

Submission ID #: 70278

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21434838>

Title: Cell-of-origin Discovery in Infant Leukemia through Integration of 3D Models and Patient Transcriptomic Data

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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. **Yes**

Current Protocol Length

Number of Steps: 19

Number of Shots: 44 (29 SC)

Interviews

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

Videographer's Note: The LED light broke during the filming of the Introduction & Conclusion. The lighting in these shots is not consistent because of this.

INTRODUCTION:

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

- 1.1. **Denise Ragusa:** Our research uses a gastruloid embryonic blood formation model for tracking the *in utero* cell of origin in pediatric leukemias.

- 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. **Denise Ragusa:** Unlike existing methods, this protocol captures the temporal and spatial complexity of embryonic hematopoiesis, tracking leukemia-mutation targets within developmental niches.

- 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. **Ayona Johns:** This protocol aims at exploring transcriptional profiles and phenotypes of leukemia-associated abnormalities within an embryonic context.

- 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. **Ayona Johns:** It is crucial to validate the chosen leukemia-associated abnormality in the initial cell culture and gastruloid conditions.

- 1.4.1. 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?~~

- 1.5. **Ayona Johns:** This protocol is compatible with high-throughput methods and standard downstream molecular, functional, and biochemical assays.

- 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. **Denise Ragusa, Postdoctoral Research Fellow, Brunel University of London**: (authors will present their testimonial statements live)

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

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

- 1.7. **Denise Ragusa, Postdoctoral Research Fellow, Brunel University of London**: (authors will present their testimonial statements live)

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

Protocol

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

Videographer's Note: The light in the lab hood was running at a different hertz to my camera. When I adjusted the shutter speed on my camera to try and compensate this, it was introducing too much motion blur when the Author moved. I had to go back to my normal shutter speed and shoot the video with the light strobing.

2. Colony Re-plating of *MNX1*-Engineered Mouse Hemogenic Gastruloids (haemGx)

Demonstrator: Ayona Johns

- 2.1. To begin, add 1.5 milliliters of prewarmed phosphate-buffered saline, or PBS (*P-B-S*) to the wells of a 6-well plate containing hemogenic gastruloid colonies in methylcellulose medium [1]. Use a P1000 (*P-one thousand*) pipette set to 500 microliters to gently resuspend the methylcellulose by slowly pipetting up and down, taking care to avoid foaming [2].

- 2.1.1. WIDE: Talent pipetting 1.5 milliliters of PBS into an individual well. ***Videographer:** Please obtain CU shot as well for this shot.*

Videographer's Note: Shot 2.1.1 has two variations (A & B). Shot 2.1.1A is a Wide shot, and Shot 2.1.1B is a CU shot.

- 2.1.2. Talent slowly pipetting the methylcellulose suspension up and down using a P1000 pipette set to 500 microliters. **Note:** Shot 2.1.2B is a CU shot of Shot 2.1.2.

- 2.2. Once the methylcellulose is fully dispersed and free of visible clumps, transfer the contents of each well to a 15-milliliter conical tube [1]. Rinse the well with an additional 1.5 milliliters of PBS to collect remaining cells [2]. Pipette slowly to resuspend any residual methylcellulose and add the suspension to the same tube [3]. After performing a final 500 microliter rinse with PBS, pool it with the previous suspensions [4].

- 2.2.1. Talent transferring the contents of the well into a 15 mL conical tube.

- 2.2.2. Talent rinsing the same well with 1.5 mL of PBS. **Note:** Shots 2.2.2, 2.2.3 and 2.2.4 were combined.

- 2.2.3. Talent adding the suspension into the same conical tube.

- 2.2.4. Talent pooling the final wash with the previous collected suspensions in the same tube.

- 2.3. Centrifuge the collected suspension at 400 *g* for 5 minutes at room temperature to pellet the cells [1]. Carefully aspirate the supernatant [2]. Wash the pellet with 5 milliliters of PBS to remove any remaining methylcellulose [3].

- 2.3.1. Talent placing the conical tube into the centrifuge. **Videographer's Note:** I had to overexpose the window to get the centrifuge exposed because the room lighting in this area of the room was not bright enough.
- 2.3.2. Talent aspirating the supernatant from the pellet after centrifugation. **Note:** Shots 2.3.2 and 2.3.3 were combined
- 2.3.3. Talent adding phosphate-buffered saline to the pellet and resuspending the cells.
- 2.4. Centrifuge the suspension again at 400 *g* for 5 minutes [1]. Discard the supernatant [2] and resuspend the final cell pellet in 200 to 1000 microliters of N2B27 (*N-two-B-twenty seven*) culture medium based on pellet size [3].
 - 2.4.1. Talent placing the tube into the centrifuge for the second centrifugation. **Videographer's Note:** This shot was filmed with the same lighting issue as with shot 2.3.1.
 - 2.4.2. Talent discarding the supernatant from the tube. **Note:** Shots 2.4.2 and 2.4.3 were combined.
 - 2.4.3. Talent resuspending the final cell pellet in N2B27 culture medium.
- 2.5. Count the viable cells using a hemocytometer [1]. Add the single-cell suspension containing 100,000 cells in 300 microliters to 3 milliliters of thawed, thoroughly mixed methyl cellulose medium, free of air bubbles [2]. After vortexing to achieve uniform cell distribution, use a 5-milliliter serological pipette to dispense the medium into 2 wells of a 6-well plate, followed by endpoint dissociation for downstream characterization [3].
 - 2.5.1. Talent loading the cell suspension onto a hemocytometer and counting viable cells. **Note:** Shot 2.5.1 was split into two shots and is shown on the clapperboard as Shot 2.5.1 and Shot 2.5.1B.
 - 2.5.2. Talent adding the cell suspension to 3 mL of pre-thawed methyl cellulose medium. **Note:** Shot 2.5.2 was split into two shots. Shot 2.5.2A and Shot 2.5.2B.
 - 2.5.3. Talent using a 5 mL serological pipette to plate the cells into the wells of 6-well plate. **Note:** Use shot 2.5.3 take 2, instead of take 1.

3. *In Silico* Temporal Mapping of Candidate Cell of Origin (CoO) via HaemGx and Patient RNA-sequencing

Demonstrator: Denise Ragusa

- 3.1. In the **UCSC Xena** (*U-C-S-C- Xena*) **Genomics Explorer** page, click on the **Datasets** tab and scroll down to locate the **GDC TARGET-AML** (*G-D-C-Target-A-M-L*) dataset [1]. Click on the link, then scroll down to the **Gene expression RNAseq** (*R-N-A-Seek*) section and click on the **STAR - TPM** (*Star-T-P-M*) dataset link to download the clinical data and ribonucleic acid, or RNA (*R-N-A*) sequencing data in transcripts per million, or TPM (*T-*

P-M) compatible with gene set enrichment analysis, or GSEA (*G-S-E-A*) [2]. Return to the main GDC TARGET-AML cohort page and click on **Phenotype** under the **Phenotype** section to obtain the link to download the phenotype data [3]. Click on the **All-Identifiers** link to view the complete list of clinical identifiers associated with the dataset and select patients for the analysis based on phenotype and clinical annotations [4].

3.1.1. SCREEN: 311_312_313.mp4. 00:11– 00:22

3.1.2. SCREEN: 311_312_313.mp4. 00:23– 00:43

Added shot: SCREEN: 311_312_313.mp4. 00:45– 00:56

3.1.3. SCREEN: 311_312_313.mp4. 01:00– 01:10

3.2. Click on the Libraries tab in the EnrichR (*enrich-R*) database and scroll down the multiple available geneset libraries [1]. Identify and download the PanglaoAugmented 2021 (*pahng-LAOW- augmented-twenty twenty one*) for cell type gene sets from PanglaoDB (*pahng-LAOW-D-B*) [2].

3.2.1. SCREEN: 321_322.mp4. 00:05–00:17

3.2.2. SCREEN: 321_322.mp4. 00:19–00:27

3.3. In the GSEA webpage, click on **Download** to obtain the link for GSEA software download and installation [1]. Prepare the files required for GSEA using a custom gene set as per the template populated in spreadsheets and saved as .txt (*dot-T-X-T*) files [2].

3.3.1. SCREEN: 331.mp4. 00:08–00:16; 00:47–00:55; 00:58–01:06

3.3.2. SCREEN: 332.mp4. 00:03–00:12

3.4. Change the file extensions of the .txt files containing the samples, genes, and expression values to. gct (*dot-G-C-T*) and click **Yes** in the **Rename** pop-up window [1]. Change the file extension of the phenotype classes file matching the .gct file from .txt to .cls (*dot-C-L-S*) phenotype label file [2]. Prepare the. gmt gene set file containing gene set names and corresponding genes from the .txt file [3].

3.4.1. SCREEN: 341.mp4. 00:07–00:15; 00:28 –00:37

3.4.2. SCREEN: 342.mp4. 00:08–00:10; 00:20–00:24; 00:37–00:45

3.4.3. SCREEN: 343.mp4. 00:10–00:14; 00:20–00:31

3.5. Use Ensembl identifiers to minimize issues caused by duplicated gene symbols [1].

3.5.1. SCREEN: 351.mp4. 00:03– 00:07. *Video Editor: Please emphasize the codes starting with “ENSG” under the column “Name”.*

3.6. In the GSEA software interface, click on **Load data**, then choose **Browse for files** [1]. Open the prepared .gmt, .cls and .gmt files and click OK in the pop-up window [2].

- 3.6.1. SCREEN: 361.mp4. 00:05–00:13
- 3.6.2. SCREEN: 361.mp4. 00:20–00:31
- 3.7. Next, select **Run GSEA** and populate the required fields to compare two phenotype classes, including the **Expression dataset** for the file containing counts for the desired samples [1]. Select the **Gene sets database** to open the local .gmt file, such as **PanglaoAugmented 2021**, to compute the gene sets [2].
- 3.7.1. SCREEN: 371_372_373.mp4. 00:05– 00:17
- 3.7.2. SCREEN: 371_372_373.mp4. 00:19– 00:31
- 3.8. Set the number of permutations to 1000 for increased statistical resolution [1]. Input the .cls phenotype label file for the comparison of categories in the phenotype labels field [2].
- 3.8.1. SCREEN: 381_382_391.mp4. 00:03–00:08
- 3.8.2. SCREEN: 381_382_391.mp4. 00:09–00:17
- 3.9. In the **Collapse/Remap to gene symbols** field, select the appropriate option based on the format of the .gct file [1].
- 3.9.1. SCREEN: 381_382_391.mp4. 00:21–00:28
- 3.10. Select the **permutation type** as gene set for RNA sequencing data with limited replicates to minimize phenotypic variability [1]. In the **Chip platform** field, click the selection area, and in the **Select a chip** pop-up window, click the **Local Chips** tab to view the locally stored chip datasets [2]. Scroll down to select the specific chip identifier and click OK [3].
- 3.10.1. SCREEN: 3101_3102.mp4. 00:03–00:09
- 3.10.2. SCREEN: 3103.mp4. 00:02–00:13
- 3.10.3. SCREEN: 3103.mp4. 00:14–00:24
- 3.11. Click **Show** to view the **Basic fields** and under the **Metric for Ranking genes** field, select **Ratio_of_classes** (*Ratio of classes*) to view absolute fold change between classes for RNA sequencing data [1].
- 3.11.1. SCREEN: 3111_3112.mp4. 00:03–00:06; 00:16–00:19; 00:24–00:30
- 3.12. Select **Run** to start the analysis [1]. Monitor the analysis progress in the status column under the GSEA reports box [2]. After analysis completion, verify by clicking the Success hyperlink in the status field, then clicking Show results folder to check the saved results in the locally specified output folder [3].
- 3.12.1. SCREEN: 3121_3122.mp4. 00:08–00:11
- 3.12.2. SCREEN: 3121_3122.mp4. 00:12–00:20
- 3.12.3. SCREEN: 3123.mp4. 00:04–00:10; 00:16–00:23

3.13. Double-click the output folder and scroll down through the newly generated dataset files, figures, and spreadsheets, then locate the main index.html (*index-dot-H-T-M-L*) file [1]. Double-click the file to open the comprehensive report summary in the web browser [2]. Click on **Snapshot of enrichment results** to view the multiple enrichment curves generated by the analysis [3]. Next, click the **enrichment results in html format** to open a detailed table of gene sets enrichment performance [4].

3.13.1. SCREEN: 3131.mp4. 00:28–00:38

3.13.2. SCREEN: 3131.mp4. 00:39–00:46

3.13.3. SCREEN: 3132_3133.mp4. 00:04– 00:12

3.13.4. SCREEN: 3132_3133.mp4. 00:15– 00:24

Results

4. Results

- 4.1. MNX1 (*M-N-X-one*)-overexpressing hemogenic gastruloid cells yield distinct, dense, and scorable hematopoietic colonies [1], easily distinguishable from non-colony cell aggregations [2]. MNX1-overexpressing hemogenic gastruloids show persistent colony-forming ability across multiple serial passages [3] and enhanced self-renewal potential across both early or 144 hours, and late or 216 hours disassembly timepoints [4].
 - 4.1.1. LAB MEDIA: Figure 3F. *Video Editor: Please emphasize the cell colonies pointed by the full arrows.*
 - 4.1.2. LAB MEDIA: Figure 3F. *Video Editor: Please emphasize the cells pointed by the dashed arrows.*
 - 4.1.3. LAB MEDIA: Figure 3G. *Video Editor: Please emphasize the blue bars and the corresponding X-axis labels PL1, PL2, PL3, PL4, and PL5 sequentially in both graphs.*
 - 4.1.4. LAB MEDIA: Figure 3G. *Video Editor: Please emphasize the blue bars and the labels "Serial plating 144 h" and "Serial plating 216 h" sequentially in both graphs.*
- 4.2. GSEA analysis of infant AML with the t-seven-twelve mutation patient transcriptomes versus other AML revealed the enrichment of gene sets corresponding to hemato-endothelial signatures like cytotoxic T cells [1], plasma cells [2], natural killer T cells and Nk (*N-K*) cells [3], and aortic and other endothelial cells [4].
 - 4.2.1. LAB MEDIA: Figure 4C: *Video Editor: Please highlight the top box with title "Lymph T" and emphasize the dark red dot to the right corresponding to label T cytotoxic cells."*
 - 4.2.2. LAB MEDIA: Figure 4C: *Video Editor: Please highlight the top box with title "Lymph B" and emphasize the dark red dot to the right corresponding to label "Plasma cells."*
 - 4.2.3. LAB MEDIA: Figure 4C: *Video Editor: Please highlight the top box with title "Lymph" and emphasize the two dark red dots corresponding to labels "Natural Killer T cells and NK cells."*
 - 4.2.4. LAB MEDIA: Figure 4C: *Video Editor: Please highlight the top box with title "Endo" and emphasize the two dark red dots corresponding to labels "Endothelial cells (Aorta) and Endothelial cells."*

- 4.3. Temporal mapping of bulk RNA-sequencing data from MNX1-overexpressing hemogenic gastruloids showed enrichment for hemato-endothelial clusters 5 and 0 and hematopoietic cluster 4 at 144-hour and 192-hour developmental windows [1]. The 144-hour and 192-hour hemato-endothelial populations matched the enrichment observed in the transcriptome of human T-seven-twelve infant leukemia patients [2]. The UMAP (*you-map*) of these enrichments showed statistically significant GSEA results superimposed to the corresponding cluster number and the enriched clusters in MNX1-overexpressing hemogenic gastruloids and human T-seven-twelve infant AML [3].
- 4.3.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the bubbles corresponding to clusters 5, 0 and 4 in the top comparison panel along with the legends for red and purple squares in the “Identity” column shown below the bubble plot.*
- 4.3.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the bubbles aligned to the 144-hour and 192-hour hemato-endothelial clusters in the middle comparison panel*

Pronunciation Guide:

1. MNX1
Pronunciation link: No confirmed link found
IPA: /ɛm ɛn ɛks wʌn/
Phonetic Spelling: em·en·ex·one
2. Hemogenic
Pronunciation link: <https://www.merriam-webster.com/medical/hemogenic>
IPA: /ˌhi:məˈdʒenɪk/
Phonetic Spelling: hee·muh·jen·ik
3. Gastruloids
Pronunciation link: No confirmed link found
IPA: /ˈgæs.trə.lɔɪdz/
Phonetic Spelling: gas·truh·loydz
4. Phosphate-buffered
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /ˈfɑːs.fert/
Phonetic Spelling: faas·fayt
5. Methylcellulose
Pronunciation link: <https://www.merriam-webster.com/dictionary/methylcellulose>
IPA: /ˌmeθ.əlˈsel.jəˌloʊs/
Phonetic Spelling: meth·uhl·sel·yuh·lohs
6. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>

IPA: /'maɪ.kroʊ.liː.tə/

Phonetic Spelling: my·kroh·lee·ter

7. Resuspend

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

IPA: /ˌriː.sə'spend/

Phonetic Spelling: ree·suh·spend

8. Conical

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

IPA: /'kɑː.nɪ.kəl/

Phonetic Spelling: kaa·nih·kuhl

9. Centrifuge

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

IPA: /'sen.trəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

10. Supernatant

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

IPA: /suː.pə'nei.tənt/

Phonetic Spelling: soo·per·nay·tuht

11. Pellet

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

IPA: /'pel.ət/

Phonetic Spelling: pel·uht

12. N2B27

Pronunciation link: No confirmed link found

IPA: /ɛn tuː biː 'twɛn.ti 'sev.ən/

Phonetic Spelling: en·two·bee·twen·tee·sev·uhn

13. Hemocytometer

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

IPA: /hiː.moʊ.sai'tɑː.mə.tə/

Phonetic Spelling: hee·moh·sy·taa·muh·ter

14. Serological

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

IPA: /ˌser.ə'lɑː.dʒɪ.kəl/

Phonetic Spelling: ser·uh·laa·jih·kuhl

15. Dissociation

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

IPA: /dɪˌsoʊ.si'eɪ.ʃən/

Phonetic Spelling: dih·soh·see·ay·shuhn

16. Characterization

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

IPA: /ˌker.ək.tə.ə'zeɪ.ʃən/

Phonetic Spelling: ker·uhk·ter·uh·zay·shuhn

17. In Silico
Pronunciation link: <https://www.merriam-webster.com/medical/in%20silico>
IPA: /ɪn ˈsɪl.ɪ.koʊ/
Phonetic Spelling: in·sil·ih·koh
18. Temporal
Pronunciation link: <https://www.merriam-webster.com/dictionary/temporal>
IPA: /ˈtem.pə.əl/
Phonetic Spelling: tem·per·uhl
19. Genomics
Pronunciation link: <https://www.merriam-webster.com/dictionary/genomics>
IPA: /dʒəˈnoʊ.mɪks/
Phonetic Spelling: juh·noh·miks
20. RNAseq
Pronunciation link: No confirmed link found
IPA: /ɑːr ɛn eɪ siːk/
Phonetic Spelling: ar·en·ay·seek
21. Ribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic%20acid>
IPA: /ˌraɪ.boʊ.nuːˈkliː.ɪk/
Phonetic Spelling: rye·boh·noo·klee·ik
22. Transcripts
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcript>
IPA: /ˈtræn.skɹɪpts/
Phonetic Spelling: tran·skɹɪpts
23. Enrichment
Pronunciation link: <https://www.merriam-webster.com/dictionary/enrichment>
IPA: /ɪnˈrɪtʃ.mənt/
Phonetic Spelling: in·rich·muhnt
24. GSEA
Pronunciation link: No confirmed link found
IPA: /dʒiː ɛs iː eɪ/
Phonetic Spelling: gee·ess·ee·ay
25. EnrichR
Pronunciation link: No confirmed link found
IPA: /ɛnˈrɪtʃ ɑːr/
Phonetic Spelling: en·rich·ar
26. PanglaoAugmented
Pronunciation link: No confirmed link found
IPA: /pæŋˈlɑʊ ɔːɡˈmen.tɪd/
Phonetic Spelling: pang·laow·awg·men·tid
27. PanglaoDB
Pronunciation link: No confirmed link found

IPA: /pæŋˈlɑʊ diː biː/

Phonetic Spelling: pang·laow·dee·bee

28. Ensembl

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

IPA: /ɛnˈsem.bəl/

Phonetic Spelling: en·sem·buhl

29. Identifiers

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

IPA: /aɪˈden.təˌfaɪ.ə/

Phonetic Spelling: eye·den·tuh·fye·er

30. Phenotype

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

IPA: /ˈfiː.nəˌtaɪp/

Phonetic Spelling: fee·nuh·type

31. Permutations

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

IPA: /ˌpɜː.mjəˈteɪ.jən/

Phonetic Spelling: per·myuh·tay·shuhn

32. Variability

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

IPA: /ˌvɛr.i.əˈbɪl.ə.ti/

Phonetic Spelling: vair·ee·uh·bil·uh·tee

33. Phenotypic

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

IPA: /ˌfiː.nəˈtɪp.ɪk/

Phonetic Spelling: fee·nuh·tip·ik

34. Remap

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

IPA: /ˌriːˈmæp/

Phonetic Spelling: ree·map

35. Hyperlink

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

IPA: /ˈhaɪ.pəˌlɪŋk/

Phonetic Spelling: hy·per·lingk

36. HTML

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

IPA: /ˌɛtʃ tiː ɛm ˈel/

Phonetic Spelling: aych·tee·em·el