

Submission ID #: 71394

Scriptwriter Name: Kathyayani Sridharan

Project Page Link: <https://review.jove.com/account/file-uploader?src=21775218>

Title: Optogenetic Analysis of Behavior in the Mosquito *Aedes aegypti*

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? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

Authors: We have added the timestamps for all the provided screen captures. Please upload the screen captures for shots 2.2.1, 2.5.5, and 2.8.3, which appear to be missing on the project page.

Authors: Please create screen capture videos for shots labelled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible:
<https://review.jove.com/account/file-uploader?src=21775218>

Videographer: Please record the computer screen for the shots labeled as SCREEN and highlighted in yellow as back-up

- 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: 25

Number of Shots: 69 (28 SC)

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**

Note: The statements have been edited for clarity

Authors: Please provide the names of the authors who delivered the interview statements at the time of filming.

INTRODUCTION:

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

- 1.1. **Trevor Sorrells:** Our research focuses on the neuronal mechanisms and evolutionary basis of mosquito host-seeking and blood-feeding behaviors.

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

NOTE: Author edited the interview answers during the shoot

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Cassie Travis:** By using optogenetics rather than genetic knockouts, this protocol enables functional characterization of mosquitoes' host-seeking neural circuits.

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. **Spruha Rami:** This protocol tests whether specific neuron types are sufficient to drive distinct steps of biting behavior.

1.3.1. 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?~~

- 1.4. **Trevor Sorrells:** Paired with studies of neural activity and neuroanatomy, these methods enable us to understand neural circuits, linking a neuron's identity and connectivity to its role in behavior.

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. **Trevor Sorrells:** Future studies will necessitate novel genetic drivers to functionally map the mosquito central nervous system.

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. Blood Blanket Assay Using Reared Optogenetic Mosquito Lines for Artificial Blood Meal Engorgement Quantification

Demonstrator: Spruha Rami

Videographer: Please record the computer screen for the shots labeled as SCREEN and highlighted in yellow as back-up

- 2.1. To begin, freshly prepare 10-milliliter minimal artificial blood meal [1-TXT] and mix the solution thoroughly by inverting [2].
 - 2.1.1. WIDE: Talent adding the stock solutions to the deionized water to prepare the minimal artificial blood meal. **TXT: Refer to text for minimal artificial blood meal composition.**
 - 2.1.2. Talent inverting the container several times to mix the solution thoroughly.
- 2.2. Configure the camera and recording settings in SpinView for video acquisition [1-TXT]. Open the **Red-light-Only_BB_.ino** (*Red-light-only-B-B-dot-ino*) Arduino (*ah-rdoo-ee-noh*) file or the desired stimulus file for the experiment [2].
 - 2.2.1. SCREEN: 71394_screenshot_1.mp4 00:24-00:28 **TXT: Refer to the text protocol for the SpinView software parameters**
 - 2.2.2. SCREEN: 71394_screenshot_2.mp4. 00:06-00:08; 00:12-00:15
- 2.3. Next, pipette 650 microliters of the minimal artificial blood meal into each well of the aluminum feeding plate, ensuring that each well is filled without overflow [1].
 - 2.3.1. Talent pipetting 650 microliters of the minimal artificial blood meal into each well of the plate.
- 2.4. Stretch a parafilm square over a 20.3-centimeter-diameter ring [1-TXT]. Place the parafilm over the plate and press the edges until it adheres [2]. Use a scalpel to trim a 1.3-centimeter-wide border around the plate [3] and tuck the excess parafilm underneath to seal the meal [4].
 - 2.4.1. Talent stretching the parafilm over the ring. **TXT: Parafilm square: 10.2 cm x 10.2 cm**
 - 2.4.2. Talent placing the parafilm over the plate and pressing the edges to secure it.
 - 2.4.3. Talent trimming the parafilm border with a scalpel
 - 2.4.4. Talent tucking the excess underneath the plate.

- 2.5. Place the plate directly on top of the polymerase chain reaction, or PCR (*P-C-R*) block to allow maximum heat transfer [1]. Place the thermocouple on the parafilm surface in the bottom-right corner of the aluminum plate [2]. Connect the camera, light-emitting diodes, and Arduino to the computer [3]. Turn on the power supply [4] and start the **BB_thermocycler_program.x50prog** program on the thermocycler [5].
 - 2.5.1. Talent placing the plate on top of the PCR block.
 - 2.5.2. SCREEN: 71394_screenshot_3.mp4. 00:15–00:20
 - 2.5.3. Talent connecting the camera, light-emitting diodes, and Arduino to the computer.
 - 2.5.4. Talent turning on the power supply.
 - 2.5.5. Talent uploads and starts **BB_thermocycler_program.x50prog** on the thermocycler. **Author's NOTE:** Videographer captured the thermocycler's screen for this as screen recording was not possible. The videographer may have mislabelled this as 2.2.1, not sure
- 2.6. Use a dark container to transfer the acrylic plate of mosquitoes onto the feeding plate under dim room lighting to minimize light exposure, ensuring that each well is aligned with the mosquitoes [1]. Ensure the thermocouple remains in the bottom-right corner of the plate and in contact with the parafilm to measure the temperature of the artificial blood meal [2].
 - 2.6.1. Talent transferring the acrylic plate of mosquitoes onto the aluminum plate under dim lighting.
 - 2.6.2. Talent confirming that the thermocouple is positioned against the parafilm in the bottom right corner of the plate.
- 2.7. Re-upload the **Red-light-Only__BB_.ino** (*Red-light-only-B-B-dot-ino*) program in the Arduino integrated development environment to start the experiment [1]. Close the serial monitor [2]. In CoolTerm, press **Connect** to display the current temperature [3]. Adjust the trigger temperature in the Arduino script to approximately 0.6 degrees Celsius below the starting temperature [4].
 - 2.7.1. SCREEN: 71394_screenshot_4.mp4. 00:00–00:03; 00:06–00:11; 00:15–00:17; 00:25–00:28
 - 2.7.2. SCREEN: 71394_screenshot_5.mp4. 00:07–00:11
 - 2.7.3. SCREEN: 71394_screenshot_5.mp4. 00:11–00:13; 00:18–00:20; 00:27–00:30
 - 2.7.4. SCREEN: 71394_screenshot_6.mp4. 00:07–00:14
- 2.8. In the CoolTerm window, select **Capture to Text/Binary file** and start data collection [1]. Start video recording in the SpinView record window after selecting the desired file location and adjusting video parameters, such as recording type, split size, and quality [2]. Select **Resume** to end the thermocycler hold [3]. Monitor the Arduino output in

CoolTerm to confirm that the heat dip was detected and that the trial has started [4].

2.8.1. SCREEN: 71394_screenshot_7.mp4. 00:03–00:06; 00:12–00:15; 00:16–00:20

2.8.2. SCREEN: 71394_screenshot_8.mp4. 00:02–00:04; 00:05–00:08; 00:09–00:16; 00:17–00:20

2.8.3. **Resume** being selected to end the thermocycler hold. **Author's NOTE:** Videographer captured this along with 2.5.5. The thermocycler's screen was recorded as screen recording was not possible

2.8.4. SCREEN: 71394_screenshot_9.mp4. 00:05–00:12

2.9. After the trial is complete, select **Stop Recording** [1]. In the CoolTerm **Capture to Text/Binary file** tab, select **Capture** and choose **Stop**, then select **Disconnect** [2].

2.9.1. SCREEN: 71394_screenshot_10.mp4. 00:04–00:07

2.9.2. SCREEN: 71394_screenshot_10.mp4. 00:09–00:13; 00:15–00:17

2.10. Remove the plate of mosquitoes from the blood blanket plate and transfer to the cold room to anesthetize the mosquitoes [1]. Count the mosquitoes with enlarged abdomens containing the artificial blood meal as engorged, including those that have partially fed [2].

2.10.1. Talent removing the mosquito plate from the blood blanket plate and transferring it to the cold room.

2.10.2. Talent examining and counting engorged mosquitoes with enlarged abdomens.

2.11. Between trials, wipe the PCR block surface with a Kimwipe moistened with 70% ethanol [1]. Remove the parafilm and rinse the aluminum plate with deionized water, repeating the procedure for additional trials and rotating daily trial schedules to minimize circadian influences [2].

2.11.1. Talent wiping the surface of the polymerase chain reaction block with a Kimwipe moistened with 70 percent ethanol.

2.11.2. Talent removing the parafilm and rinsing the aluminum plate with deionized water.

3. Opto-Membrane Assay for Quantifying Mosquito Host-Seeking and Blood-Feeding Behaviors

Demonstrator: Cassandra Travis

3.1. Place a 119-milliliter bottle of water per trial into a 45 degrees Celsius heat bath, maintaining the volume and temperature constant to keep the blood meal close to body temperature throughout the experiment [1]. Prepare 5 milliliter aliquots of defibrinated sheep blood [2] and store them at 4 degrees Celsius [3]. Invert the aliquots before use to prevent separation and prepare a fresh blood meal before each experiment [4].

3.1.1. WIDE: Talent placing 119 milliliter bottles of water into a 45 degrees Celsius heat

bath.

- 3.1.2. Talent preparing 5 milliliter aliquots of defibrinated sheep blood.
- 3.1.3. Talent storing the aliquots at 4 degrees Celsius storage.
- 3.1.4. Talent inverting the blood aliquot after removing it from 4 °C storage.
- 3.2. Place the blood aliquot into a 45 degrees Celsius heat bath for at least 20 minutes before the trial [1]. Thaw the adenosine triphosphate, or ATP (A-T-P) on ice [2]. Stretch a square of parafilm over the acrylic lid on the side with the larger flat circle to create a well for the blood meal [3-TXT].
 - 3.2.1. Talent placing the blood aliquot into the heat bath.
 - 3.2.2. Talent thawing the ATP on ice.
 - 3.2.3. Talent stretching the parafilm over the acrylic lid to form the blood meal well.
TXT: Parafilm square: 5.08 cm x 5.08 cm
- 3.3. Wipe the computer with 70 percent ethanol to remove human odors [1]. Connect the computer to an external hard drive [2]. Connect the camera and the Arduino [3]. Plug the red, green, and blue lights and infrared lights into a power source [4].
 - 3.3.1. Talent wiping the computer with 70 percent ethanol.
 - 3.3.2. Talent connecting the computer to an external hard drive.
 - 3.3.3. Talent connecting the camera and the Arduino.
 - 3.3.4. Talent plugging the red, green, and blue lights and infrared lights into a power source.
- 3.4. Open the SpinView software and the Arduino file for the appropriate stimulus [1]. Create a folder on the external hard drive for the experiment data [2]. Upload the **blue_only.io** Arduino file to provide dim blue 471 nanometer light from the red, green, and blue light-emitting diodes to the arena [3].
 - 3.4.1. SCREEN: 71394_screenshot_14. 00:00–00:04; 00:09–00:11; 00:14–00:16
 - 3.4.2. SCREEN: 71394_screenshot_15. 00:03–00:05; 00:07–00:09; 00:17–00:19
 - 3.4.3. SCREEN: 71394_screenshot_16. 00:00–00:03; 00:08–00:12. *Video Editor: Please emphasize the text “Uploading” at the bottom of the screen.*
- 3.5. Without exposing the mosquitoes to excess carbon dioxide from breath, examine the canister and record any deaths before the experiment [1]. Place the canister in the center and allow acclimation under dim blue light for 10 to 20 minutes before stimulus exposure [2].
 - 3.5.1. Talent examining the mosquito canister and recording any deaths.
 - 3.5.2. Talent placing the canister in the center for acclimation under dim blue light.

- 3.6. For video capture, select the camera in the SpinView program [1]. In the **Settings** tab, adjust the exposure and gain to maintain high contrast between the light-colored insects and the dark background [2].
- 3.6.1. SCREEN: 71394_screenshot_17. 00:00–00:06
- 3.6.2. SCREEN: 71394_screenshot_18. 00:02–00:05; 00:07–00:11
- 3.7. Select the red record button on the top bar to adjust the video capture settings [1]. Select **Browse** and navigate to the external hard drive to set the file path [2]. Create a new folder with a descriptive name for each trial without using spaces [3].
- 3.7.1. SCREEN: 71394_screenshot_19. 00:00–00:03
- 3.7.2. SCREEN: 71394_screenshot_20. 00:00–00:07
- 3.7.3. SCREEN: 71394_screenshot_21. 00:00–00:02; 00:05–00:07; 00:17–00:21; 00:27–00:30
- 3.8. In the **Save Options** tab, select **Capture 0 frames** to allow continuous recording until manually stopped [1]. Under **Recording Mode**, select **Buffered** [2]. In the **Videos** tab, set **Video Recording Type** to **MJPEG (M-J-P-G)**, **Video File Split Size** to **1000 megabytes**, and **JPEG Compression Quality** to **65** [3].
- 3.8.1. SCREEN: 71394_screenshot_22. 00:00–00:03
- 3.8.2. SCREEN: 71394_screenshot_23. 00:00–00:03
- 3.8.3. SCREEN: 71394_screenshot_24. 00:00–00:08
- 3.9. Immediately before stimulus exposure, pipette 500 microliters of 20 millimolar ATP into the prepared 5 milliliter blood aliquot to achieve a final concentration of 2 millimolar [1]. Invert the tube several times to mix [2].
- 3.9.1. Talent pipetting adenosine triphosphate into the blood aliquot.
- 3.9.2. Talent inverting the tube several times to mix the blood meal.
- 3.10. Pour the ATP blood solution into the blood meal container [1]. Place an inverted 119-milliliter bottle filled with water at 45 degrees Celsius on top of the blood meal to maintain its temperature close to human body temperature throughout the experiment [2].
- 3.10.1. Talent pouring the ATP blood solution into the blood meal container.
- 3.10.2. Talent placing the inverted warm water bottle on top of the blood meal container.
- 3.11. In the SpinView software, select **Start Recording** [1]. Without stimulating the mosquitoes with breath, place the blood meal on top of the mesh lid of the canister so that the mosquitoes can pierce the parafilm membrane through the mesh [2].
- 3.11.1. SCREEN: 71394_screenshot_25. 00:00–00:03

- 3.11.2. Talent placing the blood meal on top of the mesh lid of the canister.
- 3.12. Upload the **1sON_10sOFF.io** (*One-S-On-Ten-S-Off-dot-I-O*) Arduino program or the desired stimulus program [1] to deliver 1 second of 624-nanometer red light every 10 seconds until the **blue_only.io** (*Blue-only-dot-I-O*) file is uploaded [2].
- 3.12.1. SCREEN: 71394_screenshot_26. 00:00–00:06
- 3.12.2. Shot of the mosquito arena illuminated with intermittent red light.
- 3.13. Place the blood aliquot into the heat bath for the following trial [1]. After the trial has run for 15 minutes, select **Stop Recording** [2]. Upload the **blue_only.io** file to stop the red-light stimulus [3].
- 3.13.1. Talent placing the next blood aliquot into the heat bath. **Videographer's NOTE:**
This is same as 3.2.1.
Author's NOTE: Reuse 3.2.1 here and keep the narration the same
- 3.13.2. SCREEN: 71394_screenshot_27. 00:00–00:03
- 3.13.3. SCREEN: 71394_screenshot_28. 00:00–00:06
- 3.14. Remove the blood meal holder and the water bottle [1]. Remove the canister and anesthetize the mosquitoes at 4 degrees Celsius [2]. Visually examine the anesthetized mosquitoes and record the number that engorged on blood by identifying blood-filled abdomens, prior to proceeding with the behavior analysis [3].
- 3.14.1. Talent removing the blood meal holder and the water bottle.
- 3.14.2. Talent removing the canister and placing the mosquitoes at 4 degrees Celsius for anesthesia. **Videographer's NOTE:** This was taken as 2 shots
- 3.14.3. Talent examining the anesthetized mosquitoes and recording the number of engorged mosquitoes.

Results

4. Results

- 4.1. In the blood blanket assay, upon receiving an artificial blood meal across ten trials per genotype, control lines exhibited similar baseline engorgement [1-TXT], whereas the experimental line achieved a significantly higher average engorgement rate of 53% [2].
 - 4.1.1. LAB MEDIA: Figure 3E. *Video Editor: Please emphasize the plots corresponding to the X-axis labels "Gr3-QF2 n = 10" and "QUAS-CsChrimson n = 10" TXT: Control lines: Gr3-QF2 and QUAS-CsChrimson; Experimental line: Gr3>CsChrimson*
 - 4.1.2. LAB MEDIA: Figure 3E. *Video Editor: Please emphasize the plot corresponding to the X-axis label "Gr3>CsChrimson n = 10"*
- 4.2. Engorgement over time, identified as the change in the abdominal area enclosed by 5 nodes in the experimental line, showed a gradual increase in abdominal area [1].
 - 4.2.1. LAB MEDIA: Figure 3F. *Video Editor: Please emphasize the increase in the height of the red color line along the increasing values on the X-axis.*
- 4.3. The mean percent occupancy on the blood meal region over time, as demonstrated by the opto-feeder assay, showed that the experimental line showed higher attraction to the meal [1], compared to control lines [2].
 - 4.3.1. LAB MEDIA: Figure 4F. *Video Editor: Please emphasize the red line*
 - 4.3.2. LAB MEDIA: Figure 4F. *Video Editor: Please emphasize the black and grey lines.*
- 4.4. The control genotypes showed similar engorgement of 26% [1] and 29%, respectively [2], while the experimental line mosquitoes demonstrated a significantly higher engorgement rate than either control, at 61% on average, indicating that the blood blanket and opto-membrane feeder assays are a viable means to assess the effects of optogenetic activation of carbon dioxide neurons on mosquito attraction and engorgement on a blood meal [3].
 - 4.4.1. LAB MEDIA: Figure 4G. *Video Editor: Please emphasize the light grey violin plot*
 - 4.4.2. LAB MEDIA: Figure 4G. *Video Editor: Please emphasize the dark grey violin plot*
 - 4.4.3. LAB MEDIA: Figure 4G. *Video Editor: Please emphasize the pink violin plot*

1. Assay

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

- IPA: /'æ,seɪ/, /ə'seɪ/
Phonetic Spelling: AS·ay, uh·SAY
2. Optogenetic
Pronunciation link: No confirmed link found
IPA: /,ɒptoudʒə'netɪk/
Phonetic Spelling: op·toh·juh·NET·ik
3. Engorgement
Pronunciation link: <https://www.merriam-webster.com/dictionary/engorgement>
IPA: /ɪn'gɔrdʒmənt/
Phonetic Spelling: en·GORJ·ment
4. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkroʊ,lɪtərz/
Phonetic Spelling: MY·kroh·lee·terz
5. Parafilm
Pronunciation link: No confirmed link found
IPA: /'pærə,fɪlm/
Phonetic Spelling: PAIR·uh·film
6. Scalpel
Pronunciation link: <https://www.merriam-webster.com/dictionary/scalpel>
IPA: /'skælpəl/, /skæl'pəl/
Phonetic Spelling: SKAL·puhl, skal·PEL
7. Polymerase
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>
IPA: /pə'limə,reɪs/, /'pələmə,reɪs/, /'pələmə,reiz/
Phonetic Spelling: puh·LIM·uh·rays, PAH·luh·muh·rays, PAH·luh·muh·rayz
8. Thermocouple
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermocouple>
IPA: /'θɜmə,kʌpəl/
Phonetic Spelling: THUR·muh·kuh·puhl
9. Acrylic
Pronunciation link: <https://www.merriam-webster.com/dictionary/acrylic>
IPA: /ə'krɪlɪk/
Phonetic Spelling: uh·KRIL·ik
10. Thermocycler
Pronunciation link: No confirmed link found
IPA: /,θɜːmoʊ'saɪklər/
Phonetic Spelling: thur·moh·SY·kler
11. Deionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>
IPA: /di'arə,narzd/
Phonetic Spelling: dee·EYE·uh·nized
12. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>

- IPA: /'εθə,nɒl/, /'εθə,nouəl/
Phonetic Spelling: ETH·uh·nawl, ETH·uh·nohl
13. Circadian
Pronunciation link: <https://www.merriam-webster.com/dictionary/circadian>
IPA: /sɜː'keɪdiən/
Phonetic Spelling: sur·KAY·dee·uhn
14. Aliquots
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquots>
IPA: /'ælə,kwɒts/, /'ælə,kwɒts/
Phonetic Spelling: AL·uh·kwɒts, AL·uh·kwuhts
15. Defibrinated
Pronunciation link: No confirmed link found
IPA: /di'faɪbrə,nertɪd/
Phonetic Spelling: dee·FY·bru·h·nay·tid
16. Adenosine
Pronunciation link: <https://www.merriam-webster.com/dictionary/adenosine>
IPA: /ə'denə,sɪn/
Phonetic Spelling: uh·DEN·uh·seen
17. Triphosphate
Pronunciation link: <https://www.merriam-webster.com/dictionary/triphosphate>
IPA: /traɪ'fas,fɜt/
Phonetic Spelling: try·FOSS·fayt
18. Infrared
Pronunciation link: <https://www.merriam-webster.com/dictionary/infrared>
IPA: /,ɪnfɹə'red/
Phonetic Spelling: in·fruh·RED
19. Nanometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
IPA: /'nænə,mɪtər/
Phonetic Spelling: NAN·uh·mee·ter
20. Acclimation
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimation>
IPA: /,æklə'meɪʃən/, /,æklai'meɪʃən/
Phonetic Spelling: ak·luh·MAY·shuhn, ak·lye·MAY·shuhn
21. Millimolar
Pronunciation link: No confirmed link found
IPA: /,mɪlə'moʊlər/
Phonetic Spelling: mil·uh·MOH·ler
22. Anesthetize
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /ə'nesθə,tɑɪz/
Phonetic Spelling: uh·NES·thuh·tize
23. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>

- IPA: /əˈnɛsθəˌtaɪzd/
Phonetic Spelling: uh·NES·thuh·tized
24. Anesthesia
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthesia>
IPA: /ˌænəsˈθiːzə/
Phonetic Spelling: an·uhs·THEE·zhuh
25. Membrane
Pronunciation link: <https://www.merriam-webster.com/dictionary/membrane>
IPA: /ˈmɛmbrɛn/
Phonetic Spelling: MEM·brayn
26. Genotype
Pronunciation link: <https://www.merriam-webster.com/dictionary/genotype>
IPA: /ˈdʒiːnəˌtaɪp/, /ˈdʒɛnəˌtaɪp/
Phonetic Spelling: JEE·nuh·type, JEN·uh·type
27. Abdominal
Pronunciation link: <https://www.merriam-webster.com/dictionary/abdominal>
IPA: /æbˈdʌmənəl/, /əbˈdʌmənəl/
Phonetic Spelling: ab·DAH·muh·nuhl, ub·DAH·muh·nuhl
28. Optofeeder
Pronunciation link: No confirmed link found
IPA: /ˌɒptəʊˈfiːdər/
Phonetic Spelling: op·toh·FEE·der
29. Diodes
Pronunciation link: <https://www.merriam-webster.com/dictionary/diode>
IPA: /ˈdaɪˌoʊdɪz/
Phonetic Spelling: DYE·ohdz
30. Engorged
Pronunciation link: <https://www.merriam-webster.com/dictionary/engorged>
IPA: /ɪnˈɡɔrdʒd/
Phonetic Spelling: en·GORJD
31. Neurons
Pronunciation link: <https://www.merriam-webster.com/dictionary/neuron>
IPA: /ˈnɔːrən/, /ˈnjʊrən/
Phonetic Spelling: NOOR·onz, NYOOR·onz