

Submission ID #: 71224

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21723368>

Title: Three-Dimensional Immunolocalization in Maize Meiocytes

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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-2T

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.).

Scope shots: 2.5.1, 2.5.2, 3.3.3, 3.3.4, 3.4.1, 3.4.2, 4.17.1

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? **Yes**

If **Yes**, how far apart are the locations? The locations are within the Institute of Biotechnology (100 m).

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: 35

Number of Shots: 58

Interviews

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

Authors: Answers are slightly edited in accordance with journal guidelines. 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. **Luis Rojas:** Our research focuses on developing a 3D approach to study maize male meiocytes and visualize meiotic proteins in single cells.
 - 1.1.1. 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.~~

- 1.2. **Arnaud Ronceret:** Besides maize, this protocol can be applied to study meiocytes in rice, rye, barley, wheat, Arabidopsis thaliana, and Brassica.
 - 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. **Luis Rojas:** This protocol enables researchers to study protein co-localization and dynamics, native subcellular localization, 3D chromosomal architecture, meiotic progression, and staging.
 - 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. **Arnaud Ronceret:** The main challenge in this protocol is carefully selecting compatible combinations of primary and secondary antibodies.
 - 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. **Arnaud Ronceret**: Future studies can build on this work by comparing male and female meiosis to investigate the molecular mechanisms underlying heterochiasmy.
- 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.

Protocol

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

2. Preparation and Fixation of Maize Meiotic Anthers

Demonstrator: Luis Rojas

- 2.1. To begin, obtain maize plants at the V13 (*V-thirteen*) stage with inflorescences still growing inside the stem [1]. During the week of meiotic anther fixation, gently press the enlarged stem at the bump while wearing gloves to check if it is soft enough to cut open [2].
 - 2.1.1. WIDE: Talent walking into the room with maize plants. *Videographer: Please obtain CU shot as well for this shot.*
 - 2.1.2. Close-up of the talent pressing the stem gently while wearing gloves.
- 2.2. After preparing the required buffers, prepare a humid chamber using 5 to 10 humidified paper towels placed inside a plastic box [1]. After ensuring the lid is properly sealed, remove excess water from the chamber [2].
 - 2.2.1. Talent arranging damp paper towels in a plastic box.
 - 2.2.2. Shot of excess water being removed.
- 2.3. Using a razor blade, cut open the stem at the inflorescence level to expose tassel branches and lateral branches [1].
 - 2.3.1. Shot of the stem being cut to reveal tassel branches and lateral branches.
- 2.4. After dissecting and collecting the lateral branches, wrap them in the humid chamber towels while maintaining the chamber at room temperature [1].
 - 2.4.1. Talent wrapping the lateral branches in the paper towels of humid chamber.
- 2.5. Using a stereomicroscope, collect fresh anthers from florets into a 35 millimeter Petri dish containing 2 milliliters of 1x Buffer A or BA (*B-A*) [1]. Identify meiotic stages based on size and color [2].
 - 2.5.1. SCOPE: Shot of anthers being collected into a 35mm Petri dish containing BA.
 - 2.5.2. SCOPE: Shot of anthers being examined and sorted by size and color.
- 2.6. After transferring the anthers to a new 35 millimeter Petri dish, add 3 milliliters of fixative solution to the anthers [1]. Seal the petri dish with parafilm to prevent formaldehyde evaporation [2]. Place the 35 millimeter Petri dish on the larger Petri

dish positioned on a rotary shaker set to 40 to 60 rpm at room temperature for 45 minutes [3].

2.6.1. Talent adding fixative to the Petri dish containing anthers.

2.6.2. Talent sealing the Petri dish with parafilm.

2.6.3. Shot of the sealed Petri dish being placed on a larger Petri dish on the rotary shaker. **NOTE: Action modified**

2.7. Rinse the anthers with 3 milliliters of 1x BA [1]. After transferring the anthers to a small Petri dish, seal the Petri dish and place it on a rotary shaker at room temperature for 45 minutes, with the speed set to 40 to 60 rpm [2].

2.7.1. Talent adding BA to the dish.

2.7.2. Talent sealing the Petri dish and placing it on a rotary shaker.

2.8. Using clean forceps, transfer the anthers to a small 35 millimeter Petri dish containing 3 milliliters of 1x BA [1]. Once the Petri dish is tightly sealed with parafilm, place the small Petri dish inside a larger Petri dish [2]. After covering it with aluminum foil, store the sample at 4 degrees Celsius until use [3].

2.8.1. Close-up of the talent transferring the anthers into a Petri dish containing BA using forceps.

2.8.2. Close-up of the talent placing the small Petri dish inside a larger dish.

2.8.3. Talent placing the covered Petri dish in a refrigerator.

3. Embedding Maize Meiocytes Within Polyacrylamide Sandwich Pads

3.1. On day 1, prepare 2 milliliters of acrylamide solution by mixing equal volumes of acrylamide and 2x BA [1]. Degas the acrylamide solution under vacuum for 5 to 10 minutes before adding sodium sulfite and ammonium persulfate [2].

3.1.1. Talent adding acrylamide and BA into a tube.

3.1.2. Close-up of the acrylamide solution placed under vacuum for degassing.

3.2. Aliquot 100 microliters of a 1 to 1 acrylamide and 2x BA mixture into 1.5 milliliter tubes according to the number of slides prepared [1].

3.2.1. Close-up of adding acrylamide and BA into 1.5 mL tube.

3.3. Place 2 centimeter by 2 centimeter pieces of parafilm over a Petri dish [1] and position them under a dissecting stereoscope [2]. After trimming the pipette tip using scissors, pipette 50 microliters of BA solution with anthers onto the parafilm [3]. Pipette 25 microliters of BA solution onto another area of the parafilm [4].

3.3.1. Shot of parafilm pieces being arranged over a Petri dish.

3.3.2. Talent placing the Petri dish under stereoscope.

3.3.3. SCOPE: Shot of anther-containing solution being pipetted onto parafilm.

3.3.4. SCOPE: Shot of pipetting BA solution on parafilm.

3.4. After holding the anthers with forceps, using a scalpel, cut their tips [1]. Once the meiocytes are squeezed out [2], use a pipette to transfer them into the 25 microliters BA drop, and mix gently [3].

3.4.1. SCOPE: Shot of anther tips being cut with a scalpel.

Added shot: SCOPE: Shot of meiocytes being squeezed out of the anther.

3.4.2. SCOPE: Shot of anther being transferred using a pipette into the BA drop and mixed.

3.5. Pipette 10 microliters of the meiocyte mixture onto a separate coverslip [1].

3.5.1. Shot of meiocyte mixture being pipetted onto a coverslip.

3.6. Rapidly add 5 microliters of sodium sulfite and 5 microliters of ammonium persulfate into a 1.5 milliliter tube containing the acrylamide and 2x BA [1].

3.6.1. Talent adding NaS and APS into the 1.5 mL tube containing reagents.

3.7. Pipette 5 microliters of this mixture onto the coverslip and mix it with the meiocytes [1]. Place a poly-L-lysine-coated coverslip on top at a 45-degree angle [2].

3.7.1. Shot of mixture being added onto the coverslip and mixed with meiocytes.

3.7.2. Shot of the second coverslip being placed on top at a 45° angle.

3.8. Once the coverslip is lifted from the solution and dried, using a razor blade, carefully separate the coverslips [1]. Place them, pad-side up, on a polyacrylamide pad in a 6-well plate [2].

3.8.1. Close-up of coverslips being separated with a razor blade.

3.8.2. Shot of coverslips being placed pad side up on a polyacrylamide pad in a 6 well plate.

3.9. After placing the coverslip on a pad, wash it with 2 milliliters of phosphate-buffered saline or PBS (P-B-S) for 10 minutes at room temperature, then repeat the wash for 20 minutes [1].

3.9.1. Shot of PBS being added onto coverslips.

3.10. Rinse the coverslip with 1 milliliter of the 1X PBS supplemented with 1 millimolar ethylenediaminetetraacetic acid or EDTA (E-D-T-A) and 1 percent Triton at room temperature for 2 hours [1].

3.10.1. Shot of reagents being added onto coverslips and left undisturbed.

4. Immunolocalizations

4.1. Prepare and combine primary antibodies from different host species to ensure distinct signals during detection [1].

4.1.1. Talent adding primary antibodies in a tube.

- 4.2. Add 50 microliters of the diluted primary antibody to the center of the coverslip containing a pad [1]. After adding water between the wells, place the plate in a humid chamber overnight [2].
 - 4.2.1. Shot of primary antibody being added onto the center of the coverslip.
 - 4.2.2. Talent placing the plate inside a humid chamber.
- 4.3. On day 2, prepare 500 milliliters of wash buffer containing 1x PBS, 0.1 percent Tween-20, and 1 millimolar EDTA [1].
 - 4.3.1. Talent adding reagents into a bottle.
- 4.4. Using a pipette, remove the overnight solution from the wells [1].
 - 4.4.1. Shot of liquid being aspirated from the wells.
- 4.5. Wash the coverslip twice with 1 milliliter of wash buffer [1]. After removing the buffer, again add 1 milliliter of wash buffer and incubate for 1 hour at room temperature [2]. Repeat this washing step seven more times [3].
 - 4.5.1. Shot of wash buffer being added onto the coverslip.
 - 4.5.2. Shot of wash buffer being added onto the coverslip and kept aside.
 - 4.5.3. Time-lapse style shot indicating repeated washes.
- 4.6. After adding water between wells, cover the plate [1] and place it in a humid chamber overnight [2].
 - 4.6.1. Shot of a plate containing water being covered.
 - 4.6.2. Talent placing the plate in the humid chamber.
- 4.7. Prepare the secondary antibodies by diluting them at a ratio of 1 to 50 in blocking buffer [1]. After removing the wash solution, add 50 microliters of the diluted secondary antibody to the center of each pad [2].
 - 4.7.1. Talent adding secondary antibody into a tube containing blocking buffer.
 - 4.7.2. Close-up shot of secondary antibody being added to the center of each pad.
- 4.8. Add water between wells [1] and place the plate in a humid chamber for 2 hours at room temperature [2].
 - 4.8.1. Shot of water being added between the wells.
 - 4.8.2. Talent placing the plate inside humid chamber.
- 4.9. After removing the solution from the well, wash 4 times with 1 milliliter of wash buffer for 1 hour each at room temperature [1].
 - 4.9.1. Talent adding wash buffer to the well.
- 4.10. Wash the well with 1 milliliter of PBS for 10 minutes and repeat this process two more times [1].
 - 4.10.1. Talent adding PBS to the well.

- 4.11. Add 500 microliters of 4',6 diamidino 2 phenylindole or DAPI (*D-A-P-I*) prepared at a final concentration of 1 microgram per milliliter to the well, and incubate for 30 minutes at room temperature [1].
- 4.11.1. Talent adding DAPI solution to wells and keeping it aside for incubation.
- 4.12. After removing the ProlongGold from 4 degrees Celsius, thaw it to reach room temperature [1].
- 4.12.1. Talent thawing the tube.
- 4.13. Wash the slides with 1 milliliter of PBS for 30 minutes and repeat twice [1].
- 4.13.1. Shot of adding PBS to the slides.
- 4.14. After removing the final wash solution, add two drops of Prolong antifade in the middle of the pad [1].
- 4.14.1. Shot of Prolong antifade being added in the middle of the pad.
- 4.15. Once the coverslip is lifted from the well, mount it onto the labeled slides [1]. ~~Lower a second coverslip on top [2].~~
- 4.15.1. Shot of coverslip being mounted onto the labeled slide.
- ~~4.15.2. Shot of lowering a second cover slip~~
- 4.16. Using a vacuum, remove excess glycerol from the edges of the coverslip [1] and seal with 2 coats of nail polish [2].
- 4.16.1. Shot of glycerol being removed from the edges of coverslip using vacuum.
- 4.16.2. Shot of edges being sealed with nail polish.
- 4.17. Observe the slides under a microscope using appropriate excitation wavelengths and filters to capture images [1].
- 4.17.1. SCOPE: Fluorescent visualization of labeled cells under the microscope.

Results

5. Results

- 5.1. DAPI staining showed a clear nucleus and chromatin staining [1], and fainter native cytoplasmic staining of plastid and mitochondrial deoxyribonucleic acid [2].
 - 5.1.1. LAB MEDIA: Figure 1A and C. *Video editor: Emphasize the images under DAPI column.*
 - 5.1.2. LAB MEDIA: Figure 1C. *Video editor: Emphasize the arrowheads and the faint dots its pointing to.*
- 5.2. Absence of first division 1 or AFD1 (*A-F-D-one*), Asynaptic 1 or ASY1 (*A-S-Y-one*), and Zypper1 or ZYP1 (*Z-Y-P-one*) localized on chromosomes [1] and in the cytoplasm during pre-leptonema [2].
 - 5.2.1. LAB MEDIA: Figure 1A. *Video editor: Under MERGE column, emphasize the oval shape leaving the coloured dots scattered around.*
 - 5.2.2. LAB MEDIA: Figure 1A. *Video editor: Under MERGE column, emphasize the coloured dots scattered around the oval shape.*
- 5.3. In later stages, the signal concentrated on the chromosome [1], while substantial ZYP1 remained in the cytoplasm before complete synapsis [2].
 - 5.3.1. LAB MEDIA: Figure 1. *Video editor: Under merge column, emphasize the blue structures in all the rows.*
 - 5.3.2. LAB MEDIA: Figure 1. *Video editor: Under merge column, emphasize the coloured structures that are not in blue.*
- 5.4. The meiotic cohesin AFD1 formed a linear structure only after zygonema [1] concomitantly with the formation of linear filaments of the synaptonemal complex [2].
 - 5.4.1. LAB MEDIA: Figure 1D. *Video editor: Under merge column, emphasize the blue line like structures.*
 - 5.4.2. LAB MEDIA: Figure 1D. *Video editor: Under merge column, emphasize the red and yellow line like structures.*
- 5.5. The ASY1 signal became comparatively faint [1] after ZYP1 and AFD1 were loaded into linear structures [2].
 - 5.5.1. LAB MEDIA: Figure 1C and D. *Video editor: Emphasize the image in D row under ASY1 column.*
 - 5.5.2. LAB MEDIA: Figure 1C and D. *Video editor: Emphasize the image in D row under DAPI and AFD1.*

1. Inflorescences
Pronunciation link: <https://www.merriam-webster.com/dictionary/inflorescence>
IPA: /ˌɪnfləˈresəns/
Phonetic Spelling: in·flaw·reh·suhns
2. Meiotic
Pronunciation link: <https://www.merriam-webster.com/dictionary/meiotic>
IPA: /maɪˈɑːtɪk/
Phonetic Spelling: my·aa·tik
3. Anthers
Pronunciation link: <https://www.merriam-webster.com/dictionary/anther>
IPA: /ˈænθər/
Phonetic Spelling: an·ther
4. Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereomicroscope>
IPA: /ˌsteriəˈmaɪkrəˌskoʊp/
Phonetic Spelling: steh·ree·uh·my·kruh·skohp
5. Formaldehyde
Pronunciation link: <https://www.merriam-webster.com/dictionary/formaldehyde>
IPA: /fərˈmældəˌhaɪd/
Phonetic Spelling: fer·mal·duh·hyde
6. Acrylamide
Pronunciation link: <https://www.merriam-webster.com/dictionary/acrylamide>
IPA: /əˈkrɪləˌmaɪd/
Phonetic Spelling: uh·kril·uh·mide
7. Ammonium
Pronunciation link: <https://www.merriam-webster.com/dictionary/ammonium>
IPA: /əˈmʌniəm/
Phonetic Spelling: uh·moh·nee·uhm
8. Persulfate
Pronunciation link: <https://www.merriam-webster.com/dictionary/persulfate>
IPA: /pərˈsʌlˌfeɪt/
Phonetic Spelling: per·sul·fate
9. Meiocytes
Pronunciation link: <https://www.howtopronounce.com/meiocyte>
IPA: /ˈmaɪəˌsaɪts/
Phonetic Spelling: my·uh·sytes
10. Polyacrylamide
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyacrylamide>
IPA: /ˌpɑːliəˈkrɪləˌmaɪd/
Phonetic Spelling: pol·ee·uh·kril·uh·mide
11. Phosphate-buffered
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /ˈfɑːsˌfeɪt/
Phonetic Spelling: foss·fate

12. Ethylenediaminetetraacetic
 Pronunciation link: No confirmed link found
 IPA: /,eθəˈliːn daɪˌæmɪn ˌtetrəˈsiːtɪk/
 Phonetic Spelling: eth·uh·leen·dy·am·uh·neen·teh·truh·uh·see·tik
13. Triton
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Triton>
 IPA: /ˈtrɪtɑːn/
 Phonetic Spelling: try·ton
14. Immunolocalizations
 Pronunciation link: No confirmed link found
 IPA: /ɪˌmjuːnoʊ ˌloʊkələˈzeɪʃənz/
 Phonetic Spelling: ih·myoo·noh·loh·kuh·luh·zay·shunz
15. Antibodies
 Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>
 IPA: /ˈæntɪˌbaːdi/, /ˈænˌtaɪˌbaːdi/
 Phonetic Spelling: an·tee·baa·dee; an·tye·baa·dee
16. Diamidino
 Pronunciation link: No confirmed link found
 IPA: /daɪˌæməˈdiːnoʊ/
 Phonetic Spelling: dye·am·uh·dee·noh
17. Phenylindole
 Pronunciation link: No confirmed link found
 IPA: /ˌfiːnəlˈɪndoʊl/
 Phonetic Spelling: fee·nuhl·in·dohl
18. Deoxyribonucleic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/deoxyribonucleic%20acid>
 IPA: /diˌɑːksiˌraɪboʊnuˈkliːɪk/
 Phonetic Spelling: dee·ok·see·rye·boh·noo·klee·ik
19. Cytoplasm
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cytoplasm>
 IPA: /ˈsaɪtəˌplæzəm/
 Phonetic Spelling: sy·tuh·plaz·uhm
20. Synaptonemal
 Pronunciation link: <https://www.howtopronounce.com/synaptonemal>
 IPA: /ˌsɪnæptəˈniːməl/
 Phonetic Spelling: sin·ap·tuh·nee·muhl
21. Zygonema
 Pronunciation link: <https://www.howtopronounce.com/zygonema>
 IPA: /ˌzaɪɡoʊˈniːmə/
 Phonetic Spelling: zye·goh·nee·muh
22. Concomitantly
 Pronunciation link: <https://www.merriam-webster.com/dictionary/concomitantly>
 IPA: /kənˈkɑːmətəntli/
 Phonetic Spelling: kun·kaa·muh·tunt·lee

23. Synopsis

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

IPA: /sɪˈnæpsɪs/

Phonetic Spelling: sih·nap·suhs

24. Chromosomes

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

IPA: /ˈkroʊməˌsoʊm/

Phonetic Spelling: kroh·muh·sohm

25. Cohesin

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

IPA: /koʊˈhiːsɪn/

Phonetic Spelling: koh·hee·sin