

Submission ID #: 72039

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Project Page Link: https://review.jove.com/files_upload.php?src=21971943

Title: Circadian Rhythms in a Petri Dish: Synchronizing Mouse Hypothalamic mHypoE-42 Cells for Circadian Clock Gene Expression Analysis

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, all done**
- 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: 38

Number of Shots: 66 (8 SCs)

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. **Laetitia Gaspar:** Our research focus is circadian rhythms and their role in disease, exploring biological clocks as potential biomarkers for disease detection and progression.
 - 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. **Laetitia Gaspar:** Existing circadian studies require overnight sampling every 2 to 6 hours, whereas this staggered protocol enables daytime-only sampling, reducing the need for overnight experiments.
 - 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. **Ana Rita Álvaro:** This protocol enables measurement of circadian clock gene expression, *Bmal1* and *Per2*, as example, and quantification of rhythmicity, MESOR, amplitude, and phase. **NOTE: Authors changed the statement during the shoot**
 - 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. **Ana Rita Álvaro:** The most important consideration here is maintaining consistent culture conditions, seeding density, and synchronization timing across staggered plates to minimize technical variability.
 - 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. **Ana Rita Álvaro:** Future studies can validate this workflow in additional cell lines and extend it to disease models and pharmacological screening.
 - 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. Setting Up mHypoE-42 Cell Culture

Demonstrators: Bárbara Ramalho, Cristiana Fernandes

- 2.1. To begin, culture the adherent mHypoE-42 (*M-Hypo-E-Forty-Two*) cells in DMEM (*D-M-E-M*) high glucose [1], supplemented with the presented components [2].
 - 2.1.1. WIDE: Talent culturing the adherent mHypoE-42 cells in DMEM.
 - 2.1.2. TEXT ON A PLAIN BACKGROUND
Heat-inactivated FBS: 10% (v/v)
Sodium bicarbonate: 3.7 g/L
Antibiotic solution: 1%
- 2.2. Incubate the cells at 37 degrees Celsius in a humidified environment with 95% air and 5% carbon dioxide, using T75 flasks with a 10-milliliter volume [1].
 - 2.2.1. Talent placing the T75 culture flasks containing the cells in the humidified incubator.
- 2.3. Subculture the cells once they reach approximately 80 to 90% confluence [1]. Wash the cells with sterile PBS [2], add 3 milliliters of 0.5% trypsin solution [2], and incubate for 3 to 5 minutes at 37 degrees Celsius to detach the cells [3].
 - 2.3.1. LAB MEDIA: 2.3.1_2.jpg
 - 2.3.2. Talent washing the cells with sterile PBS.
 - 2.3.3. Talent adding 3 milliliters of 0.5% trypsin solution to the cells.
 - 2.3.4. Talent placing the cells in the incubator. **Author's NOTE:** This was same as shot 2.2.1
- 2.4. Add at least twice the trypsin volume of the complete culture medium containing 10% FBS to neutralize the trypsin [1].
 - 2.4.1. Talent adding the complete culture medium containing 10% volume per volume FBS to neutralize the trypsin.
- 2.5. Dilute the cells with the fresh growth medium at a ratio of up to 1 to 2 [1].
 - 2.5.1. Talent diluting the cells with the fresh growth medium at a ratio of up to 1:2.

3. Synchronization of mHypoE-42 Cell Line

- 3.1. After trypsinization, centrifuge the cells at 700 g for 5 minutes [1].

- 3.1.1. Talent placing the cells in the centrifuge.
- 3.2. Discard the supernatant [1] and resuspend the cell pellet in 10 milliliters of the fresh culture medium [2].
 - 3.2.1. Talent discarding the supernatant.
 - 3.2.2. Talent resuspending the cell pellet in 10 milliliters of the fresh culture medium.
- 3.3. Mix the cell suspension with the trypan blue dye in an equal ratio for a total volume of 20 microliters [1] and determine the cell concentration using a hemocytometer [2].
 - 3.3.1. Talent mixing the cell suspension with the trypan blue dye at a 1:1 ratio for a total volume of 20 microliters.
 - 3.3.2. Talent adding the cells on the hemocytometer
- 3.4. Divide the culture plates into two sets. Use the first set for circadian time points 0, 4, 8, 24, 28, and 32, and the second set for time points 12, 16, 20, 36, 40, 44, and 48. [1].
 - 3.4.1. Talent randomly divides the culture plates into 2 experimental sets and marks them.
- 3.5. Plate 2.0×10^5 cells per well in 6-well plates [1] and incubate at 37 degrees Celsius for 24 hours [2].
 - 3.5.1. Talent plating 2.0×10^5 cells per well in either 6-well plates.
 - 3.5.2. Talent placing the cultures in the incubator.
- 3.6. Then, aspirate the medium from the first set of plates, add 1 milliliter of serum-free DMEM [1], and incubate for 12 hours at 37 degrees Celsius to induce serum starvation [3].
 - 3.6.1. Talent aspirating the medium from the first set of plates and adding DMEM.
 - 3.6.2. Talent placing the cultures in the incubator.
- 3.7. After aspirating the starvation medium from the first set, expose the cells to 1 milliliter of the freshly prepared 50% horse serum solution [1], and incubate for 2 hours at 37 degrees Celsius [2].
 - 3.7.1. Talent adding 1 milliliter of the freshly prepared 50% horse serum solution to the cells.
 - 3.7.2. Talent placing the cultures in the incubator. **Author's NOTE: This was same as shot 3.6.2**
- 3.8. Simultaneously, induce starvation in the second set of plates as demonstrated previously [1].
 - 3.8.1. Reuse 3.6.1 and 3.6.2 **Author's NOTE: This steps was same as 3.6.1 and 3.6.2. Modify the narration as "Simultaneously, induce starvation in the second set of plates as previously described"**

~~3.8.2. Talent aspirating the medium from the second set of plates and adding 1ml serum free DMEM~~

~~3.8.3. Talent placing the cultures in the incubator.~~

3.9. After the 2-hour serum shock, replace the medium with 1 milliliter of high-glucose DMEM supplemented with 10% heat-inactivated FBS, and designate this time point as circadian time 0 [1].

3.9.1. Talent aspirating the medium and adding 1 milliliter of high-glucose DMEM.

~~3.9.2. Talent marking the plate.~~ **NOTE: Plates were already marked in shot 3.4.1.**

4. Sample Collection

4.1. Place the culture plates on ice [1]. After aspirating the medium, wash the cells twice with 1 milliliter of cold sterile PBS, and discard the wash solution [2]. Mechanically lyse the cells using up to 1 milliliter of the lysis reagent [3].

4.1.1. Talent placing the culture plates on ice.

4.1.2. Talent washing the cells twice with 1 milliliter of cold sterile 1× phosphate-buffered saline, and discarding the wash solution.

4.1.3. Talent mechanically lysing the cells using up to 1 milliliter of the lysis reagent.

4.2. Transfer each lysate to a sterile tube [1] and store the samples at minus 80 degrees Celsius until RNA extraction [2].

4.2.1. Talent transferring each lysate to a sterile tube.

4.2.2. Talent placing the samples in storage at -80 degrees Celsius.

4.3. Similarly, collect samples at circadian time 4 and circadian time 8 [1].

4.3.1. Talent collects samples at circadian time 4 and circadian time 8 into a sterile tube. **Author's NOTE: We only shot circadian time 4 as it is exactly the same procedure**

4.4. At circadian time 8, induce synchronization in the second set of plates as demonstrated previously [1].

4.4.1. Reuse 3.7.1 and 3.7.2 **Author's NOTE: This steps was same as 3.6.1 and 3.6.2. Modify the narration as "At circadian time 8, induce synchronization in the second set of plates as previously described"**

~~4.4.2. Talent adding 1 milliliter of the freshly prepared 50% horse serum solution to the cells.~~

~~4.4.3. Talent places the cells in the incubator.~~

4.5. After 2 hours of serum shock, terminate synchronization of the second experimental set by replacing the medium with 1 milliliter of high-glucose DMEM supplemented with 10% heat-inactivated FBS [1].

4.5.1. Talent replacing the medium in the second experimental set with 1 milliliter of high-glucose DMEM supplemented with 10% heat-inactivated FBS. **Author's NOTE: Same shot as 3.9.1.**

4.6. Collect samples alternately from the two experimental sets at circadian time points of 12, 16, 20, 24, 28, and 32 hours. Repeat the procedure the next day for 36, 40, 44, and 48 hours [1].

4.6.1. Talent collecting samples from the 2 experimental sets.

5. RNA Extraction

5.1. After thawing the samples, re-homogenize the lysates and incubate them at room temperature for 5 minutes [1].

5.1.1. Talent re-homogenizing the lysates and keeping them aside.

5.2. Add 200 microliters of chloroform for every 1 milliliter of the lysis reagent [1].

5.2.1. Talent adding 200 microliters of chloroform for every 1 milliliter of the lysis reagent.

5.3. Shake the tubes vigorously for 15 seconds and incubate them at room temperature for 3 minutes [1].

5.3.1. Talent vigorously shakes the tubes and keeps them aside.

5.4. Centrifuge the samples at 12,000 *g* for 15 minutes at 4 degrees Celsius [1]. Allow the samples to separate into three phases: a lower phenol-chloroform layer with proteins, a white interphase with DNA, and an upper aqueous layer enriched in RNA [2].

5.4.1. Talent placing the samples in the centrifuge.

5.4.2. Shot of the samples separated into the lower phenol-chloroform phase, the white interphase, and the upper aqueous phase. **Authors: Please point out the different phases**

5.5. Carefully transfer the aqueous phase to a new sterile tube without disturbing the interphase [1]. Store the organic phase at 4 degrees Celsius if subsequent protein or DNA isolation is planned [2].

5.5.1. Talent carefully transferring the aqueous phase to a new sterile tube without disturbing the interphase.

5.5.2. Talent placing the organic phase in storage at 4 degrees Celsius.

5.6. Add an equal volume of 70% ethanol prepared with RNAase-free water to the aqueous phase [1] and vortex twice for 15 seconds to promote RNA binding [2].

5.6.1. Talent adding an equal volume of 70% ethanol prepared with ribonuclease-free water to the aqueous phase.

- 5.6.2. Talent vortexing the sample twice for 15 seconds.
- 5.7. Load the sample onto a silica spin column and purify the RNA according to the column purification workflow [1].
 - 5.7.1. Talent loading the sample onto a silica spin column.
- 5.8. After DNase treatment, wash the column using the provided wash buffers, followed by an additional wash with 100 microliters of the wash buffer [1] and centrifuge at 11,000 g for 2 minutes to dry the silica membrane [2].
 - 5.8.1. Talent washing the column with 100 microliters of the wash buffer.
 - 5.8.2. Talent placing the column in the centrifuge.
- 5.9. Once the spin column is transferred to a clean microcentrifuge tube, apply 40 to 60 microliters of RNase-free water directly to the center of the silica membrane [1]. Centrifuge at 11,000 g for 1 minute to elute the RNA [2].
 - 5.9.1. Talent applying 40 to 60 microliters of ribonuclease-free water directly to the center of the silica membrane.
 - 5.9.2. Talent placing the spin column in the centrifuge. **Author's NOTE: We only shot the settings in the centrifuge. Add shot 5.8.2. to complete**
- 5.10. Evaluate RNA integrity using 1.5% agarose or capillary electrophoresis, ensuring distinct 28S and 18S bands are present [2].
 - 5.10.1. LAB MEDIA: 5.10.1. *Video editor: Please crop and show only the gel image*
- 5.11. Determine the RNA concentration and purity by measuring the absorbance with a spectrophotometer [1-TXT].
 - 5.11.1. Talent measuring the absorbance with a spectrophotometer to determine the ribonucleic acid concentration and purity. **TXT: Store purified RNA at -80 °C until cDNA synthesis**

6. cDNA Synthesis

- 6.1. After thawing the RNA samples on ice, prepare a 20-microliter reverse transcription reaction [1].
 - 6.1.1. Talent prepares the 20-microliter reverse transcription reaction with the specified components.
 - AND
 - TEXT ON A PLAIN BACKGROUND
 - Total RNA: 1 µg
 - 2× Master Mix: 10 µL
 - Enzyme mix (containing reverse transcriptase): 2 µL
 - DEPC-treated water: upto 20 µL *Video editor: Please use split screen to show*

these shots side by side as a table

- 6.2. Incubate the reaction in a thermal cycler under the presented conditions [1].
 - 6.2.1. TEXT ON A PLAIN BACKGROUND.
Primer annealing: 25 °C, 10 min
cDNA synthesis: 50 °C, 30 min
Reverse transcriptase inactivation: 85 °C, 5 min
- 6.3. Add 1 microliter of RNase H to the reaction [1] and incubate for 20 minutes at 37 degrees Celsius to degrade the RNA strand of the RNA-DNA hybrid [2].
 - 6.3.1. Talent adding 1 microliter of ribonuclease H to the reaction.
 - 6.3.2. Talent placing the reaction for incubation at 37 degrees Celsius in the thermal cycler.
- 6.4. Dilute the cDNA preparation up to 1 to 20 with sterile water according to the assay optimization requirements [1] and store the diluted cDNA at minus 20 degrees Celsius until qRT-PCR analysis [2].
 - 6.4.1. Talent diluting the complementary deoxyribonucleic acid preparation up to 1:20 with sterile water.
 - 6.4.2. Talent placing the diluted complementary deoxyribonucleic acid in storage at -20 degrees Celsius.
- 6.5. Quantify circadian gene expression using validated qRT-PCR [1 and 2].
ADDED SHOT: Talent pipetting a PCR plate.
 - 6.5.1. Talent in front of the computer looking at the RT-PCR data.

7. Data Analysis

- 7.1. To analyze rhythmicity and circadian parameters using Online CircaCompare (*Circa-Compare*) analysis, prepare a CSV file with time, group, and outcome variables [1].
 - 7.1.1. SCREEN: 72039_screenshot_1.mp4: 00:08-00:15
- 7.2. Upload the CSV file to the online CircaCompare application [1]. Assign the time, group, and outcome variables to the corresponding columns [2]. Then, select **Run** to perform the analysis [3].
 - 7.2.1. SCREEN: 72039_screenshot_1.mp4: 00:40-00:42, 00:45-00:53
 - 7.2.2. SCREEN: 72039_screenshot_1.mp4: 00:55-01:05
 - 7.2.3. SCREEN: 72039_screenshot_1.mp4: 01:06-01:08, 01:20
- 7.3. To perform CircaCompare analysis in R, install the required R packages [1] and upload

the input CSV file [2].

7.3.1. SCREEN: 72039_screenshot_2.mp4: 01:22-01:24.

7.3.2. SCREEN: 72039_screenshot_2. mp4: 00:54-01:03

7.4. Set alpha_threshold to 1 [1]. Add the names of the input data file, desired result file, and plot file. Adjust the y-axis limits and any additional plotting parameters as needed based on the dataset. Then execute the CircaCompare analysis [2].

7.4.1. SCREEN: 72039_screenshot_2. mp4: 01:11-01:15

7.4.2. SCREEN: 72039_screenshot_2. mp4: 01:19-01:30, 01:46-01:56

Results

8. Results

- 8.1. Under basal conditions, *Bmal1* (*B-M-A-L-One*) and *Per2* (*Per-Two*) exhibited statistically significant rhythmicity [1].
 - 8.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the values in the "Rhythmicity (p-value)" column for the rows "Bmal1" and "Per2"*
- 8.2. *Bmal1* and *Per2* oscillated in opposite phases [1], with a significant phase difference of minus 10.9356 hours [2].
 - 8.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the light Per2 curve and the dark Bmal1 curve*
 - 8.2.2. LAB MEDIA: Table 2. *Video editor: Highlight the value in the "Phase difference estimate (p-value)" column*
- 8.3. Following exposure to Compound A, *Bmal1* no longer exhibited statistically significant rhythmicity [1].
 - 8.3.1. LAB MEDIA: Table 1. *Video editor: Highlight the value in the "Rhythmicity (p-value)" column for the row "Bmal1 + Compound A"*
- 8.4. The midline estimating statistic of rhythm, amplitude, and peak time for *Bmal1* was not reported after exposure to compound A [1].
 - 8.4.1. LAB MEDIA: Table 1. *Video editor: Highlight the dash entries in the "MESOR," "Amplitude," and "Peak time (h)" columns for the row "Bmal1 + Compound A"*

1. mHypoE-42
Pronunciation link: No confirmed link found
IPA: /ˌɛm ˌhaɪpoo ˈiː ˌfɔrti ˈtuː/
Phonetic Spelling: em·hy·poh·ee·for·tee·too
2. DMEM
Pronunciation link: No confirmed link found
IPA: /ˌdiː ˌɛm ˌiː ˈɛm/
Phonetic Spelling: dee·em·ee·em
3. Sodium bicarbonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium%20bicarbonate>

- IPA: /'soʊdiəm bɑː'kɑːrbənət/
Phonetic Spelling: soh·dee·uhm·bye·kar·buh·nuht
4. Confluence
Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
IPA: /'kɑːnfluəns/
Phonetic Spelling: kon·floo·uhns
5. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /'trɪpsɪn/
Phonetic Spelling: trip·sin
6. Trypsinization
Pronunciation link: No confirmed link found
IPA: /,trɪpsɪnə'zeɪʒən/
Phonetic Spelling: trip·sin·uh·zay·shuhn
7. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
8. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /,suːpər'neɪtənt/
Phonetic Spelling: soo·per·nay·tuht
9. Resuspend
Pronunciation link: No confirmed link found
IPA: /,riːsə'spend/
Phonetic Spelling: ree·suh·spend
10. Trypan blue
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypan%20blue>
IPA: /'traɪpən 'bluː/
Phonetic Spelling: try·puhn·bloo
11. Hemocytometer
Pronunciation link: No confirmed link found
IPA: /,hiːməsaɪ'tɑːmɛtər/
Phonetic Spelling: hee·muh·sy·tom·uh·ter
12. Circadian
Pronunciation link: <https://www.merriam-webster.com/dictionary/circadian>
IPA: /sər'keɪdiən/
Phonetic Spelling: ser·kay·dee·uhn
13. Phosphate-buffered saline
Pronunciation link: No confirmed link found
IPA: /'fɑːsfet 'bʌfərd 'seɪliːn/
Phonetic Spelling: fos·fayt·buff·erd·say·leen
14. Lysate
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysate>

- IPA: /'laɪ, sɛɪt/
Phonetic Spelling: ly·sayt
15. Chloroform
Pronunciation link: <https://www.merriam-webster.com/dictionary/chloroform>
IPA: /'klɔːrə, fɔːrm/
Phonetic Spelling: klor·uh·form
16. Phenol
Pronunciation link: <https://www.merriam-webster.com/dictionary/phenol>
IPA: /'fiː, nɔːl/
Phonetic Spelling: fee·nawl
17. Phenol-chloroform
Pronunciation link: No confirmed link found
IPA: /'fiː, nɔːl 'klɔːrə, fɔːrm/
Phonetic Spelling: fee·nawl·klor·uh·form
18. Interphase
Pronunciation link: <https://www.merriam-webster.com/dictionary/interphase>
IPA: /'ɪntər, feɪz/
Phonetic Spelling: in·ter·fayz
19. Aqueous
Pronunciation link: <https://www.merriam-webster.com/dictionary/aqueous>
IPA: /'eɪkwɪəs/
Phonetic Spelling: ay·kwee·uhs
20. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'εθə, nɔːl/
Phonetic Spelling: eth·uh·nawl
21. Ribonuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonuclease>
IPA: /, raɪboʊ'nuːkli, eɪs/
Phonetic Spelling: rye·boh·noo·klee·ays
22. Silica
Pronunciation link: <https://www.merriam-webster.com/dictionary/silica>
IPA: /'sɪlɪkə/
Phonetic Spelling: sil·ih·kuh
23. DNase
Pronunciation link: No confirmed link found
IPA: /'diː, ɛn, eɪs/
Phonetic Spelling: dee·en·ays
24. Microcentrifuge
Pronunciation link: No confirmed link found
IPA: /, maɪkroʊ'sentrə, fjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
25. RNase
Pronunciation link: No confirmed link found

- IPA: /'ɑːr ɛn ɛɪs/
Phonetic Spelling: ar·en·ays
26. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /'æɡəˌroʊs/
Phonetic Spelling: ag·uh·rohs
27. Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /'kæpəˌlɛri/
Phonetic Spelling: kap·uh·lair·ee
28. Electrophoresis
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>
IPA: /ɪˌlɛktroʊfəˈriːsɪs/
Phonetic Spelling: ih·lek·troh·fuh·ree·sis
29. Spectrophotometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
IPA: /ˌspektroʊfoʊˈtɑːmɛtər/
Phonetic Spelling: spek·troh·foh·tom·uh·ter
30. Ribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic>
IPA: /ˌraɪboonuˈkliːk/
Phonetic Spelling: rye·boh·noo·klee·ik
31. cDNA
Pronunciation link: No confirmed link found
IPA: /ˌsiːˌdiːˌɛnˈeɪ/
Phonetic Spelling: see·dee·en·ay
32. Reverse transcription
Pronunciation link: No confirmed link found
IPA: /rɪˈvɜːs trænˈskrɪpʃən/
Phonetic Spelling: rih·vurs·tran·skrip·shuhn
33. Reverse transcriptase
Pronunciation link: No confirmed link found
IPA: /rɪˈvɜːs trænˈskrɪpˌteɪs/
Phonetic Spelling: rih·vurs·tran·skrip·tays
34. DEPC
Pronunciation link: No confirmed link found
IPA: /ˌdiːˌiːˌpiːˈsiː/
Phonetic Spelling: dee·ee·pee·see
35. Annealing
Pronunciation link: <https://www.merriam-webster.com/dictionary/annealing>
IPA: /əˈniːlɪŋ/
Phonetic Spelling: uh·nee·ling
36. Deoxyribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/deoxyribonucleic>

- IPA: /diˌɑːksɪrəboʊnuˈkliːɪk/
Phonetic Spelling: dee·ok·see·rye·boh·noo·klee·ik
37. qRT-PCR
Pronunciation link: No confirmed link found
IPA: /ˌkjuːˌɑːrˌtiːˌpiːsiːˈɑːr/
Phonetic Spelling: cue·ar·tee·pee·see·ar
38. Rhythmicity
Pronunciation link: No confirmed link found
IPA: /rɪðˈmɪsəti/
Phonetic Spelling: rith·miss·uh·tee
39. CircaCompare
Pronunciation link: No confirmed link found
IPA: /ˈsɜːkə kəmˈpɛr/
Phonetic Spelling: sur·kuh·kuhm·pair
40. Bmall
Pronunciation link: No confirmed link found
IPA: /ˌbiːˌɛmˌeɪˌɛlˈwʌn/
Phonetic Spelling: bee·em·ay·el·wun
41. Per2
Pronunciation link: No confirmed link found
IPA: /ˈpɜːˈtuː/
Phonetic Spelling: per·too
42. Oscillated
Pronunciation link: <https://www.merriam-webster.com/dictionary/oscillate>
IPA: /ˈɑːsəˌleɪtɪd/
Phonetic Spelling: oss·uh·lay·tid
43. MESOR
Pronunciation link: No confirmed link found
IPA: /ˈmiːzɔːr/
Phonetic Spelling: mee·zor