

Submission ID #: 70879

Scriptwriter Name: Kathyayani Sridharan

Project Page Link: <https://review.jove.com/account/file-uploader?src=21618143>

Title: CRISPR/Cas9-mediated Endogenous Fluorescent Tagging of Germline-specific Genes in *Caenorhabditis elegans*

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**

SCOPE shots: 3.4.1, 3.4.2, 3.6.3, 3.6.4, 3.7.1, 3.7.2, 3.7.4, 3.7.5, 3.8.1, 3.8.2, 4.1.1.

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

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

3. Filming location: Will the filming need to take place in multiple locations? **Yes. We will need to film in two laboratories and one conference room, and they are all located in the same building.**

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: 14

Number of Shots: 50

Introduction

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

INTRODUCTION:

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

- 1.1. **Xinyue Lu:** We use CRISPR-based endogenous tagging to study the localization and dynamics of germline proteins in *C. elegans*.

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. **Xinyue Lu:** Conventional transgenes may affect germline expression, but this protocol tags proteins at the endogenous locus under native control.

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. **Lin Cheng:** This protocol enables *in vivo* analysis of protein localization and dynamics in the *C. elegans* germline.

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. **Lin Cheng:** Careful tag design and thorough validation are essential to preserve protein function and minimize false-positive candidates.

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. **Lin Cheng:** Future studies can use this workflow to track protein localization and explore potential interactions during germline development.

- 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. Peng Wang, Professor of Fuyang Normal University: (authors will present their testimonial statements live)

What value do you expect this video article to have for other researchers working in your field?

- 1.7. Peng Wang, Professor of Fuyang Normal University: (authors will present their testimonial statements live)

Protocol

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

2. Germline Target Gene Selection and CRISPR Component Design in *Caenorhabditis elegans*

Demonstrator: Xiaowen Sun

- 2.1. To begin, identify the candidate genes with predominant expression in the gonad or germ cells using public databases and published literature [1]. After opening the UniProt entry for the target protein, click **Structure** to evaluate structural features and protein domains, and identify an appropriate fluorescent tagging site [2]. Scroll down and open the **AlphaFold DB entry** to verify the model confidence values [3]. Click on **Domains** for specific structural classifications [4]. Next, open the target gene page in WormBase [5], scroll down and click on **Sequences** widget and the **primary transcript link** to open the transcript page [6]. Scroll down to the **Sequences** section and click on the specific option to reveal the spliced sequence of the selected transcript [7]. **Note:** The numbers and narration have been adjusted for the added shots.

2.1.1. WIDE: Talent analyzing literature to identify target genes.

2.1.2. LAB MEDIA: 70879_screenshot_1.mp4. 00:00 – 00:15 *Video Editor: Please emphasize the clicking on Structure option*

Added shot: LAB MEDIA: 70879_screenshot_1.mp4. 00:16 – 00:25 Video Editor: Please emphasize the clicking on AlphaFold DB option

*Added shot: LAB MEDIA: 70879_screenshot_1.mp4. 00:26 – 00:29 Video Editor: Please emphasize the text “Domain 1”, “High confidence **” and “Average pLDDT 85.09”*

2.1.3. LAB MEDIA: 70879_screenshot_2.mp4. 00:00 – 00:06 *Video Editor: Please emphasize the entry of text “nkb-2” in the search bar*

Added shot: LAB MEDIA: 70879_screenshot_2.mp4. 00:07 – 00:20 Video Editor: Please emphasize the arrow clicking on the Sequences tab

Added shot: LAB MEDIA: 70879_screenshot_2.mp4. 00:21 – 00:28

- 2.2. In the IDT (*I-D-T*) CRISPR (*kris-puh*)- Cas 9 (*kaz-nine*) guide RNA design tool webpage [1], copy and paste the first exon sequence of the target gene from the **Wormbase** page, and select *C. elegans* from the **Species** dropdown menu for guide RNA, or gRNA (*G-R-N-A*) design [2]. Click on the **Design** button to obtain and review the top recommended gRNA sequence and its corresponding PAM sequence [3].

2.2.1. LAB MEDIA: 70879_screenshot_3.mp4. 00:00 – 00:11

2.2.2. LAB MEDIA: 70879_screenshot_3.mp4. 00:12 – 00:20

2.2.3. LAB MEDIA: 70879_screenshot_3.mp4. 00:21 – 00:38

- 2.3. On **SnapGene**, select **Sequence** to identify the cloning point between the CelU6 (*Cell-U-six*) promoter and the gRNA scaffold regions [1]. Copy the gRNA sequence from the **IDT tool** page, paste it in the insertion dialog box and click **Insert** to integrate the spacer into the CRISPR expression plasmid [2]. To confirm the accuracy of gRNA insertion, select the sequence and click on **Show alignments** [3] to compare the Sanger sequencing result base-by-base with the expected sequence [4]. To construct the donor plasmid, copy the homology arms' genomic sequences from **WormBase** [5]. In **SnapGene**, paste the sequence and click **Insert** [6]. Next, right-click the insertion site to open the **Set DNA Color** window and change it to orange to mark the junction [7]. Copy the green fluorescent protein, or GFP (*G-F-P*) insert sequence and paste it directly at the marked position, then click **Add Feature** [8]. Name the feature 'GFP', change its display color to green, and click **OK** to complete the *in silico* donor plasmid assembly [9]. To introduce a silent substitution into the donor plasmid template for preventing Cas9 recutting after integration, copy the twenty-nucleotide gRNA spacer from the expression vector [10]. In the donor plasmid tab, paste the sequence into the **Find DNA sequence** search bar to locate the target site, and select the specific nucleotide position to mutate [11]. In **Replace base**, enter new nucleotide and click **Insert** [12]. Right-click the modified base and use **Set DNA Color** to change its color to red [13]. Align the donor plasmid Sanger sequencing results with the expected sequence to confirm integrity [14]. **Note: The numbers and narration have been adjusted for the added shots.**

2.3.1. LAB MEDIA: 70879_screenshot_4.mp4. 00:00 – 00:03

Added shot: LAB MEDIA: 70879_screenshot_4.mp4. 00:04 – 00:16

2.3.2. LAB MEDIA: 70879_screenshot_5.mp4. 00:00 – 00:06

Added shot: LAB MEDIA: 70879_screenshot_5.mp4. 00:07 – 00:15

2.3.3. LAB MEDIA: 70879_screenshot_6.mp4. 00:00 – 00:05

Added shot: LAB MEDIA: 70879_screenshot_6.mp4. 00:06 – 00:09

Added shot: LAB MEDIA: 70879_screenshot_6.mp4. 00:07 – 00:19

Added shot: LAB MEDIA: 70879_screenshot_6.mp4. 00:20 – 00:30

Added shot: LAB MEDIA: 70879_screenshot_6.mp4. 00:31 – 00:35

2.3.4. LAB MEDIA: 70879_screenshot_7.mp4. 00:00 – 00:04

Added shot: LAB MEDIA: 70879_screenshot_7.mp4. 00:05 – 00:14

Added shot: LAB MEDIA: 70879_screenshot_7.mp4. 00:15 – 00:17

Added shot: LAB MEDIA: 70879_screenshot_7.mp4. 00:18 – 00:21

2.3.5. LAB MEDIA: 70879_screenshot_8.mp4. 00:04 – 00:10

3. Gonadal Microinjection

Demonstrator: Xiaowen Sun

3.1. Prepare the injection [1] mix [2] and centrifuge at $10,000 \times g$ (*ten-thousand-gee*) for ten to twenty minutes to pellet out particulate material and prevent clogging of microinjection capillary [3].

3.1.1. Talent preparing the injection mix

3.1.2. TEXT ON A PLAIN BACKGROUND

Component	Working concentration (ng/ μ L)
pDD162 (CRISPR/Cas9 plasmid)	50-100
pPD95.75 (Donor plasmid)	50-100
pRF4 (Co-injection marker)	50-100
pPD122.11 (Co-injection marker)	5-10
Sterile ddH ₂ O	Adjust to final volume

3.1.3. Talent centrifuging the injection mix to obtain clean supernatant.

3.2. Use a needle puller to create microinjection needles from glass capillaries with a long taper and sharp tip suitable for gonadal injection, while optimizing the puller settings empirically [1-TXT]. Heat a microcapillary pipette and snap it into two pieces to create a loading tool [2]. Attach a rubber aspirator bulb to the wide end of the shortened microcapillary pipette [3].

3.2.1. Talent creating microinjection needles from glass capillaries using glass pullers.
TXT: Puller settings: Heat: 540, Pull: 20, Velocity: 100

3.2.2. Talent creating a loading tool by heating and snapping a microcapillary pipette.

3.2.3. Talent attaching a rubber bulb to the wide end of microcapillary pipette.

3.3. Aspirate approximately one microliter of injection mix into the loader [1]. Insert the loader into the back end of the injection needle [2] and gently dispense the injection mix [3]. Place the loaded needle horizontally for ten minutes to form a continuous column and allow the solution to settle toward the tip [4].

3.3.1. Talent aspirating 1 μ L injection mix into the loader.

3.3.2. Talent inserting the loader into back end of injection needle.

3.3.3. Talent dispensing the injection mix.

3.3.4. Talent placing the loaded needle horizontally.

- 3.4. Mount the needle on the injection microscope and bring the tip into focus using brightfield optics [1]. Open the needle tip by gentle contact with the edge of a coverslip in halocarbon oil and verify flow [2].
 - 3.4.1. SCOPE: Focusing the mounted needle tip using brightfield optics.
 - 3.4.2. SCOPE: The flow of injection mix via the needle being verified.
- 3.5. To culture the worms for injection, maintain the N2 (*N-two*) wildtype worms on OP50 (*O-P-fifty*) bacteria-seeded media plates [1]. Select hermaphrodites at the L4 (*L-four*) stage from a healthy plate and incubate at twenty degrees Celsius for ten hours to obtain young adults with well-developed germlines [2]. Transfer worms to an unseeded plate to reduce bacterial carryover [3].
 - 3.5.1. Talent maintaining the N2 worms on OP50 bacteria-seeded media plates.
 - 3.5.2. Talent selecting and incubating the hermaphrodites at the L4 stage for 10 h.
 - 3.5.3. Talent transferring the young worms to OP50-unseeded plate.
- 3.6. Prepare a dry two-percent agarose pad on a glass slide [1] and apply a drop of halocarbon oil [2]. Use eyelash picker to transfer a worm onto the agarose pad and immobilize it [3]. Use a low-magnification objective to orient the gonad for injection [4].
 - 3.6.1. Talent preparing 2% agarose pad on glass slide.
 - 3.6.2. Talent applying a drop of halocarbon oil to the agarose pad.
 - 3.6.3. SCOPE: The worm being transferred to the agarose pad using eyelash picker for immobilizing.
 - 3.6.4. SCOPE: A low-magnification objective being used to orient the gonad for injection.
- 3.7. Use micromanipulator controls to position the needle at a twenty-to-forty-degree angle relative to the worm [1]. Use high magnification to focus on the distal germline [2]. Advance the worm onto the needle [3] and position the tip within the gonadal syncytium [4]. Inject the solution until gonad swelling is visible, while setting the injection pressure at 30-80 (*thirty to eighty*) psi (*sigh*); duration to 0.1-0.3 (*zero-point one to zero-point three*) seconds and compensation pressure at 0.1-0.5 (*zero-point one to zero-point five*) psi [5].
 - 3.7.1. SCOPE: Show micromanipulator controls being used to position the needle at an angle relative to the worm.
 - 3.7.2. SCOPE: Show switching to high magnification to focus on distal germline.
 - 3.7.3. SCOPE: Show advancement of the worm onto the needle.
 - 3.7.4. SCOPE: Show the needle tip within the gonadal syncytium.
 - 3.7.5. SCOPE: Show the solution being injected until gonadal swelling is observed.

3.8. Withdraw the needle and reposition for subsequent injections [1]. Add M9 (*M-nine*) buffer to soften and partially break up the agarose pad to release worms [2]. Transfer each worm to an individual recovery plate, then repeat injections for twenty to twenty-five worms [3-TXT].

3.8.1. SCOPE: The needle being withdrawn and repositioned for subsequent injections.

3.8.2. SCOPE: Show addition of M9 buffer to release worms.

3.8.3. Talent transferring each worm to an individual recovery plate. **TXT: Incubation and Recovery: 20 °C; 2–3 days**

4. Screening F1 and F2 Progeny

Demonstrator: Zihan Wen

4.1. Screen F1 (*F-one*) progeny for co-injection markers and select marker-positive animals [1]. Transfer each F1 worm to an individual plate and allow for one to two days for egg laying [2]. Lyse each F1 worm in proteinase K buffer to prepare PCR (*P-C-R*) templates [3]. Perform junction PCR using one genomic primer outside the homology arm and one internal tag primer [4] and retain corresponding plates from PCR-positive F1 animals [5].

4.1.1. SCOPE: Show screening and selection of marker-positive F1 progeny.

4.1.2. Talent transferring each F1 worm onto individual plate for egg laying.

4.1.3. Talent preparing PCR templates by lysing each worm.

4.1.4. Talent performing junction PCR.

4.1.5. Talent retaining plates corresponding to PCR-positive worms.

4.2. Isolate F2 (*F-two*) progeny from PCR-positive F1 lines onto individual plates to produce progeny [1]. Lyse adults for genotyping [2]. Perform PCR using primers flanking the homology arms to distinguish wild-type and knock-in alleles by product size [3].

4.2.1. Talent isolating F2 progeny into individual plates for progeny production.

4.2.2. Talent lysing the adult worms for genotyping.

4.2.3. Talent performing PCR.

4.3. Use the **Snapgene** tool to verify and validate correct integration at both the left and right insertion junctions by aligning Sanger sequencing results with the expected edited genomic sequence [1]. Use fluorescence microscopy to verify germline expression and localization [2]. Expand confirmed homozygous lines, maintain stocks and cryopreserve validated lines for long-term storage [3].

4.3.1. LAB MEDIA: 70879_screenshot_9.mp4. 00:00 – 00:21

- 4.3.2. Talent performing fluorescence microscopy to confirm fluorescence localization in germline cells.
- 4.3.3. Talent expanding homozygous stocks.

Results

5. Results

- 5.1. PCR-based screening of F1 progeny revealed successful knock-ins, identified by 1550 bp (*one thousand five hundred and fifty base pairs*) junction PCR amplicon—using one flanking genomic and one internal GFP primer [1]. The absence of the 1550 bp band in most F1 animals indicated no detectable target integration, consistent with non-integrative DNA transmission rather than homology-directed repair [2].
 - 5.1.1. LAB MEDIA: Figure 3A: *Video Editor: Please emphasize the lanes 5 and 18 showing the 1550 bp band.*
 - 5.1.2. LAB MEDIA: Figure 3A: *Video Editor: Please emphasize the other areas excluding the lanes M, 1 5 and 18.*
- 5.2. Zygosity determination in F2 progeny derived from junction-PCR-positive lines distinguishes alleles by band size at 1345 bp (*one thousand three hundred and forty five base pairs*) as wildtype allele [1] and 2344 bp (*two thousand three hundred and forty four base pairs*) as knock-in allele [2].
 - 5.2.1. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the text 1345 bp on the right side of the image.*
 - 5.2.2. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the text 2344 bp on the right side of the image.*
- 5.3. Heterozygotes show both bands [1], homozygous knock-in samples lack the WT-sized band [2] and the samples with failed integration show only the WT-sized product [3]. Additional validation of homozygous GFP knock-in line was done using confocal microscopic imaging where GFP fluorescence in the germline cells with signal enriched in the spermatogenic region was observed [4].
 - 5.3.1. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the lanes labelled as 2,3, 4, 5, 7, 8, 11, 12, 13, 16*
 - 5.3.2. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the lanes labelled as 9, 15 and 21.*
 - 5.3.3. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the lanes labelled as 6, 10, 14, 17, 18, 19 and 20.*
 - 5.3.4. LAB MEDIA: Figure 4: *Video Editor: Please emphasize the green region in the GFP and Merge panels.*

Pronunciation Guide:

1. Germline
Pronunciation link: <https://www.merriam-webster.com/dictionary/germline>
IPA: /'dʒɜːmˌlaɪn/
Phonetic Spelling: jurm·line
2. Caenorhabditis
Pronunciation link: <https://www.howtopronounce.com/caenorhabditis>
IPA: /ˌsiː.nəʊˌræbˈdaɪ.tɪs/
Phonetic Spelling: see·noh·rab·dy·tis
3. elegans
Pronunciation link: <https://www.merriam-webster.com/dictionary/elegans>
IPA: /'el.ə.gænz/
Phonetic Spelling: el·uh·ganz
4. Gonad
Pronunciation link: <https://www.merriam-webster.com/dictionary/gonad>
IPA: /'ɡoʊ.næd/
Phonetic Spelling: goh·nad
5. UniProt
Pronunciation link: <https://www.uniprot.org/>
IPA: /'juː.ni.proʊt/
Phonetic Spelling: yoo·nee·proht
6. AlphaFold
Pronunciation link: <https://www.alphafold.ebi.ac.uk/>
IPA: /'æɫ.fə.foʊld/
Phonetic Spelling: al·fuh·fohd
7. Transcript
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcript>
IPA: /'trænˌskɹɪpt/
Phonetic Spelling: tran·skript
8. Spliced
Pronunciation link: <https://www.merriam-webster.com/dictionary/splice>
IPA: /splaɪst/
Phonetic Spelling: splyst
9. CRISPR
Pronunciation link: <https://www.merriam-webster.com/dictionary/CRISPR>
IPA: /'kɹɪs.pə/
Phonetic Spelling: kris·per
10. Guide RNA
Pronunciation link: <https://www.merriam-webster.com/dictionary/RNA>
IPA: /ɡaɪd ɑːr.en'eɪ/
Phonetic Spelling: guide·ar·en·ay
11. Exon
Pronunciation link: <https://www.merriam-webster.com/dictionary/exon>

IPA: /'ek.sɑ:n/

Phonetic Spelling: ek·saan

12. Plasmid

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

IPA: /'plæz.mɪd/

Phonetic Spelling: plaz·mid

13. Scaffold

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

IPA: /'skæf.əʊld/

Phonetic Spelling: skaf·ohld

14. Alignment

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

IPA: /ə'laɪn.mənt/

Phonetic Spelling: uh·line·muhnt

15. Sanger

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

IPA: /'sæŋ.ə/

Phonetic Spelling: sang·er

16. Homology

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

IPA: /hə'mɑ:l.ə.dʒi/

Phonetic Spelling: huh·maa·luh·jee

17. Genomic

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

IPA: /dʒə'noʊ.mɪk/

Phonetic Spelling: juh·noh·mik

18. Fluorescent

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

IPA: /flʊ:'res.ənt/

Phonetic Spelling: flor·ess·uhnt

19. Substitution

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

IPA: /sʌb.stə'tu:ʃən/

Phonetic Spelling: sub·stuh·too·shuhn

20. Centrifuge

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

IPA: /'sen.trə.fju:dʒ/

Phonetic Spelling: sen·truh·fyooj

21. Particulate

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

IPA: /pɑ:r'tɪk.jə.lət/

Phonetic Spelling: par·tik·yuh·luht

22. Microinjection
Pronunciation link: <https://www.merriam-webster.com/dictionary/microinjection>
IPA: /ˌmaɪ.kroʊ.ɪnˈdʒek.ʃən/
Phonetic Spelling: my·kroh·in·jek·shuhn
23. Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /ˈkæp.ə.lər.i/
Phonetic Spelling: kap·uh·lair·ee
24. Microcapillary
Pronunciation link: No confirmed link found
IPA: /ˌmaɪ.kroʊˈkæp.ə.lər.i/
Phonetic Spelling: my·kroh·kap·uh·lair·ee
25. Aspirator
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirator>
IPA: /ˈæs.pə.reɪ.tər/
Phonetic Spelling: ass·puh·ray·ter
26. Magnification
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnification>
IPA: /ˌmæɡ.nə.fəˈkeɪ.ʃən/
Phonetic Spelling: mag·nuh·fuh·kay·shuhn
27. Hermaphrodites
Pronunciation link: <https://www.merriam-webster.com/dictionary/hermaphrodite>
IPA: /həˈmæf.rəˌdarts/
Phonetic Spelling: her·maf·ruh·dyts
28. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /ˈæg.ə.roʊs/
Phonetic Spelling: ag·uh·rohs
29. Halocarbon
Pronunciation link: No confirmed link found
IPA: /ˌhæl.əʊˈkɑːr.bən/
Phonetic Spelling: hal·oh·kaar·buhn
30. Immobilize
Pronunciation link: <https://www.merriam-webster.com/dictionary/immobilize>
IPA: /ɪˈmoʊ.bəˌlaɪz/
Phonetic Spelling: ih·moh·buh·lyze
31. Micromanipulator
Pronunciation link: <https://www.merriam-webster.com/medical/micromanipulator>
IPA: /ˌmaɪ.kroʊ.məˈnɪp.jə.leɪ.tər/
Phonetic Spelling: my·kroh·muh·nip·yuh·lay·ter
32. Distal
Pronunciation link: <https://www.merriam-webster.com/dictionary/distal>

IPA: /'dɪs.təl/

Phonetic Spelling: dis·tuhl

33. Syncytium

Pronunciation link: <https://www.merriam-webster.com/medical/syncytium>

IPA: /sɪn'sɪf.i.əm/

Phonetic Spelling: sin·sish·ee·uhm

34. Progeny

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

IPA: /'prɑː.dʒə.ni/

Phonetic Spelling: praaj·uh·nee

35. Proteinase

Pronunciation link: <https://www.merriam-webster.com/medical/proteinase>

IPA: /proʊ'tiː.neɪs/

Phonetic Spelling: proh·tee·nays

36. Genotyping

Pronunciation link: <https://www.merriam-webster.com/medical/genotype>

IPA: /'dʒiː.nə.taɪ.pɪŋ/

Phonetic Spelling: jee·nuh·ty·ping

37. Homozygous

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

IPA: /ˌhoʊ.moʊ'zɑɪ.gəs/

Phonetic Spelling: hoh·moh·zy·guhs

38. Cryopreserve

Pronunciation link: <https://www.merriam-webster.com/medical/cryopreservation>

IPA: /ˌkraɪ.oʊ.pri'zɜːv/

Phonetic Spelling: kry·oh·prih·zerv