

Submission ID #: 71681

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21862753>

Title: Hybridization Chain Reaction FISH in the Developing *Drosophila* Optic Lobe

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 SMZ1500 (has a camera port + Nikon Digital Sight 1000 but the camera lacks an SD Card).

SCOPE SHOTS:

2.2.2, 2.2.3, 2.2.4, 2.3.1, 4.2.1, 4.3.2

Videographer: Please film the above-mentioned shots using the scope kit

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. **Yes**

Current Protocol Length

Number of Steps: 25

Number of Shots: 50 (**6 Scope**)

Interviews

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. **Jennifer Malin:** Our laboratory studies the development of the visual system using the fruit fly *Drosophila melanogaster* as a model.
 - 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. **Jennifer Malin:** Traditional in situ hybridization has limited use in the *Drosophila* visual system. This HCR-FISH protocol improves tissue penetration, signal amplification, and detection reliability.
 - 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. **Jennifer Malin:** HCR-FISH allows researchers to detect where specific genes are transcribed in situ, with multiplexing providing enhanced sensitivity and simultaneous analysis of multiple targets.
 - 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. **Jennifer Malin:** Successful protocol implementation requires RNase-free conditions and careful dissection, ensuring brains remain fully submerged in buffer to prevent tissue dehydration.

- 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. **Jennifer Malin:** Researchers can combine HCR-FISH with GFP reporter lines or HCR-IHC against GFP to improve positional accuracy and cell identification.

- 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.

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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

1.6. **Jennifer Malin, Assistant Professor**: (authors will present their testimonial statements live)

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

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

1.7. **Jennifer Malin, Assistant Professor**: (authors will present their testimonial statements live)

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

Protocol

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

Authors: During filming, please point out the organs or veins before dissecting them.

2. Dissection and Fixation of Third-Instar *Drosophila* Larval Optic Lobes

Demonstrator: Jennifer Malin

2.1. To begin, obtain a clean dissecting dish [1] and add approximately 250 microliters of nuclease-free PBS into the dish [2].

2.1.1. WIDE: Talent placing the dissecting dish on the work bench. . ***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. Talent aliquoting nuclease-free PBS onto a dissecting dish.

2.2. Transfer a wandering third instar *Drosophila* larva into the PBS using forceps [1]. Hold the abdomen with one pair of forceps [2] and grasp the mouth hooks with a second pair [3]. Pull the mouth hooks away from the body to isolate the brain and ventral nerve cord [4].

2.2.1. Talent transferring a wandering third instar larva into the PBS using forceps.

2.2.2. SCOPE: holding the abdomen with one pair of forceps.

2.2.3. SCOPE: grasping the mouth hooks with a second pair of forceps.

2.2.4. SCOPE: pulling the mouth hooks away from the body to isolate the brain and ventral nerve cord.

Videographer's NOTE: 2.2.2- 2.3.1 Please use VID00002 in the Scope folder

VID00002

2.2.1 - 00-:15

2.2.2 - :27-:37

2.2.3. - :30 -:40

2.2.4. - :40 -1:30

2.3.1. 2:30 - finished frame

- 2.3. Next, remove fat bodies and salivary glands while retaining the ventral nerve cord [1]. Transfer the dissected brain into a microcentrifuge tube containing 600 microliters of ice-cold nuclease-free PBS [2].
 - 2.3.1. SCOPE: removing fat bodies and salivary glands while retaining the ventral nerve cord.
 - 2.3.2. Talent transferring the dissected brain into a MCT tube containing ice-cold nuclease-free PBS.
- 2.4. Repeat the dissection procedure [1] to pool 6 brains in total [2].
 - 2.4.1. *Reuse 2.3.4*
 - 2.4.2. Talent adding a couple of more brains to the same tube. **Authors: Keep a couple of more brains ready**
- 2.5. Within 20 minutes after dissection, add paraformaldehyde to the sample tube to achieve a final concentration of 4% [1]. Gently invert the tube to mix [2], and incubate for 15 minutes at room temperature [3].
 - 2.5.1. Talent adding 16% paraformaldehyde to the tube.
 - 2.5.2. Talent gently inverting the tube to mix.
 - 2.5.3. Talent placing the tube at room temperature for incubation.
- 2.6. Then, remove the fixative and discard it in the designated chemical waste container [1]. Wash the samples 3 times with 600 microliters of cold nuclease-free PBS [2].
 - 2.6.1. Talent removing the fixative from the sample and discarding it in the designated chemical waste container.
 - 2.6.2. Talent adding cold nuclease-free PBS to the tube.

3. Probe Hybridization and Signal Amplification

- 3.1. Transfer the brains into a PCR tube containing 100 microliters of Probe Hybridization Buffer tempered at 37 degrees Celsius [1]. Mix gently by flicking the tube [2] and incubate for 10 minutes at room temperature [3].

- 3.1.1. Talent transferring the brains into a polymerase chain reaction tube containing prewarmed Probe Hybridization Buffer.
 - 3.1.2. Talent gently flicking the polymerase chain reaction tube to mix.
 - 3.1.3. Talent placing the polymerase chain reaction tube at room temperature for incubation.
- 3.2. Dilute each HCR-FISH probe set in Probe Hybridization Buffer to a final concentration of 4 nanomolar in a total volume of 100 microliters [1-TXT].
 - 3.2.1. Talent preparing a probe solution by adding the probe to the Probe Hybridization Buffer. **TXT: HCR-FISH: Hybridization Chain Reaction Fluorescence In Situ Hybridization**
- 3.3. Remove the buffer from the PCR tube containing the brains without disturbing the tissue [1] and add the diluted probe solution to each sample [2].
 - 3.3.1. Talent removing the prehybridization buffer without disturbing the tissue.
 - 3.3.2. Talent adding the diluted probe solution to each sample.
- 3.4. Incubate the samples at 37 degrees Celsius on a nutator for 16 to 24 hours [1].
 - 3.4.1. Talent placing the samples on a nutator for incubation.
- 3.5. Before probe amplification, prepare approximately 200 microliters of Amplification Buffer per sample at room temperature [1]. Warm the probe wash buffer to 37 degrees Celsius [2].
 - 3.5.1. Talent preparing the Amplification Buffer for each sample.
 - 3.5.2. Talent placing the Probe Wash Buffer in a waterbath set to 37 degrees Celsius.
- 3.6. After incubation, remove the probe solution from the sample tube [1] and store the used solution at minus 20 degrees Celsius for reuse [2].
 - 3.6.1. Talent removing the probe solution from the sample and transferring it into a fresh tube.
 - 3.6.2. Talent placing the probe solution at minus 20 degrees Celsius. **Author's NOTE:**
This was filmed, but its better to delete this shot

3.7. Wash the samples 4 times for 10 minutes each by adding the Probe Wash Buffer tempered to 37 degrees Celsius under a fume hood [1]. Then, wash the samples twice for 5 minutes each at room-temperature with 5x SSCT [2-TXT].

3.7.1. Talent washing the samples by adding preheated Probe Wash Buffer inside a fume hood. **Videographer's NOTE:** use B112_B487_0612L0_001

3.7.2. Talent removing the previous wash solution and adding room-temperature 5-fold SSCT to the tube. **TXT: SSCT: Saline-Sodium Citrate buffer with Tween-20**

3.8. After removing the wash solution [1], add 100 microliters of Amplification Buffer to the sample [2] and incubate for 30 minutes at room temperature [3].

Added shot : 3.7.3. Removing SSCT wash

3.8.1. Talent adding Amplification Buffer without hairpins.

3.8.2. Talent placing the samples aside at room temperature for incubation.

3.9. Add 2 microliters of each 3 micromolar hairpin stock to separate PCR tubes [1] and incubate them at 95 degrees Celsius for 90 seconds in a thermocycler [2]. Then, cover the tubes with aluminum foil and allow them to cool at room temperature for at least 30 minutes [2].

3.9.1. Talent adding the hairpin stock to separate polymerase chain reaction tubes.

3.9.2. Talent placing the hairpin tubes in the thermocycler for incubation.

3.9.3. Talent covering the tubes with aluminum foil and keeping them aside.

3.10. Next, mix the hairpins with the Amplification Buffer to a final volume of 100 microliters per sample [1].

3.10.1. Talent combining the hairpins in Amplification Buffer. **Videographer's NOTE:** use B112_B468_0612MP_

3.11. Remove the pre-amplification buffer from the samples [1]. Add hairpin-containing Amplification Buffer [2] and incubate the samples overnight at room temperature on a nutator, protected from light [3].

3.11.1. Talent removing the pre-amplification buffer from the samples.

3.11.2. Talent adding hairpin-containing Amplification Buffer.

3.11.3. Talent placing the samples on a nutator placed inside a dark chamber.

3.12. After incubation, remove the amplification solution [1]. Wash the samples with 5x SSCT [2-TXT] and perform 2 brief washes in nuclease-free PBS to remove the detergent [3].

3.12.1. Talent removing the amplification solution.

3.12.2. Talent adding 5-fold SSCT to the sample. **TXT: 5 min in 150 μ L (2x); 10 min in 150 μ L (3x) on a nutator**

3.12.3. Talent removing the SSCT wash solution and adding PBS. **Videographer's NOTE: no slate, use B112_B477_06124X**

4. Mounting and Imaging Preparation

4.1. Use a razor blade to cut an imaging spacer sticker into four equal pieces [1]. Adhere the cut sticker to a glass slide [2] and press gently to remove the trapped air bubbles [3].

Added shot: Cutting the image spacer sticker with a blade

4.1.1. Talent adhering an imaging spacer sticker to a glass slide.

4.1.2. Talent gently pressing the imaging spacer sticker to remove trapped air bubbles.

4.2. Bisect the brain through the ventral nerve cord using forceps [1].

4.2.1. SCOPE: bisecting the brain through the ventral nerve cord using forceps. **Videographer's NOTE: VID00003 in Scope folder**

NOTE: Show step 4.4 here, before step 4.3

4.3. Place the brain in the center of the spacer well [1] and orient the tissue laterally with the optic lobe facing upward [2].

4.3.1. Talent placing the brain in the center of the spacer well.

4.3.2. SCOPE: orienting the tissue laterally with the optic lobe facing upward. **Videographer's NOTE: VID00005 in Scope folder**

4.4. Add sufficient antifade mounting medium to fill the well, avoiding air bubbles [1], and distribute it evenly using forceps [2]. **NOTE: Move step 4.4 before step 4.3.**

4.4.1. Talent adding antifade mounting medium to fill the well without introducing air bubbles.

4.4.2. Talent distributing the mounting medium evenly using forceps.

4.5. Finally, place a coverslip over the sample [1] and seal with nail polish before imaging [2].

4.5.1. Talent placing a coverslip over the sample.

4.5.2. Talent sealing the coverslip with nail polish.

Results

5. Results

5.1. HCR-FISH against Fas2 (*Fas-2*), Distal-less, and hamlet labeled the Dm2 (*D-M-2*) neurons at the posterior edge of the developing optic lobe [1].

5.1.1. LAB MEDIA: Figure 2C. Video editor: Highlight the cells marked by arrows

5.2. HCR-FISH against abrupt labeled the zfh1-positive Dm4 neurons within the predicted neurogenic region [1]. Additionally, abrupt expression was detected in the fenestrated glia near the brk (*B-R-K*) and dpp (*D-P-P*) expression domains [2].

5.2.1. LAB MEDIA: Figure 2D. Video editor: Highlight the arrow pointing to the cells labeled with abrupt and zfh1

5.2.2. LAB MEDIA: Figure 2E. Video editor: Highlight the dotted outlined regions showing blue signals

5.3. HCR-FISH against wishful thinking demonstrated broad expression within the developing optic neuroepithelium and the newborn neurons [1].

5.3.1. LAB MEDIA: Figure 3B-B'. Video editor: Highlight the purple signals

5.4. The BMP type II (2) receptor punt displayed stronger expression in the neurons of the medulla and lobula neuropils [1].

5.4.1. LAB MEDIA: Figure 3D-D'. Video editor: Highlight the bright purple signals

5.5. Although both wishful thinking and punt were broadly expressed, their expression domains were not identical [1].

5.5.1. LAB MEDIA: Figure 3D''.

5.6. The co-Smad Medea exhibited broader expression throughout the optic lobe [1].

5.6.1. LAB MEDIA: Figure 3E.

1. Drosophila

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

IPA: /drə'sɑ:fələ/

Phonetic Spelling: druh·sah·fuh·luh

2. Larval
Pronunciation link: <https://www.merriam-webster.com/dictionary/larval>
IPA: /'lɑːrvəl/
Phonetic Spelling: lar·vuhl
3. Optic
Pronunciation link: <https://www.merriam-webster.com/dictionary/optic>
IPA: /'ɑːptɪk/
Phonetic Spelling: op·tik
4. Nuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
IPA: /'nuːkliːəs/
Phonetic Spelling: noo·klee·ays
5. Abdomen
Pronunciation link: <https://www.merriam-webster.com/dictionary/abdomen>
IPA: /'æbdəmən/
Phonetic Spelling: ab·duh·muhn
6. Ventral
Pronunciation link: <https://www.merriam-webster.com/dictionary/ventral>
IPA: /'ventrəl/
Phonetic Spelling: ven·truhl
7. Microcentrifuge
Pronunciation link: <https://www.merriam-webster.com/medical/microcentrifuge>
IPA: /ˌmaɪkroʊ'sentrɪfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
8. Paraformaldehyde
Pronunciation link: <https://www.howtopronounce.com/paraformaldehyde>
IPA: /ˌpærəfɔːr'mældəˌhaɪd/
Phonetic Spelling: par·uh·for·mal·duh·hyde
9. Hybridization
Pronunciation link: <https://www.merriam-webster.com/dictionary/hybridization>
IPA: /ˌhaɪbrədaɪ'zeɪʃən/
Phonetic Spelling: hy·bruh·duh·zay·shuhn
10. Amplification
Pronunciation link: <https://www.merriam-webster.com/dictionary/amplification>
IPA: /ˌæmpləfə'keɪʃən/
Phonetic Spelling: am·pluh·fuh·kay·shuhn
11. Nanomolar
Pronunciation link: <https://www.merriam-webster.com/medical/nanomolar>
IPA: /ˌnænoʊ'moʊlər/
Phonetic Spelling: nan·oh·moh·ler
12. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'resəns/
Phonetic Spelling: floo·res·uhns

13. Thermocycler
Pronunciation link: <https://www.howtopronounce.com/thermocycler>
IPA: /'θɜːrmouˌsaɪklər/
Phonetic Spelling: ther·moh·sy·kler
14. Neurogenic
Pronunciation link: <https://www.merriam-webster.com/medical/neurogenic>
IPA: /ˌnʊrəˈdʒenɪk/
Phonetic Spelling: nyur·uh·jen·ik
15. Neuroepithelium
Pronunciation link: <https://www.merriam-webster.com/medical/neuroepithelium>
IPA: /ˌnʊroʊˌepəˈθiːliəm/
Phonetic Spelling: nyur·oh·ep·uh·thee·lee·uhm
16. Neuropils
Pronunciation link: <https://www.howtopronounce.com/neuropil>
IPA: /ˌnʊrəˌpɪl/
Phonetic Spelling: nyur·uh·pil
17. Fenestrated
Pronunciation link: <https://www.merriam-webster.com/medical/fenestrated>
IPA: /ˌfenəˌstreɪtəd/
Phonetic Spelling: fen·uh·stray·tuhd
18. Antifade
Pronunciation link: <https://www.howtopronounce.com/antifade>
IPA: /ˌæntɪˈfeɪd/
Phonetic Spelling: an·tee·fayd
19. Medulla
Pronunciation link: <https://www.merriam-webster.com/dictionary/medulla>
IPA: /məˈdʌlə/
Phonetic Spelling: muh·dul·uh
20. Lobula
Pronunciation link: <https://www.howtopronounce.com/lobula>
IPA: /ˈləːbjʊlə/
Phonetic Spelling: lob·yuh·luh