

Submission ID #: 69791

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21286303>

Title: Chromatin Accessibility Profiling in Whole *Caenorhabditis elegans* L4 Larvae

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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**

Videographer: Please film the computer screen for all shots labelled as SCREEN

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: 26

Number of Shots: 52

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. **Julian Valdes:** Our research focuses on how epigenetic regulation shapes gene expression, specifically how chromatin accessibility controls gene activity across different biological conditions.
 - 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. **Julian Valdes:** ATAC-seq in *C. elegans* is limited by its protective cuticle; Our protocol overcomes this barrier to enable reliable chromatin accessibility analysis.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.8.1*

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Julian Valdes:** Our protocol will enable genome-wide chromatin accessibility profiling in whole worms to understand how environments influence gene regulation
 - 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. **Julian Valdes:** The key challenge is balancing worm input and Tn5 activity to avoid insufficient or excessive tagmentation, ensuring optimal chromatin accessibility profiling.

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. **Julian Valdes:** This approach opens opportunities to study epigenetic variation across wild isolates, environmental conditions, and even other nematode species.

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. Isolation and Dissociation of *Caenorhabditis elegans* into Single-Cell Suspension

Demonstrator: Nallely Cano

- 2.1. To begin, obtain a synchronous population of L1 worms and allow them to grow for 26 hours at 20 degrees Celsius [1]. Confirm under the microscope that most of the worms are in the L4 stage [2].
 - 2.1.1. WIDE: Talent removing the worm plates from the incubator.
 - 2.1.2. Talent checking the worms under the microscope to confirm that the majority are in the L4 stage. **Video editor: Show 2.1.2-L4-C_elegans.png as an inset**
- 2.2. Recover all the worms from the plates with M9 buffer [1] and transfer them into a 1.5-milliliter centrifuge tube [2].
 - 2.2.1. Talent collecting all the worms from the plates with M9 buffer.
 - 2.2.2. Talent transferring the worms into a 1.5 milliliter centrifuge tube.
- 2.3. Wash the worms 5 times with 1 milliliter of sterile water by letting them settle by gravity [1].
 - 2.3.1. Talent adding water to the tube and placing it on the rack to settle the worms.
- 2.4. Next, transfer the tube containing the worms to a 25 degrees Celsius incubator for 30 minutes to remove intestinal food remains [1].
 - 2.4.1. Talent placing the tube containing the worms into the 25 degrees Celsius incubator.
- 2.5. Then, take 50 microliters of the worm pellet and place it into a new tube [2].
 - 2.5.1. Talent collecting 50 microliters of the worm pellet and adding it into a new tube.
- 2.6. To lyse the worms, add 200 microliters of fresh SDS-DTT (*S-D-S-D-T-T*) buffer [1] and mix in a tube rotator for exactly 5 minutes [2].
 - 2.6.1. Talent adding 200 microliters of fresh SDS-DTT buffer to the tube. **NOTE: Show 2.6.2-elegans-in-SDS-(1).mp4: 00:07-00:09 using split screen**
 - 2.6.2. Talent placing the tube in a tube rotator.
- 2.7. Next, add 1 milliliter of cold egg buffer [1]. Then, spin on a picofuge to remove the supernatant and repeat 6 times [2].

- 2.7.1. Talent adding 1 milliliter of cold egg buffer to the tube.
- 2.7.2. Talent placing the tube in the picofuge.
- 2.8. After that, add 80 microliters of freshly prepared pronase [1]. Using a 200-microliter pipette with filtered tips, mix the worm-pronase mixture 70 times [2] and incubate it at room temperature for 15 minutes [3].
 - 2.8.1. Talent adding 80 microliters of freshly prepared pronase to the tube.
 - 2.8.2. Talent mixing the worms-pronase mixture repeatedly using a 200 microliter pipette with filtered tips.
 - 2.8.3. Talent placing the tube on the bench for incubation at room temperature.
- 2.9. Add 800 microliters of L-15 10 percent fetal bovine serum media [1] and centrifuge at 500 g for 5 minutes at 4 degrees Celsius [2].
 - 2.9.1. Talent adding 800 microliters of L-15 10 percent fetal bovine serum media to the tube.
 - 2.9.2. Talent placing the tube into the centrifuge.
- 2.10. Following supernatant removal, add 1 milliliter of L-15 10 percent fetal bovine serum media and centrifuge as demonstrated previously [1].
 - 2.10.1. Talent adding 1 milliliter of L-15 10 percent fetal bovine serum media to the tube.
- 2.11. Once the pellet is washed 3 times, remove the supernatant [1] and resuspend the pellet in 900 microliters of L-15 10 percent fetal bovine serum media [2]. Then, filter the suspension using a 20-micrometer filter [3].
 - 2.11.1. Talent removing the supernatant from the tube.
 - 2.11.2. Talent resuspending the pellet in 900 microliters of L-15 10 percent fetal bovine serum media.
 - 2.11.3. Talent filtering the suspension through a 20-micrometer filter.

3. Nuclei Isolation and Chromatin Tagmentation for ATAC Sequencing

- 3.1. Centrifuge the filtered cell suspension at 500 g for 5 minutes at 4 degrees Celsius [1]. Then, remove the supernatant, leaving approximately 50 microliters [2].
 - 3.1.1. WIDE: Talent placing the tube containing the filtered cell suspension into the centrifuge.
 - 3.1.2. Talent removing the supernatant from the tube while leaving approximately 50 microliters behind.
- 3.2. Add 45 microliters of freshly prepared lysis buffer [1] and carefully resuspend the

sample [2].

3.2.1. Talent adding 45 microliters of freshly prepared lysis buffer to the tube.

3.2.2. Talent gently resuspending the sample in the tube.

3.3. Then, carefully add 50 microliters of wash buffer [1]. Centrifuge at 500 *g* for 5 minutes at 4 degrees Celsius [2]. Remove the supernatant and keep the sample on ice [3].

3.3.1. Talent carefully adding 50 microliters of wash buffer to the tube without mixing.

3.3.2. Talent placing the tube into the centrifuge.

3.3.3. Talent removing the supernatant and placing the tube on ice.

3.4. While centrifuging, prepare the Tn5 (*T-N-Five*) mix per sample by combining 25 microliters of Tn5 Buffer, 24.8 microliters of nuclease-free water, and 0.2 microliters of Tn5 enzyme [1].

3.4.1. Talent pipetting and combining the components to prepare the Tn5 mix in a tube.

3.5. After centrifugation, remove the supernatant [1] and keep the nuclei on ice [2]. Add 50 microliters of Tn5 mix to the nuclear pellet, then pipette it up and down 7 times to mix [3]. Incubate at 37 degrees Celsius for 30 minutes on a thermoblock and gently tap the tube every 10 minutes [4].

3.5.1. Talent removing the supernatant from the tube.

3.5.2. Talent placing the tube containing nuclei on ice.

3.5.3. Talent adding 50 microliters of Tn5 mix to the nuclei pellet and slowly pipetting the mixture up and down seven times.

3.5.4. Talent placing the tube in a thermoblock for incubation.

3.6. After Tn5 incubation, add 300 microliters of PB buffer [1] and mix by vortexing at maximum speed for 25 seconds [2].

3.6.1. Talent adding 300 microliters of PB buffer to the tube.

3.6.2. Talent vortexing the tube at maximum speed.

3.7. Now, transfer the entire volume from the tube into the spin column stored at 4 degrees Celsius [1]. Centrifuge at room temperature for 1 minute at maximum speed [2].

3.7.1. Talent transferring the entire sample volume into the spin column.

3.7.2. Talent placing the spin column into the centrifuge.

3.8. Add 750 microliters of PE buffer to the spin column and centrifuge again [1]. Then, discard the supernatant with the collection tube [2].

3.8.1. Talent adding 750 microliters of PE buffer to the spin column.

- 3.8.2. Talent discarding the supernatant along with the collection tube.
- 3.9. Following an empty spin, place the column into a new labeled 1.5 milliliter tube [1]. Add 10 microliters of EB buffer at 37 degrees Celsius to the center of the column without touching it [2]. Leave it at room temperature for 1 minute [3].
 - 3.9.1. Talent placing the spin column into a new labeled 1.5 milliliter tube.
 - 3.9.2. Talent adding 10 microliters of EB buffer to the center of the spin column without touching it.
 - 3.9.3. Talent leaving the tube on the bench at room temperature.
- 3.10. Then, centrifuge for 1 minute at maximum speed at room temperature, then repeat the elution steps to obtain a final volume of 20 microliters in EB buffer [1-TXT].
 - 3.10.1. Talent placing the tube into the centrifuge and closes the lid.

4. ATAC Library Amplification and Cycle Optimization Using qPCR

- 4.1. Perform a real-time PCR to estimate the proper cycle amplification number for ATAC (A-T-A-C) library amplification [1].
 - 4.1.1. LAB MEDIA: Table 2
- 4.2. Estimate the final amplification cycle number by selecting the cycle at which the amplification signal reaches one-third of its maximum for each sample [1]. **NOTE: The VO is edited for the deleted shot**
 - ~~4.2.1. Show the real time polymerase chain reaction amplification curve and identify the cycle at the first third of the curve for one sample. **NOTE: TALENT FILMED SEEING THE SCREEN** **NOTE: Redundant information, do not use this shot**~~
Videographer: Please film the computer screen for all shots labelled as SCREEN
 - 4.2.2. SCREEN: Show the selected cycle number for each sample and confirm that it does not exceed 15. **NOTE: Use unedited: Amplification curves.png**. And look into the reference image (4.2.2 bis.png) for labeling/highlighting the required area.
- 4.3. Perform the final amplification for the ATAC (A-T-A-C) library [1] and assign a different reverse primer to each sample and record it [2]. Amplify each sample for the estimated cycle number for that sample [1].
 - 4.3.1. Talent setting up the samples for final ATAC library amplification.
 - 4.3.2. SCREEN: Talent assigning a different reverse primer to each sample and taking notes. **NOTE: Not a screen shot, TALENT FILMED SEEING BOOK NOTE**
 - 4.3.3. SCREEN: Talent setting the amplification cycle number for each sample. **NOTE:**

Not a screen shot, TALENT FILMED SEEING BOOK NOTE

4.4. Perform the presented polymerase chain reaction in 200 microliter tubes using 8 microliters of tagmented DNA and 50 microliters of amplified ATAC libraries **[1-TXT]**.

4.4.1. LAB MEDIA: Table 3

4.5. Store the samples at minus 20 degrees Celsius if pausing the protocol **[1]**. After purifying the amplified library, perform capillary electrophoresis to see the fragment size distribution pattern **[2]**.

4.5.1. Talent placing the samples into storage at minus 20 degrees Celsius.

4.5.2. LAB MEDIA: Figure 1B

Results

5. Results

5.1. The amplification curve reaching a plateau before 15 cycles indicated good-quality material suitable for continuation [1]. The amplification curve, showing a delayed increase, indicated poor-quality material and should not be continued [2].

5.1.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the amplification curve on the left that rises and levels off before the 15 cycles mark.*

5.1.2. LAB MEDIA: Figure 1A. *Video editor: Highlight the amplification curve on the right that rises later after 15 cycles.*

5.2. Fragment length distribution after amplification showed nucleosomal patterns at approximately 150 base pairs [1] and 300 base pairs in valid samples [2].

5.2.1. LAB MEDIA: Figure 1B–D. *Video editor: Highlight the peaks or bands around 150 base pairs in samples 1 and 2.*

5.2.2. LAB MEDIA: Figure 1B–D. *Video editor: Highlight the peaks or bands around 300 base pairs in samples 1 and 2.*

5.3. A smeared fragment pattern indicated overtagmented DNA requiring repetition of the sample [1].

5.3.1. LAB MEDIA: Figure 1B and 1E. *Video editor: Highlight the continuous smear pattern in sample 3 instead of distinct bands.*

1. Centrifuge

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

IPA: /'sen.trəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

2. Incubator

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

IPA: /'ɪŋ.kjəˌbeɪ.tər/

Phonetic Spelling: ing·kyuh·bay·ter

3. Microscope

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

- IPA: /'maɪ.krə.skəʊp/
Phonetic Spelling: my·kruh·skohp
4. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪ.kroʊ.li.t̬ə/
Phonetic Spelling: my·kroh·lee·ter
 5. Centrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifugation>
IPA: /,sen.trə.fjuː'geɪ.fən/
Phonetic Spelling: sen·truh·fyoo·gay·shuhn
 6. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /,suː.pə'nei.tənt/
Phonetic Spelling: soo·per·nay·tuhnt
 7. Pronase
Pronunciation link: <https://www.howtopronounce.com/pronase>
IPA: /'proʊ.neɪs/
Phonetic Spelling: proh·nays
 8. Pellet
Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>
IPA: /'pel.ət/
Phonetic Spelling: পেঁল·uht
 9. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /,riː.sə'spend/
Phonetic Spelling: ree·suh·spend
 10. Tagmentation
Pronunciation link: <https://www.howtopronounce.com/tagmentation>
IPA: /,tæg.mən'teɪ.fən/
Phonetic Spelling: tag·muhn·tay·shuhn
 11. Sequencing
Pronunciation link: <https://www.merriam-webster.com/dictionary/sequencing>
IPA: /'siː.kwən.sɪŋ/
Phonetic Spelling: see·kwuhn·sing
 12. Lysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysis>
IPA: /'laɪ.sɪs/
Phonetic Spelling: ly·suhs
 13. Nuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
IPA: /'nuː.kli.eɪs/
Phonetic Spelling: noo·klee·ays
 14. Enzyme
Pronunciation link: <https://www.merriam-webster.com/dictionary/enzyme>

- IPA: /'en.zaim/
Phonetic Spelling: en·zyme
15. Thermoblock
Pronunciation link: <https://www.howtopronounce.com/thermoblock>
IPA: /'θɜː.moʊ.blæk/
Phonetic Spelling: thur·moh·blok
16. Vortexing
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /'vɔːr.teks/
Phonetic Spelling: vor·teks
17. Elution
Pronunciation link: <https://www.merriam-webster.com/dictionary/elution>
IPA: /iˈluː.ʃən/
Phonetic Spelling: ee·loo·shuhn
18. Amplification
Pronunciation link: <https://www.merriam-webster.com/dictionary/amplification>
IPA: /,æm.plə.fə'keɪ.ʃən/
Phonetic Spelling: am·pluh·fuh·kay·shuhn
19. Polymerase
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>
IPA: /pə'lim.ə.reɪs/
Phonetic Spelling: puh·lim·uh·rays
20. Electrophoresis
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>
IPA: /iˌlek.troʊ.fə'riː.sɪs/
Phonetic Spelling: ee·lek·troh·fuh·ree·suhs
21. Nucleosomal
Pronunciation link: <https://www.howtopronounce.com/nucleosomal>
IPA: /,nuː.kli.ʊs'soʊ.məl/
Phonetic Spelling: noo·klee·oh·soh·muhl
22. Overtagmented
Pronunciation link: <https://www.howtopronounce.com/overtagmented>
IPA: /,oʊ.və'tæg.mən.tɪd/
Phonetic Spelling: oh·ver·tag·muhn·tid
23. Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /'kæp.ə.lər.i/
Phonetic Spelling: kap·uh·lair·ee
24. Distribution
Pronunciation link: <https://www.merriam-webster.com/dictionary/distribution>
IPA: /,dɪs.trə'bjuː.ʃən/
Phonetic Spelling: dis·truh·byoo·shuhn
25. Chromatin
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatin>

IPA: /'kroʊ.mə.tɪn/

Phonetic Spelling: kroh·muh·tin