

Submission ID #: 72960

Scriptwriter Name: Vipasha Sharma

Project Page Link: https://review.jove.com/files_upload.php?src=22252848

Title: Optimized Methods of Investigating Developmental Protein and Transcript Expression in Whole-Mount Mouse Embryos

Authors and Affiliations:

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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?

No

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

Nikon SMZ-2B

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 (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Step 5.1.2

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, done**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? 2.7 miles

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. **Yes**

If **Yes**, please provide the **full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author willing to participate.

Victoria Rashbrook, Postdoctoral Research Scientist

Current Protocol Length

Number of Steps: 18

Number of Shots: 35

Interviews

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

Authors: Statements have been edited for length and clarity. 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. **Victoria Rashbrook:** Our research focuses on understanding how environmental factors influence congenital heart disease risk and identifying potentially targetable underlying mechanisms.
 - 1.1.1. 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?**~~

- 1.2. **Victoria Rashbrook:** This protocol allows researchers to examine the expression and spatial distribution of multiple genes and proteins and quantify their abundance or intensity within individual cells.
 - 1.2.1. 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.**~~

- 1.3. **Victoria Rashbrook:** A key consideration is optimizing permeabilization and antigen retrieval to ensure effective antibody or probe penetration while preserving sample integrity.
 - 1.3.1. 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.**~~

- 1.4. **Victoria Rashbrook:** Future studies can build on this work by optimizing these techniques for larger embryos and other complex tissue types.
 - 1.4.1. 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.

Testimonial Questions (OPTIONAL):-

~~*Videographer: Please capture all testimonial shots in a wide angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.*~~

~~What is the key takeaway you hope viewers remember after watching your video article?~~

- ~~1.5. **Victoria Rashbrook, Postdoctoral Research Scientist:** (authors will present their testimonial statements live)~~

~~How does the video format of your article help you to share your work with the broader scientific community?~~

- ~~1.6. **Victoria Rashbrook, Postdoctoral Research Scientist:** (authors will present their testimonial statements live)~~

Videographer's NOTE: Did not film as the authors changed their mind

Ethics Title Card

This research has been approved by the University of Oxford Animal Welfare Review Board and the Home Office

Protocol

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

2. Embryo Rehydration and Antigen Retrieval

Demonstrator: Victoria Rashbrook

Videographer's NOTE: Victoria (the author) told me that the University (Oxford) requested the protocol to be shot without showing the author's face. They would like to see shots of just the arms and hands. Victoria showed me an email on her phone from the University confirming this. Aside from the wide shot for the first shot, I framed the shots in a way that met with the University's request.

- 2.1. To begin, retrieve the E8.5 and E9.5 embryos harvested from pregnant female mice from minus 20 degrees Celsius storage and allow them to reach room temperature at 20 to 26 degrees Celsius for 10 to 20 minutes [1]. Select embryos with similar somite numbers to compare similar developmental stages [2].
 - 2.1.1. WIDE: Talent retrieving the embryos from -20 °C storage and keeping them aside for 10 to 20 minutes
 - 2.1.2. Talent selecting embryos with similar somite numbers
- 2.2. Bleach and permeabilize the embryos in a solution of 100% methanol, DMSO, and 30% hydrogen peroxide in a 4:1:1 ratio for 2 hours [1]. Rehydrate the E8.5 and E9.5 embryos by rinsing them in 70% methanol, followed by washing in 50% methanol, 25% methanol, and PBS for 10 minutes each at room temperature with gentle rocking [2].
 - 2.2.1. Talent placing the embryos in the methanol, dimethyl sulfoxide, and hydrogen peroxide solution
 - 2.2.2. Talent washing the samples sequentially in 70% methanol, 50% methanol, 25% methanol, and phosphate-buffered saline with gentle rocking
- 2.3. Preheat the block to 98 degrees Celsius [1]. Prepare the citrate unmasking solution by diluting 100 microliters of the citrate unmasking solution in 10.7 milliliters of distilled water [2].
 - 2.3.1. Talent switching the heating block ON and setting the temperature to 98°C.
Videographer's NOTE: Shots 2.3.1 & 2.3.2 were combined together
 - 2.3.2. Talent preparing the citrate unmasking solution by diluting with 100 µL of the

citrate unmasking solution in 10.7 mL of distilled water

Author: I have rearranged the steps to improve the narration flow by placing the rehydration step before the preheating step.

- 2.4. For E9.5 embryos, perform antigen retrieval by preheating the citrate unmasking solution to 98 degrees Celsius using the preheated heat block for at least 20 minutes [1]. Wash the embryos in the citrate unmasking solution 3 times for 10 minutes each at room temperature, keeping the washes stationary to prevent embryo clumping and damage [2].

- 2.4.1. Talent placing the citrate unmasking solution in the preheated heat block

Videographer's NOTE: Shots 2.4.1 & 2.4.2 were combined together, take 2 is better

- 2.4.2. Talent washing the embryos in the citrate unmasking solution

- 2.5. After the room-temperature washes, add the preheated citrate unmasking buffer to the embryos [1] and heat them in a hot block at 98 degrees Celsius for 10 minutes [2]. Remove the embryos from the heat and let them cool for at least 40 minutes [3].

- 2.5.1. Talent adding the preheated citrate unmasking buffer to the embryos

Videographer's NOTE: Shots 2.5.1 & 2.5.2 were combined together, take 2 is better

- 2.5.2. Talent placing the embryos in the hot block

- 2.5.3. Talent removing the embryos from the hot block and keeping them aside for cooling down

3. Blocking and Primary Antibody Incubation

- 3.1. Wash the embryos 3 times for 10 minutes each in 1 milliliter of PBS containing 0.1% Triton-X at room temperature with rocking [1]. Block the embryos in a minimum of 150 microliters per tube of PBS containing 0.1% Triton-X and 10% normal donkey serum for 16 to 24 hours at 4 degrees Celsius with rocking [2].

- 3.1.1. Talent washing the embryos in 1 mL of PBS containing 0.1% Triton-X with rocking **Videographer's NOTE:** take 2 is better

- 3.1.2. Talent placing the embryos in a minimum of 150 μ L per tube of the blocking solution

3.2. Prepare primary antibodies 1 hour before application by diluting them to the recommended concentrations in PBS containing 0.1% Triton-X and 10% normal donkey serum [1]. Keep the diluted primary antibodies at 4 degrees Celsius until use [2].

3.2.1. Talent preparing the primary antibody solution **Videographer's NOTE: Take 1 shouldn't be used. Victoria forgot the timer was going off. She has asked for Take 2 of this shot to be used instead.**

3.2.2. Talent placing the diluted primary antibodies at 4 °C

3.3. Remove the blocking solution from the embryos [1] and add a minimum of 150 microliters of the diluted primary antibody solution to each tube [2]. Incubate the embryos for 1 to 3 days at 4 degrees Celsius with rocking [3].

3.3.1. Talent removing the blocking solution from the embryos

Videographer's NOTE: Shots 3.3.1 & 3.3.2 were combined together,

3.3.2. Talent adding a minimum of 150 µL of diluted primary antibody solution to each tube

3.3.3. Talent placing the embryos for incubation at 4 °C

3.4. After primary antibody incubation, rinse the embryos in PBS containing 0.1% Triton-X [1], then wash them 6 times for 30 minutes each in 1 milliliter of PBS containing 0.1% Triton-X at room temperature [2].

3.4.1. Talent rinsing the embryos in PBS containing 0.1% Triton-X

Videographer's NOTE: Shots 3.4.1 & 3.4.2 were combined together. Take 1 of this shot is not usable. The camera started acting weird and threw the focus out even though I was filming in manual focus mode. Take 3 is the best take from this shot and the one I would recommend using

3.4.2. Talent washing the embryos in 1 mL of PBS containing 0.1% Triton-X

4. Secondary Antibody and Nuclear Staining

4.1. Prepare the secondary antibody solution 1 hour before application by diluting the appropriate fluorescently conjugated secondary antibodies and DAPI (*Da-pee*) to the recommended concentrations in PBS containing 0.1% Triton-X and 10% normal donkey serum [1]. Keep the solution at 4 degrees Celsius in the dark until use [2].

4.1.1. Talent preparing the fluorescently conjugated secondary antibody and nuclear marker solution **Videographer's NOTE: use take 2, not take 1**

4.1.2. Talent placing the secondary antibody solution at 4 °C in the dark until use

4.2. Remove the wash solution [1] and add the diluted secondary antibody solution to the embryos [2]. Incubate the embryos for 16 to 24 hours at 4 degrees Celsius with gentle rocking, protected from light [3].

4.2.1. Talent removing the wash solution from the embryos **Videographer's NOTE: Shots 4.2.1 & 4.2.2 were combined together**

4.2.2. Talent adding the diluted secondary antibody solution to the embryos

4.2.3. Talent placing the embryos at 4 °C in the dark

4.3. Rinse the embryos in PBS containing 0.1% Triton-X [1], then wash them 6 times for 30 minutes each in 1 milliliter of PBS containing 0.1% Triton-X at room temperature with rocking in the dark [2]. Store the embryos in PBS containing 0.1% Triton-X at 4 degrees Celsius in the dark for up to 4 weeks [3].

4.3.1. Talent rinsing the embryos in PBS containing 0.1% Triton-X

Videographer's NOTE: Shots 4.3.1 & 4.3.2 were combined together

4.3.2. Talent washing the embryos in 1 mL of PBS containing 0.1% Triton-X with rocking in the dark.

4.3.3. Talent placing the embryos in PBS containing 0.1% Triton-X at 4 °C in the dark

5. Whole Mount Embryo Preparation

5.1. After staining, using an LDPE (L-D-P-E) Pasteur pipette, gently pipette up the stained embryo and transfer it into a multi-well imaging slide without letting the embryo dry out [1]. If needed, gently orient the embryo as desired without damaging the sample [2].

5.1.1. Talent using an LDPE Pasteur pipette to gently transfer the stained embryo into a multi-well imaging slide

5.1.2. SCOPE: Talent gently orienting the embryo as desired without damaging the sample. **Videographer's NOTE: I could not take the scope kit for shoot.**

Authors provided some images and video. take 3 is the best example." Victoria's footage for Shot 5.1.2 (the scope shot) can be found in the folder called 'Footage Supplied From Victoria Susan Rashbrook - Shot 5.1.2'.

5.2. For tissue clearing, gently remove the last wash from the embryo [1]. Add up to 200 microliters of Cubic-R solution to the embryo at room temperature [2]. Cover the slide with a lid and incubate the embryo in Cubic-R solution for at least 40 minutes [3].

5.2.1. Talent gently removing the last wash from the embryo **Videographer's NOTE:**
Shots 5.2.1 & 5.2.2 and 5.2.3 were combined together

5.2.2. Talent adding up to 200 μ L of Cubic-R solution to the embryo

5.2.3. Talent covering the slide with a lid and keeping it aside for 40 minutes

5.3. Place the prepared slide on the confocal microscope stage [1] and acquire images [2].

5.3.1. Talent placing the slide on the microscope stage

5.3.2. **SCREEN:** 72960_screenshot-1_step-5.4.2.mp4 04:22-04:26

Videographer's NOTE: Use take 2 if using my footage

Author: Step 5.3 has been added to bridge the protocol with the Results section.

Results

6. Results

6.1. In E8.5 embryos, cells in the G2/M (*G-two-M*) phase of the cell cycle, indicated by phospho-histone H3 (*H-three*) staining in green [1], were visualized within migrating neural crest cells, indicated by SOX10 (*Sox-ten*) staining in magenta [2].

6.1.1. LAB MEDIA: Figure 2A. Video editor: Please highlight the green-stained cells in the 4th image from the left

6.1.2. LAB MEDIA: Figure 2A. Video editor: Please highlight the magenta-stained cells surrounding the green-stained cells in the 4th image from the left

6.2. Residual yolk sac in some embryos produced strong non-specific staining [1], while an additional non-specific streak was observed adjacent to the heart tube due to the use of anti-mouse antibodies [2].

6.2.1. LAB MEDIA: Figure 2A'. Video editor: Please point to the brightly stained yellow area marked by the left red arrowhead in the 4th image from the left

6.2.2. LAB MEDIA: Figure 2A'. Video editor: Please point to the narrow yellow streak marked by the upper red arrowhead in the 4th image from the left

6.3. In E9.5 embryos, cardiac neural crest cells forming part of the aortic arch were indicated by SRY-box transcription factor 10 staining in yellow [1], while second heart field progenitor cells were indicated by ISLET1 (*eye-let-one*) staining in green [2]. Endomucin staining further visualized venous and capillary endothelial cells [3].

6.3.1. LAB MEDIA: Figure 2B. Video editor: Please highlight the yellow-stained cells in the 5th image

6.3.2. LAB MEDIA: Figure 2B. Video editor: Please highlight the green-stained cells in the 5th image

6.3.3. LAB MEDIA: Figure 2B. Video editor: Please highlight the purple-stained cells in the 5th image

1. Embryos

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

IPA: /'em.bri.ouz/

Phonetic Spelling: em·bree·ohz

2. Somite

Pronunciation link: No confirmed link found

- IPA: /'soʊ.mart/
Phonetic Spelling: soh·mite
3. Methanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/methanol>
IPA: /'mɛθ.ə.nɔ:l/, /'mɛθ.ə.noʊl/
Phonetic Spelling: meth·uh·nawl / meth·uh·nohl
 4. DMSO
Pronunciation link: <https://www.merriam-webster.com/dictionary/dimethyl%20sulfoxide>
IPA: /,di:.ɛm.ɛs'oo/
Phonetic Spelling: dee·em·ess·oh
 5. Hydrogen peroxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrogen%20peroxide>
IPA: /'haɪ.drə.dʒən pə'rɑ:k.said/
Phonetic Spelling: hy·druh·juhn puh·rok·side
 6. Rehydrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/rehydrate>
IPA: /,ri:'haɪ,dreit/
Phonetic Spelling: ree·hy·drayt
 7. Phosphate-buffered saline
Pronunciation link: No confirmed link found
IPA: /'fɑ:s.fɛrt 'bʌf.əd 'seɪ.li:n/
Phonetic Spelling: fahs·fayt buff·erd say·leen
 8. Citrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/citrate>
IPA: /'sɪ,treit/
Phonetic Spelling: sih·trayt
 9. Antigen
Pronunciation link: <https://www.merriam-webster.com/dictionary/antigen>
IPA: /'æn.tɪ.dʒən/, /'æn.tɪ.dʒən/
Phonetic Spelling: an·tih·juhn / an·tih·jen
 10. Triton-X
Pronunciation link: No confirmed link found
IPA: /'traɪ.tən ɛks/
Phonetic Spelling: try·tuhn·eks
 11. Antibody
Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>
IPA: /'æn.tɪ.bɑ:.di/
Phonetic Spelling: an·tih·baa·dee
 12. Incubation
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubation>
IPA: /,ɪŋ.kjə'beɪ.fən/
Phonetic Spelling: ing·kyuh·bay·shuhn
 13. Fluorescently
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>

- IPA: /flʊ'res.ənt.li/, /flʊ:'res.ənt.li/
Phonetic Spelling: floo·ress·uhnt·lee / flaw·ress·uhnt·lee
14. Conjugated
Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugated>
IPA: /'kɑ:n.dʒə,geɪ.tɪd/
Phonetic Spelling: kon·juh·gay·tid
15. DAPI
Pronunciation link: No confirmed link found
IPA: /'dæ.pi/
Phonetic Spelling: da·pee
16. LDPE
Pronunciation link: No confirmed link found
IPA: /,ɛl.diː.pi:'iː/
Phonetic Spelling: el·dee·pee·ee
17. Pasteur
Pronunciation link: No confirmed link found
IPA: /pæ'stɜː/
Phonetic Spelling: pa·stur
18. Pipette
Pronunciation link: <https://www.merriam-webster.com/dictionary/pipet>
IPA: /paɪ'pet/
Phonetic Spelling: pie·pet
19. CUBIC-R
Pronunciation link: No confirmed link found
IPA: /'kjuː.bɪk ɑːr/
Phonetic Spelling: kyoo·bik·ar
20. Confocal
Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
IPA: /kɑ:n'foʊ.kəl/
Phonetic Spelling: kon·foh·kuhl
21. Phospho-histone
Pronunciation link: No confirmed link found
IPA: /'fɑːs.foʊ 'hɪs,təʊn/
Phonetic Spelling: fahs·foh hiss·tohn
22. Histone
Pronunciation link: <https://www.merriam-webster.com/dictionary/histone>
IPA: /'hɪs,təʊn/
Phonetic Spelling: hiss·tohn
23. Neural
Pronunciation link: <https://www.merriam-webster.com/dictionary/neural>
IPA: /'nɔr.əl/, /'njɔr.əl/
Phonetic Spelling: noor·uhl / nyoor·uhl
24. SOX10
Pronunciation link: No confirmed link found

- IPA: /sɑːks tən/
Phonetic Spelling: socks·ten
25. Yolk sac
Pronunciation link: No confirmed link found
IPA: /'jɒk ,sæk/
Phonetic Spelling: yohk·sak
26. Aortic
Pronunciation link: <https://www.merriam-webster.com/medical/aortic>
IPA: /eɪ'ɔːr.tɪk/
Phonetic Spelling: ay·or·tik
27. Progenitor
Pronunciation link: <https://www.merriam-webster.com/dictionary/progenitor>
IPA: /proʊ'dʒen.ə.tə/, /prə'dʒen.ə.tə/
Phonetic Spelling: proh·jen·uh·ter / pruh·jen·uh·ter
28. ISLET1
Pronunciation link: No confirmed link found
IPA: /'aɪ.lət wʌn/
Phonetic Spelling: eye·luht·wun
29. Endomucin
Pronunciation link: No confirmed link found
IPA: /,ɛn.doʊ'mjuː.sɪn/
Phonetic Spelling: en·doh·myoo·sin
30. Venous
Pronunciation link: <https://www.merriam-webster.com/dictionary/venous>
IPA: /'viː.nəs/
Phonetic Spelling: vee·nuhs
31. Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /'kæp.ə,lər.i/, /'kæp.ə,lər.i:/
Phonetic Spelling: kap·uh·lair·ee
32. Endothelial
Pronunciation link: No confirmed link found
IPA: /,ɛn.doʊ'θiː.li.əl/
Phonetic Spelling: en·doh·thee·lee·uhl