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Title: Genome Editing in Primary Mammalian Cells via Electroporation of Editor RNA

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: 21

Number of Shots: 52

Interviews

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

Videographer's Note: Did each interview question at least twice. Most shots of the author are cropped a little closer than usual.

INTRODUCTION:

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

- 1.1. **Maria Viskadourou:** Our research focuses on the development of genome editor therapeutics for the treatment of genetic diseases.

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. **Maria Viskadourou:** Genome editor delivery by plasmid is often inefficient in primary cells. Editor mRNAs enable comparisons among different editors and the study of resulting phenotypes.

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

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.3. **Maria Viskadourou:** A critical challenge lies in preserving mRNA integrity, as it degrades rapidly upon cellular exposure or due to RNase contamination.

1.3.1. 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.

Videographer's Note: Most of the slates should be accurate. In this case most shots were single steps, but we combined steps into a single shot where appropriate. A couple times we added an insert close up to better show the detail or if the talent's arm blocked the shot for a moment. Those takes were labeled as the shot with (a) at the end.

2. Editor RNA Electroporation into Induced Pluripotent Stem Cells

Demonstrator: Maria Viskadourou

- 2.1. To begin, use *in vitro* transcription, or IVT (*I-V-T*), to prepare high-quality editor messenger RNA, or mRNA (*m-R-N-A*), encoding the genome editor from a uniform double-stranded DNA-PCR template containing a T7 (*T-seven*) promoter and 120-nucleotide polyA (*poly-A*) tail [1]. To keep the mRNA pellet sterile during purification, resuspend it in a biosafety cabinet [2]. After quantification and normalization to 2 microgram per microliter, aliquot and store at minus 80 degrees and thaw on ice before use. [3].
 - 2.1.1. WIDE: Talent assembling the reaction tubes and components for IVT reaction.
Videographer: Please obtain CU shot as well for this shot.
 - 2.1.2. Talent resuspending the RNA pellet in the biosafety cabinet
 - 2.1.3. Talent aliquoting mRNA into PCR strip tubes
- 2.2. Use **Benchling** software tool to design target-specific guide RNAs, or gRNAs (*G-R-N-Aze*), with one or more editors for testing in the KOLF2.1J (*kolf-two-point-one-jay*) cells [1]. After obtaining the chemically modified, lyophilized synthetic gRNAs, dissolve them in RNase-free water on ice [2].
 - 2.2.1. Representative shot of talent designing the guide RNA using Benchling software tool.
 - 2.2.2. Talent dissolving a lyophilized gRNA tube from a manufacturer on ice.
- 2.3. Trypsinize the cells for electroporation with editor RNAs [1], then wash the resuspended cells with Dulbecco's phosphate-buffered saline, or DPBS (*D-P-B-S*), to remove serum and RNases [2]. Next, resuspend the cells to a density of 200 thousand per reaction in 5 microliters of cold electroporation buffer, freshly formulated by combining the buffer and supplement tubes [3]. Maintain the cells in electroporation cuvettes on ice until use [4].
 - 2.3.1. Talent adding trypsin solution to the adherent cells.

- 2.3.2. Talent placing the tube with PBS-resuspended cells in the centrifuge.
- 2.3.3. Talent resuspending the washed cells in 5 μL of cold electroporation buffer.
- 2.3.4. Talent placing the cells in electroporation cuvettes on ice
- 2.4. Prepare a 1.2X (*one-point-two-X*) master mix of RNA cargo and electroporation buffer on ice in PCR strip tubes to account for pipetting loss [1]. Combine the editor mRNA and gRNA in electroporation buffer [2] for the number of reactions intended [3].
- 2.4.1. Talent arranging the RNA cargo and buffer components on ice in PCR strip tubes.
- 2.4.2. Talent combining the editor mRNA and gRNA in electroporation buffer.
- 2.4.3. TEXT ON A PLAIN BACKGROUND
- Electroporation Solution per sample (17 μL)
- For Base Editors and Nucleases

3 μg base editor mRNA	1.5 μL
50 picomoles gRNA	0.5 μL
Electroporation Buffer	15 μL
 - For Prime Editors

1 μg prime editor mRNA	0.5 μL
150 picomoles gRNA	1.5 μL
Electroporation Buffer	15 μL
- 2.5. Add 17 microliters of mRNA cargo to 5 microliters of cell suspension placed in the electroporation cuvettes on ice [1]. Mix the suspension by pipetting it 3 times, avoiding the introduction of bubbles [2].
- 2.5.1. Talent adding RNA cargo in the electroporation buffer to the cells.
- 2.5.2. Talent pipetting the suspension three times to mix.
- 2.6. Within 2 minutes of mixing the cells and RNA, run the DN-100 (*D-N-hundred*) electroporation program optimized for the selected cell type in the nucleofection device to complete the electroporation [1]. Confirm successful pulse delivery for each well using the instrument readout [2].
- 2.6.1. Talent placing the electroporation cuvettes in the nucleofection device.
Videographer: Please capture the device's digital display showing DN100.
- 2.6.2. SCREEN: Show the instrument readout confirming successful pulse delivery for each well. *Videographer: Please capture the device's digital display showing the readout.*

2.7. After electroporation, add 80 microliters of pre-warmed recovery medium supplemented with CEPT (*C-E-P-T*) cocktail to each cuvette [1], allowing the cells to recover undisturbed for 10 minutes in a 37 degrees incubator [2]. Transfer the cells to pre-equilibrated and appropriately coated 12-well culture plates [3]. Incubate the cells at 37 degrees Celsius in 5 percent carbon dioxide [4]. Examine the cells daily after plating to monitor cell health and recovery [5].

2.7.1. Talent adding pre-warmed recovery medium to each cuvette.

2.7.2. Talent placing the electroporation well in the incubator.

2.7.3. Talent transferring the cells to prepared culture plates.

2.7.4. Talent placing the culture plates for incubation.

2.7.5. Talent examining the plated cells under the microscope to assess health and recovery.

3. Genomic DNA Extraction from Electroporated Cells

Demonstrator: D. Mark Eckley

3.1. At least 2 days after electroporation, wash the adhered cells once with PBS [1]. Remove the buffer completely [2].

3.1.1. Talent washing the cells with Dulbecco's phosphate-buffered saline.

3.1.2. Talent removing the buffer completely.

3.2. Next, to lyse the cells, add 400 microliters of complete lysis buffer per well [1], prepared by freshly adding Proteinase K at a 1 to 1000 dilution to previously prepared incomplete lysis buffer [2]. Incubate the samples at 37 degrees Celsius for 60 minutes to complete cell lysis and proteinase K digestion [3].

3.2.1. Talent adding complete lysis buffer to the cells.

3.2.2. TEXT ON A PLAIN BACKGROUND

Incomplete Lysis Buffer (1 liter)

1M Tris-HCl (pH 8.0)	10 mL
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10% w/v SDS	5 mL
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Nuclease-free water	Adjust to a final volume of 1 L
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3.2.3. Talent placing the samples in the incubator.

3.3. Transfer the lysates to PCR tubes [1]. Incubate the samples at 80 degrees Celsius for 30 minutes to inactivate the proteinase K [2]. Store the lysates at minus 20 degrees Celsius until PCR amplification [3].

- 3.3.1. Talent transferring lysates to PCR tubes.
- 3.3.2. Talent placing the samples for incubation.
- 3.3.3. Talent storing the lysates at minus 20 degrees Celsius.

4. Targeted Amplicon Sequencing and Analysis of Genome Editing Outcomes

Demonstrator: J Noah Workman

- 4.1. Visualize the genomic locus of the annotated pegRNA spacer and primers on Snapgene [1]. Design locus-specific primers to generate approximately 300-basepair amplicons, ensuring the primers flank the target edit site by spanning at least 25 basepairs upstream and downstream of the intended modification [2]. Copy the forward and reverse primer sequences [3] and combine the Illumina adapter sequence and locus-specific primer sequences to generate the final primer sequences to be ordered [4].

- 4.1.1. SCREEN: 71449_4.1.1.mp4. 00:05–00:07
- 4.1.2. SCREEN: 71449_4.1.1.mp4. 00:34–00:42
- 4.1.3. SCREEN: 71449_4.1.1.mp4. 00:48–00:51; 00:58–01:05
- 4.1.4. SCREEN: 71449_4.1.1.mp4. 01:20–01:24; 01:26–01:28

- 4.2. Amplify the target genomic locus by performing the first polymerase chain reaction, or PCR1 (*P-C-R-one*) [1], using the specific reaction components and thermocycling conditions [2].

- 4.2.1. Talent preparing the PCR-1 components for amplification of the target genomic locus.
- 4.2.2. LAB MEDIA: Table 6 and Table 7. *Video Editor: Please add the headings “PCR1 reagents for MiSeq library preparation” and “PCR1 cycling conditions” for Table 6 and Table 7 respectively. Additionally, please add the text from Table 6_additional info at the bottom of table 6 content.*

- 4.3. Use the PCR1 product as the template to add unique sample indices [1] and perform 9 cycles of PCR amplification [2] under specific conditions to generate PCR 2 (*P-C-R-two*) products [3].

- 4.3.1. Talent adding unique sample indices using the first polymerase chain reaction product as the template.
- 4.3.2. SCREEN: Show the amplification setup with 9 cycles selected. **Note:** This shot was captured by the videographer as per the author’s e-mail confirmation.

- 4.3.3. **TEXT ON A PLAIN BACKGROUND**

- **PCR-2 Master Mix Composition**

2x polymerase master mix

12.5uL

Unprocessed PCR1	0.5uL
10uM forward primer	1uL
10uM reverse primer	1uL
Nuclease-free water	10uL

- **PCR-2 Amplification Conditions**

Initial denaturation at 98°C	30 seconds
Denaturation at 98°C	10 seconds
Annealing at 62°C	15 seconds
Extension at 72°C	30 seconds
Final extension at 72°C	1 minute
Hold at 4°C	

4.4. Run the PCR1 and PCR2 products on a 1.5 percent agarose gel [1] to confirm the presence of single bands at the expected sizes [2].

4.4.1. Talent loading polymerase chain reaction products onto the agarose gel.

4.4.2. Representative shot of the image showing single bands of PCR1 and PCR2 products.

4.5. Pool the PCR2 products in equimolar amounts [1]. Use bead-based cleanup to remove adapter dimers under specific conditions [2].

4.5.1. Talent pooling the second polymerase chain reaction products.

4.5.2. Talent removing adapter dimers using the selected cleanup method.

4.6. Use fluorometric double-stranded deoxyribonucleic acid or dsDNA (*D-S-D-N-A*) assay to quantify the final pooled library [1]. Assess the fragment size distribution and purity of the final pooled library using capillary electrophoresis, where an ideal, clean library shows a single sharp peak at 461 base pairs [2], while a bad library profile shows a large peak around 584 base pairs [3] along with lower-weight adapter dimers around 280 base pairs [4].

4.6.1. Talent quantifying the pooled library using a fluorometric double-stranded deoxyribonucleic acid assay. *Videographer: Please capture the reading on the instrument's digital display.*

4.6.2. SCREEN: 71449_4.6.2_GOOD-example.PNG. *Video Editor: Please emphasize the single, clean peak labelled 461 bp.*

4.6.3. SCREEN: 71449_4.6.2_BAD-example.PNG. *Video Editor: Please emphasize the peak labelled 584 bp.*

- 4.6.4. SCREEN: 71449_4.6.2_BAD-example.PNG. *Video Editor: Please emphasize the short peak labelled 280 bp.*
- 4.7. Initiate a new sequence run planning template in the **BaseSpace** panel and download the required formatting spreadsheet [1]. Open the file in a spreadsheet editor to organize the target parameters [2]. Populate the document columns by entering the specific sample names categorized by genomic locus and index layouts [3]. Save the progress, return to the platform interface, and upload the completed sample sheet to establish automated data demultiplexing [4].
- 4.7.1. SCREEN: 71449_4.8.1.mp4. 00:06–00:11; 00:56–01:00
- 4.7.2. SCREEN: 71449_4.8.1.mp4. 01:04–01:09
- 4.7.3. SCREEN: 71449_4.8.1.mp4. 01:29–01:33; 01:53–01:58
- 4.7.4. SCREEN: 71449_4.8.1.mp4. 03:39–03:45; 03:50–03:56
- 4.8. Load and run the finalized pooled library on an Illumina MiSeq (*my-seek*) instrument, configuring it to execute single-end, 300-cycle reads [1]. Monitor the sequencing run performance metrics on the Illumina BaseSpace platform [2]. Navigate to the Indexing QC (*Q-C*) dashboard to verify that the final sequence data achieves high-quality scores and that the total read count falls within the optimal range across the individual pooled samples [3].
- 4.8.1. Talent preparing the libraries for sequencing on the Illumina MiSeq instrument. *Videographer: Please capture the reading on the instrument's digital display.*
- 4.8.2. SCREEN: 71449_4.7.2.mp4. 00:20–00:23; 00:30–00:34
- 4.8.3. SCREEN: 71449_4.7.2.mp4. 00:35–00:38; 00:48–00:51; 01:10–01:13; 01:19–01:23
- 4.9. Download the FASTQ (*fast-Q*) files from the **Basespace** panel and select the storage directory folder [1]. Create a new folder [2] and use the search bar to isolate and aggregate all the nested .fastq.gz (*dot-fast-queue-dot-gee-zee*) compressed data entries scattered across the download folder [3]. Select and copy the data files into the new folder to consolidate them for direct batch analysis execution in CRISPResso2 (*Crispresso-two*) [4].
- 4.9.1. SCREEN: 71449_4.8.2.mp4. 00:17–00:19; 00:23–00:30
- 4.9.2. SCREEN: 71449_4.8.2.mp4. 01:32–01:37
- 4.9.3. SCREEN: 71449_4.8.2.mp4. 01:43–01:51
- 4.9.4. SCREEN: 71449_4.8.2.mp4. 02:00–02:03; 02:07–02:09; 02:14–02:18
- 4.10. Install CRISPResso2 according to the instructions provided in the official GitHub repository [1]. Verify the FASTQ paths and window parameters in the Excel batch file [2] and run the CRISPResso2 batch analysis using the specific commands [3]. Monitor

the live interface logs until the run displays a successful completion confirmation message [4].

4.10.1. SCREEN: 71449_4.9.1.mp4. 00:02–00:04; 00:22–00:25

4.10.2. SCREEN: 71449_4.9.2.mp4. 00:05–00:08; 00:11–00:15

4.10.3. SCREEN: 71449_4.9.2.mp4. 00:50–00:57

4.10.4. SCREEN: 71449_4.9.2.mp4. 01:38–01:42

4.11. To quickly overview the reports, open the HTML (*H-T-M-L*) output report [1]. Confirm successful sample processing [2].

4.11.1. SCREEN: 71449_4.10.1.mp4. 00:08–00:12; 00:20–00:22

4.11.2. SCREEN: 71449_4.10.2.mp4. 00:07–00:12

4.12. Identify the target edit site position in the HTML report [1], open the raw data file in Excel [2]. After applying filtering parameters to selectively visualize the target nucleotide rows [3], calculate the final conversion frequency [4].

4.12.1. SCREEN: 71449_4.11.1.mp4. 00:03–00:05; 00:39–00:41

4.12.2. SCREEN: 71449_4.11.1.mp4. 00:15–00:22

4.12.3. SCREEN: 71449_4.11.1.mp4. 00:53–00:59

4.12.4. SCREEN: 71449_4.11.1.mp4. 02:30–02:37

4.13. After opening the raw file in Excel [1], extract indel proportions and percentages of the negative control and the edited samples [2] and quantify the allelic editing outcomes using the allele frequency table [3].

4.13.1. SCREEN: 71449_4.11.2.mp4. 00:17–00:22

4.13.2. SCREEN: 71449_4.11.2.mp4. 01:39–01:44; 02:40–02:45

4.13.3. SCREEN: 71449_4.11.3.mp4. 00:17–00:22

Results

5. Results

- 5.1. Representative agarose gel electrophoresis of IVT-generated base editor [1] and prime editor mRNAs shows sharp, single bands with minimal degradation, confirming high integrity for downstream electroporation [2].
 - 5.1.1. LAB MEDIA: Figure 2_updated. *Video Editor: Please emphasize the single mRNA bands in the two lanes labelled "ABE8e" and "CBE6b"*
 - 5.1.2. LAB MEDIA: Figure 2_updated. *Video Editor: Please emphasize the mRNA band in lane labelled "PE2max"*
- 5.2. Representative editing efficiencies in induced pluripotent stem cells, or iPSCs (*I-P-S-sees*), showed significant variation ranging from minimal to highly successful by buffer [1] and program combination, highlighting the necessity of empirical optimization [2].
 - 5.2.1. LAB MEDIA: Figure 3. *Video Editor: Please emphasize the first 3 bars and the 5th bar along with their corresponding X-axis labels.*
 - 5.2.2. LAB MEDIA: Figure 3. *Video Editor: Please emphasize the 4th bar along with its corresponding X-axis label "NEON(1600V,20ms, 1pulse)"*
- 5.3. Editing optimization of seven pulse programs [1] across buffers P2 and P3 in primary fibroblasts revealed variable efficiencies [2], peaking sharply with the Buffer P2 and DS-150 combination [3].
 - 5.3.1. LAB MEDIA: Figure 4. *Video Editor: Please highlight the X-axis labels "CA-137, CM-138, DS-150, EH-100, EN-150, EO-114, FF-113"*
 - 5.3.2. LAB MEDIA: Figure 4. *Video Editor: Please emphasize all the blue bars, followed by all orange bars.*
 - 5.3.3. LAB MEDIA: Figure 4. *Video Editor: Please emphasize the height of the blue bar with the X-axis label DS-150.*

Pronunciation Guide:

1. Electroporation

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

IPA: /ɪˌlek.troʊ.pəˈreɪ.ʃən/

Phonetic Spelling: ih-lek-troh-puh-ray-shuhn

2. Induced

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

IPA: /ɪnˈduːst/

Phonetic Spelling: in-doost

3. Pluripotent
Pronunciation link: <https://www.merriam-webster.com/dictionary/pluripotent>
IPA: /ˌplʊr.əˈpʊʊ.tənt/
Phonetic Spelling: ploor·uh·poh·tuhnt
4. Messenger RNA (mRNA)
Pronunciation link: <https://www.merriam-webster.com/dictionary/messenger%20RNA>
IPA: /ˈmes.ən.dʒər ˌɑːr.enˈeɪ/
Phonetic Spelling: mess·en·jer ar·en·ay
5. In vitro
Pronunciation link: <https://www.merriam-webster.com/dictionary/in%20vitro>
IPA: /ɪn ˈviː.troʊ/
Phonetic Spelling: in vee·troh
6. Transcription
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcription>
IPA: /trænˈskrɪp.ʃən/
Phonetic Spelling: tran·skrip·shuhn
7. Genome
Pronunciation link: <https://www.merriam-webster.com/dictionary/genome>
IPA: /ˈdʒiː.noʊm/
Phonetic Spelling: jee·nohm
8. Double-stranded
Pronunciation link: <https://www.merriam-webster.com/dictionary/double-stranded>
IPA: /ˌdʌb.əl ˈstræn.dɪd/
Phonetic Spelling: dub·uhl stran·did
9. Nucleotide
Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleotide>
IPA: /ˈnuː.kli.ə.taɪd/, /ˈnjuː.kli.ə.taɪd/
Phonetic Spelling: noo·klee·uh·tyde, nyoo·klee·uh·tyde
10. PolyA
Pronunciation link: No confirmed link found
IPA: /ˌpɑː.liˈeɪ/
Phonetic Spelling: pol·ee·ay
11. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /ˈæl.ɪ.kwɑːt/
Phonetic Spelling: al·ih·kwaht
12. Benchling
Pronunciation link: <https://www.howtopronounce.com/benchling>
IPA: /ˈbentʃ.lɪŋ/
Phonetic Spelling: bench·ling
13. Guide RNA (gRNA)
Pronunciation link: No confirmed link found

IPA: /gaɪd ˌɑːr.en'et/

Phonetic Spelling: guide ar·en·ay

14. KOLF2.1J

Pronunciation link: No confirmed link found

IPA: /koʊ el ef tuː pɔɪnt wʌn dʒet/

Phonetic Spelling: koh·el·ef two point one jay

15. Lyophilized

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

IPA: /laɪ'ɑː.fəˌlaɪzd/

Phonetic Spelling: lye·off·uh·lyzed

16. RNase

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

IPA: /'ɑːr.en.ets/

Phonetic Spelling: ar·en·ace

17. Dulbecco's

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

IPA: /dʊl'bek.ʊ/

Phonetic Spelling: dul·bek·oh

18. Phosphate-buffered saline (DPBS)

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

IPA: /'fɑːs.feɪt 'bʌf.əd sə'liːn/

Phonetic Spelling: foss·fayt buf·erd suh·leen

19. Cuvette

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

IPA: /kjuː'vet/

Phonetic Spelling: kyoo·vet

20. Nucleofection

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

IPA: /ˌnuː.kli.ʊs'fek.jən/

Phonetic Spelling: noo·klee·oh·fek·shuhn

21. Trypsinize

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

IPA: /'trɪp.sɪ.naɪz/

Phonetic Spelling: trip·sih·nyze

22. Genomic

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

IPA: /dʒə'noʊ.mɪk/

Phonetic Spelling: juh·noh·mik

23. Lysate

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

IPA: /'laɪ.set/

Phonetic Spelling: lye·sayt

24. Proteinase
Pronunciation link: <https://www.merriam-webster.com/dictionary/proteinase>
IPA: /proʊˈtiː.nɪs/
Phonetic Spelling: proh-tee-nace
25. Tris-HCl
Pronunciation link: No confirmed link found
IPA: /ˈtrɪs ɛrtʃ siː el/
Phonetic Spelling: tris aych see el
26. Nuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
IPA: /ˈnuː.kli.ɛs/, /ˈnjuː.kli.ɛs/
Phonetic Spelling: noo-kee-ace, nyoo-kee-ace
27. Amplicon
Pronunciation link: <https://www.howtopronounce.com/amplicon>
IPA: /ˈæm.plɪ.kən/
Phonetic Spelling: am-plih-kon
28. SnapGene
Pronunciation link: <https://www.howtopronounce.com/snapgene>
IPA: /ˈsnæp.dʒiːn/
Phonetic Spelling: snap-jeen
29. Illumina
Pronunciation link: <https://www.howtopronounce.com/illumina>
IPA: /ɪˈluː.mɪ.nə/
Phonetic Spelling: ih-loo-mih-nuh
30. Thermocycling
Pronunciation link: <https://www.howtopronounce.com/thermocycling>
IPA: /ˌθɜː.moʊˈsaɪ.klɪŋ/
Phonetic Spelling: thur-moh-sy-king
31. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /ˈæg.ə.roʊs/
Phonetic Spelling: ag-uh-rohs
32. Equimolar
Pronunciation link: <https://www.howtopronounce.com/equimolar>
IPA: /iː.kwɪˈmoʊ.lə/
Phonetic Spelling: ee-kwih-moh-ler
33. Fluorometric
Pronunciation link: <https://www.howtopronounce.com/fluorometric>
IPA: /ˌflɔr.oʊˈmet.rɪk/
Phonetic Spelling: floor-oh-met-rik
34. Capillary electrophoresis
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrophoresis>

IPA: /'kæp.ə.lər.i.ɪ.lek.troʊ.fə'ri:.sɪs/

Phonetic Spelling: cap·uh·lair·ee ih·lek·troh·fuh·ree·sis

35. MiSeq

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

IPA: /'maɪ.sɪ:k/

Phonetic Spelling: my·seek

36. BaseSpace

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

IPA: /'beɪsˌspeɪs/

Phonetic Spelling: base·space

37. Indexing QC

Pronunciation link: No confirmed link found

IPA: /'ɪn.dek.sɪŋˌkjuː'si:/

Phonetic Spelling: in·dek·sing cue·see

38. FASTQ

Pronunciation link: No confirmed link found

IPA: /fæstˈkju:/

Phonetic Spelling: fast·cue

39. CRISPResso2

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

IPA: /kriˈspres.əʊ tu:/

Phonetic Spelling: krih·spress·oh two

40. Demultiplexing

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

IPA: /ˌdiːˌmʌl.tɪˈplek.sɪŋ/

Phonetic Spelling: dee·mul·tih·plek·sing

41. Allelic

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

IPA: /əˈliː.lɪk/

Phonetic Spelling: uh·lee·lik

42. Indel

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

IPA: /'ɪn.del/

Phonetic Spelling: in·del