliliters of 0.5 times SSC to rinse the embryos. **TXT: Repeat rinse 1x, if required**
- 3.6. Then, add 2 milliliters of TBST into the glass vial [1]. Place the glass vial on a rotator and rotate the embryos at a low speed for 1 hour [2]. Remove the liquid from the glass vial after rotation [3-TXT].

- 3.6.1. Talent adding 2 milliliters of 1 times TBST into the glass vial.
- 3.6.2. Talent placing the glass vial on a rotator for rotation at a low speed for 1 hour.
- 3.6.3. Talent removing the liquid from the glass vial with a pipette. **TXT: Wash with TBST 5x followed by 2x with TBS**
- 3.7. Next, in a 1.5-milliliter microcentrifuge tube, add reagents to prepare the click reaction mixture [1] [2]. Add the ascorbic acid to the reaction mixture last, then pipette up and down to mix thoroughly [3].
 - 3.7.1. Talent adding components in a 1.5 ml tube. *Video editor: Use split screen to show 3.9.1 and 3.9.2 side by side*
 - 3.7.2. TEXT ON A PLAIN BACKGROUND
 - 100 mM Tris-HCl (pH 8.5)
 - 1 mM CuSO₄
 - 25 µM TAMRA-azide
 - 100 mM ascorbic acid
 - 3.7.3. Talent adding ascorbic acid last to the reaction mixture in the microcentrifuge tube and pipetting the mixture
- 3.8. Now, add 200 microliters of the reaction mixture to the embryos in the glass vial [1]. Ensure that the reaction mixture volume is sufficient to submerge all embryos in the vial [2]. Incubate the embryos for 12 hours at room temperature in the dark [3].
 - 3.8.1. Talent adding 200 microliters of the click reaction mixture to the embryos in the glass vial.
 - 3.8.2. Shot showing the embryos fully submerged in the reaction mixture in the glass vial.
 - 3.8.3. Talent placing the glass vial in a dark location for incubation at room temperature for 12 hours.
- 3.9. Afterwards, carefully remove the click reaction mixture from the glass vial [1]. Quickly rinse the embryos twice with TBST [2]. Then wash the embryos with TBST at least 5 times, changing the solution every 1 hour [3].
 - 3.9.1. Talent carefully removing the click reaction mixture from the glass vial with a pipette.
 - 3.9.2. Talent rinsing the embryos with 1 times TBST.
 - 3.9.3. Talent placing the glass vial on a rotator to perform repeated washes with 1 times TBST.
- 3.10. Continue washing the embryos by replacing TBST every 2 hours [1]. Leave the embryos overnight at 4 degrees Celsius on a rotator [2-TXT].

3.10.1. Talent replacing the TBST solution in the glass vial.

3.10.2. Talent placing the glass vial on a rotator at 4 degrees Celsius. **TXT: Stop washing when non-specific fluorescence is hardly visible**

4. Clearing Embryos before Confocal Imaging

4.1. After the final wash, carefully remove the buffer from the glass vial [1] and add 2 milliliters of 100 percent methanol to the glass vial [2]. Wrap it with aluminium foil and place it on a rotator to rotate the embryos at a low speed for 5 minutes [3]. Remove the liquid from the glass vial after rotation [4-TXT].

4.1.1. Talent carefully removing the buffer from the glass vial with a pipette.

4.1.2. Talent adding 2 milliliters of 100 percent methanol to the glass vial.

4.1.3. Talent wrapping the glass vial in aluminum foil and placing it on a rotator.

4.1.4. Talent removing the methanol from the glass vial after rotation. **TXT: Repeat dehydration with 100% methanol 5x**

4.2. To completely dehydrate the embryos, add 2 milliliters of 100 percent anhydrous methanol to the glass vial [1]. Place the vial on a rotator and rotate the embryos at a low speed for 5 minutes [2]. Remove the methanol after rotation and repeat the dehydration at least 5 times [3].

4.2.1. Talent adding 2 milliliters of 100 percent anhydrous methanol to the glass vial.

4.2.2. Talent placing the glass vial on a rotator for rotation at a low speed for 5 minutes.

4.2.3. Talent removing the methanol from the glass vial.

4.3. Carefully remove as much methanol as possible from the glass vial [1].

4.3.1. Talent carefully removing the remaining methanol from the glass vial with a pipette.

4.4. Using a glass pipette, slowly add 500 microliters of the clearing reagent benzyl alcohol benzyl benzoate into the glass vial [1]. To completely clear the embryos, keep them in benzyl alcohol benzyl benzoate for 1 to 2 days before imaging [2]. Once the embryos settle at the bottom [3], carefully remove the solution without damaging them, then proceed with imaging [4-TXT].

4.4.1. Talent slowly adding 500 microliters of benzyl alcohol benzyl benzoate into the glass vial using a glass pipette.

4.4.2. Talent leaving the embryos in benzyl alcohol benzyl benzoate on the bench.

4.4.3. Close up shot of the embryos settled at the bottom of the vial.

4.4.4. Talent carefully removes the benzyl alcohol benzyl benzoate solution from the

glass vial. **TXT: Repeat clearing step 2x or until no schlieren lines are visible**

NOTE: 4.5-4.7 are not filmed

- ~~4.5. Using scissors, cut a small square from very high bond tape with a thickness of approximately 1.1 to 1.2 millimeters [1]. Then, cut a piece approximately 10 millimeters by 10 millimeters and adhere it onto a 24 millimeter by 40 millimeter coverglass to form an imaging chamber [2].~~
 - ~~4.5.1. Talent cutting a small square from very high bond tape using scissors.~~
 - ~~4.5.2. Talent adhering the cut tape piece onto a 24 millimeter by 40 millimeter coverglass to form the imaging chamber.~~
- ~~4.6. Carefully transfer the cleared embryos into the chamber [1]. Cover the embryos with another cover glass so that the embryos are sandwiched between the cover glasses for imaging from both sides [2].~~
 - ~~4.6.1. Talent transferring the cleared embryos into the prepared imaging chamber.~~
 - ~~4.6.2. Talent placing another cover glass over the embryos to form a coverglass sandwich.~~
- ~~4.7. Perform imaging under a confocal microscope [1].~~
 - ~~4.7.1. Talent placing the prepared chamber containing embryos onto the stage of a confocal microscope for imaging.~~

Results

5. Results

- 5.1. Nascent 5-ethynyl uridine-RNAs were detected in the nuclei of embryonic cells during large-scale zygotic genome activation [1], with histone H3 used as a nuclear marker [2] and DNA labeling confirming nuclear localization [3].

5.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the red signal labeled "EU-RNA"*

5.1.2. LAB MEDIA: Figure 1. *Video editor: Highlight the green signal labeled "H3" showing green dots across the embryo.*

5.1.3. LAB MEDIA: Figure 1. *Video editor: Highlight the purple signal labeled "DNA"*

- 5.2. The merged image showed that 5-ethynyl uridine -RNA signals overlapped with histone H3 and DNA signals within the nuclei of embryonic cells [1].

5.2.1. LAB MEDIA: Figure 1. *Video editor: Show the panel labeled "Merge" where the red, green, and purple signals appear together in the embryo.*

1. Paraformaldehyde
Pronunciation link: <https://www.merriam-webster.com/dictionary/paraformaldehyde>
IPA: /ˌpɛr.əˈfɔːrˈmæl.dəˌhaɪd/
Phonetic Spelling: peh·ruh·for·mal·duh·hyde
2. Scintillation
Pronunciation link: <https://www.merriam-webster.com/dictionary/scintillation>
IPA: /ˌsɪn.təˈleɪʃən/
Phonetic Spelling: sin·tuh·lay·shuhn
3. Ethynyl
Pronunciation link: <https://www.howtopronounce.com/ethynyl>
IPA: /ˈɛθmaɪl/
Phonetic Spelling: eth·ih·nile
4. Uridine
Pronunciation link: <https://www.merriam-webster.com/dictionary/uridine>
IPA: /ˈjʊr.əˌdiːn/
Phonetic Spelling: yoor·uh·deen
5. Microinjected
Pronunciation link: <https://www.howtopronounce.com/microinjected>

- IPA: /ˌmaɪ.kroʊ.ɪnˈdʒɛk.tɪd/
Phonetic Spelling: my·kroh·in·jekt·id
6. Rotator
Pronunciation link: <https://www.merriam-webster.com/dictionary/rotator>
IPA: /ˈroʊ.teɪ.tə/
Phonetic Spelling: roh·tay·ter
 7. Fixative
Pronunciation link: <https://www.merriam-webster.com/dictionary/fixative>
IPA: /ˈfɪk.sə.tɪv/
Phonetic Spelling: fik·suh·tiv
 8. Methanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/methanol>
IPA: /ˈmɛθ.əˌnɔːl/
Phonetic Spelling: meth·uh·nawl
 9. Dehydrated
Pronunciation link: <https://www.merriam-webster.com/dictionary/dehydrated>
IPA: /ˌdiːˈhaɪ.dreɪ.tɪd/
Phonetic Spelling: dee·hy·dray·tid
 10. Conjugating
Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugating>
IPA: /ˈkɑːn.dʒəˌgeɪ.tɪŋ/
Phonetic Spelling: kon·juh·gay·ting
 11. Fluorophore
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorophore>
IPA: /ˈflɒr.əˌfɔːr/
Phonetic Spelling: floor·uh·for
 12. Rehydrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/rehydrate>
IPA: /ˌriːˈhaɪ.dreɪt/
Phonetic Spelling: ree·hy·drayt
 13. Sequentially
Pronunciation link: <https://www.merriam-webster.com/dictionary/sequentially>
IPA: /sɪˈkwɛn.fə.li/
Phonetic Spelling: si·kwen·shuh·lee
 14. Formamide
Pronunciation link: <https://www.merriam-webster.com/dictionary/formamide>
IPA: /ˈfɔːr.məˌmaɪd/
Phonetic Spelling: for·muh·mide
 15. Hydrogen
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrogen>
IPA: /ˈhaɪ.drə.dʒən/
Phonetic Spelling: hy·druh·juhn
 16. Aluminum
Pronunciation link: <https://www.merriam-webster.com/dictionary/aluminum>

- IPA: /əˈluː.mə.nəm/
Phonetic Spelling: uh·loo·muh·nuhm
17. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /ˈm.kjuː.bert/
Phonetic Spelling: in·kyoo·bayt
18. Microcentrifuge
Pronunciation link: <https://www.howtopronounce.com/microcentrifuge>
IPA: /ˌmaɪ.kroʊˈsɛn.trɪ.fjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
19. Ascorbic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ascorbic>
IPA: /əˈskɔːr.bɪk/
Phonetic Spelling: uh·skor·bik
20. Anhydrous
Pronunciation link: <https://www.merriam-webster.com/dictionary/anhydrous>
IPA: /ænˈhaɪ.drəs/
Phonetic Spelling: an·hy·druhs
21. Benzyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/benzyl>
IPA: /ˈbɛn.zɪl/
Phonetic Spelling: ben·zil
22. Benzoate
Pronunciation link: <https://www.merriam-webster.com/dictionary/benzoate>
IPA: /ˈbɛn.zoʊ.eɪt/
Phonetic Spelling: ben·zoh·ayt
23. Confocal
Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
IPA: /kɑːnˈfoʊ.kəl/
Phonetic Spelling: kon·foh·kuhl
24. Embryonic
Pronunciation link: <https://www.merriam-webster.com/dictionary/embryonic>
IPA: /ˌɛm.briˈɑː.nɪk/
Phonetic Spelling: em·bree·on·ik
25. Zygotic
Pronunciation link: <https://www.merriam-webster.com/dictionary/zygotic>
IPA: /zɑɪˈɡɑː.tɪk/
Phonetic Spelling: zy·got·ik
26. Histone
Pronunciation link: <https://www.merriam-webster.com/dictionary/histone>
IPA: /ˈhɪs.toʊn/
Phonetic Spelling: his·tohn
27. Nuclei
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclei>

IPA: /'nu:.kli.aɪ/

Phonetic Spelling: noo·klee·eye

28. Localization

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

IPA: /,loo.kə.lə'zeɪ.ʃən/

Phonetic Spelling: loh·kuh·luh·zay·shuhn