

Submission ID #: 70886

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21620278>

Title: Visualization and Quantification of Intermolecular RNA Base Pairing in In Vitro RNA Clusters using Split Broccoli RNA Reporters

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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? **Yes**

Next to each other.

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: 33

Number of Shots: 58 (5 SCs)

Introduction

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

INTRODUCTION:

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

- 1.1. **Tatjana Trcek:** We apply the split Broccoli RNA reporter assay to detect and quantify intermolecular base pairing in RNA clusters in vitro.
 - 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. **Tatjana Trcek:** Chemical probing and simulations suggest RNA interactions but cannot directly measure them; this protocol enables direct, accurate assessment within RNA clusters.
 - 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. **Tatjana Trcek:** The most important consideration is that robust signal generation may require crowding reagents, while sensitivity depends on efficient dimerization and folding of split Broccoli RNAs
 - 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. **Tatjana Trcek:** This assay complements RNA pull-down and gel electrophoresis approaches for studying intermolecular base pairing in RNA clusters.
 - 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. **Tatjana Trcek:** This assay can examine the effects of RNA sequences, helicases, and ions on intermolecular base pairing within RNA clusters.

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. Preparation of In Vitro RNA Transcription Reaction

Demonstrator: Siran Tian

- 2.1. After preparing the DNA templates for in vitro RNA transcription, wipe the working surface, tube racks, and pipettes with ribonuclease decontamination solution [1]. Use ribonuclease-free, filtered tips for all pipetting steps
 - 2.1.1. WIDE: Talent wiping the laboratory working surface, tube racks, and pipettes with ribonuclease decontamination solution.
 - 2.1.2. Talent places a box of filtered tips on the working area.
- 2.2. Thaw the reaction buffer and nucleotide solutions supplied with the transcription kit, along with uridine triphosphate-Cyanine 5 or UTP-Cy5 (*U-T-P-Sigh-Five*) on ice [1]. Centrifuge all tubes at 2000 g for 2 seconds before use [2].
 - 2.2.1. Talent placing reagent tubes on ice to thaw.
 - 2.2.2. Talent placing tubes into a centrifuge.
- 2.3. Then, calculate the number of thymine bases in the DNA [1]. Determine the required volumes of UTP and UTP-Cy5 for a 20-microliter transcription reaction while maintaining consistent labelling density across RNA species [2].
 - 2.3.1. Talent analyzing the DNA sequence for thymine base count on the computer screen.
 - 2.3.2. Talent calculating and noting reagent volumes.
- 2.4. Next, prepare a 20-microliter transcription reaction by combining the presented reagents [1]. Mix thoroughly by pipetting up and down [2] and centrifuge at 2000 g for 2 seconds [3].
 - 2.4.1. TEXT ON A PLAIN BACKGROUND
ATP, CTP, and GTP: 2 μ L (each)
UTP and UTP-Cy5: As per the calculations
10 $\times$ reaction buffer: 2 μ L enzyme mix,
Enzyme mix: 2 μ L

200 ng DNA template

Nuclease-free water

2.4.2. Talent pipetting the mixture up and down.

2.4.3. Talent placing the tube into a centrifuge.

2.5. After incubating the reaction at 37 degrees Celsius for 6 hours, add 1 microliter of deoxyribonuclease supplied with the kit, mix thoroughly by pipetting, and centrifuge again [1].

2.5.1. Talent adding deoxyribonuclease into the reaction tube and pipetting it to mix the contents.

2.6. Then, incubate at 37 degrees Celsius for an additional 15 minutes [1].

2.6.1. Talent placing the reaction tube into an incubator.

2.7. Transfer the reaction to a 1.5-millilitre tube [1]. Add 115 microliters of nuclease-free water and 15 microliters of ammonium acetate stop solution supplied with the kit [2]. After mixing, centrifuge the solution at 2000 g for 2 seconds [3].

2.7.1. Talent transferring the reaction mixture into a 1.5 milliliter tube.

2.7.2. Talent adding nuclease-free water and ammonium acetate stop solution into the tube.

2.7.3. Talent placing the tube into a centrifuge.

2.8. Next, add 150 microliters of phenol-chloroform to the tube and mix gently to combine the phases [2].

2.8.1. Talent adding phenol chloroform to the tube and gently mixing the tube.

2.9. Centrifuge at 16000 g for 10 minutes at 4 degrees Celsius [1]. Then, transfer the upper aqueous phase to a new tube [2].

2.9.1. Talent placing the tube into a centrifuge.

2.9.2. Talent carefully transferring the upper aqueous layer into a new tube.

2.10. Add an equal volume of chloroform to the transferred solution and mix thoroughly to ensure proper phase interaction [1]. Now, centrifuge at 16000 g for 2 minutes at 4 degrees Celsius and repeat the phase separation process once again [2].

2.10.1. Talent adding chloroform to the tube and mixing the solution thoroughly.

2.10.2. Talent placing the tube into a centrifuge.

2.11. Then, transfer the aqueous layer of approximately 100 to 150 microliters to a new tube [1], add 900 microliters of isopropanol, and mix thoroughly [2].

2.11.1. Talent transferring the aqueous layer into a new tube.

2.11.2. Talent adding isopropanol and mixing thoroughly.

2.12. After aliquoting the solution in separate tubes, store the samples at minus 20 degrees Celsius overnight or up to one month [1].

2.12.1. Talent placing the prepared aliquots into a freezer.

3. Purification of In Vitro Transcribed RNA

3.1. Centrifuge the RNA sample in isopropanol at 16000 *g* for 15 minutes at 4 degrees Celsius [1]. Carefully remove the isopropanol without disturbing the RNA pellet [2].

3.1.1. Talent placing the tube containing the ribonucleic acid sample into a centrifuge.

3.1.2. Talent carefully aspirating and removing isopropanol while leaving the pellet undisturbed.

3.2. Then, add 1 millilitre of 70 percent ethanol prepared in nuclease-free water to the pellet [1]. Centrifuge at 16000 *g* for 2 minutes at 4 degrees Celsius [1].

3.2.1. Talent adding 70 percent ethanol into the tube containing the pellet.

3.2.2. Talent placing the tube into a centrifuge.

3.3. After removing the ethanol, add 1 milliliter of 70 percent ethanol prepared in nuclease-free water to the tube and repeat centrifugation [1].

3.3.1. Talent adding 70 percent ethanol into the tube.

3.4. During the final ethanol wash, remove most of the ethanol using a 1000 microliter pipette [1]. Briefly centrifuge at 2000 *g* for 2 seconds in a benchtop minicentrifuge [2] and remove any remaining ethanol using a 20-microliter pipette [3]. NOTE: The VO is edited for the additional shot

3.4.1. Talent removing most of the ethanol using a 1000 microliter pipette.

3.4.2. Talent placing the tube into a benchtop minicentrifuge(This requires two shots to complete, see below)

Added shot: Talent removing the residual ethanol with a 20 microliter pipette.

- 3.5. Once the RNA pellet is dried, resuspend it in 20 microliters of nuclease-free water [1]. Keep the sample on ice and protect it from light [2].
 - 3.5.1. Talent adding nuclease-free water and resuspending the pellet by pipetting.
 - 3.5.2. Talent placing the tube on ice away from light.
- 3.6. Using a microvolume spectrophotometer, measure RNA concentration and purity [1]. Ensure that the absorbance ratio at 260 over 280 nanometers is approximately 2.0 and the ratio at 260 over 230 nanometers is greater than 2.0 [2].
 - 3.6.1. Talent loading the sample into a microvolume spectrophotometer for measurement.
 - 3.6.2. Display the spectrophotometer readout showing A260/A280 and A260/A230 values.

4. Preparation of In Vitro RNA Clustering Reaction

- 4.1. Thaw a tube of 100 millimolar spermine solution and 50 percent polyethylene glycol 8000 on ice [1].
 - 4.1.1. Talent placing tubes of spermine solution and polyethylene glycol 8000 on ice to thaw.
- 4.2. Prepare 500 microliters of freshly made 10 times RNA refolding buffer [1]. After mixing the components thoroughly by pipetting, centrifuge at 2000 *g* for 2 seconds in a benchtop minicentrifuge [2].
 - 4.2.1. Talent pipetting potassium chloride, magnesium chloride into a tube.
AND
TEXT ON A PLAIN BACKGROUND
2M KCl: 50 µL ,
1M MgCl₂: 50 µL
1M Tris (pH 7.0): 50 µL
Nuclease-free water: 350 µL *Video editor: Use split screen to show both the shots side by side*
 - 4.2.2. Talent placing the tube into a benchtop minicentrifuge.
- 4.3. For the co-folding condition, in a 200 microliter PCR tube, combine in vitro transcribed RNA carrying either top or bottom sequence, RNA refolding buffer, and nuclease-free water [1]. Mix thoroughly by pipetting up and down and centrifuge as demonstrated earlier [2].

- 4.3.1. Talent pipetting ribonucleic acid samples, refolding buffer in a PCR tube.
- 4.3.2. Talent pipetting the mixture up and down.
- 4.4. Heat the sample at 90 degrees Celsius for 2 minutes [1], immediately transfer the sample to room temperature, protect from light, and incubate for 1 hour [2]. **NOTE: The VO is edited for the additional shot**
Added shot: Talent placing the tube into a heating device.
 - 4.4.1. Talent removing the tube and placing it at room temperature in dark.
- 4.5. For the separate folding condition, heat the RNA sample in a 200 microliter PCR tube [1] and then immediately place it on ice for at least 15 minutes [2].
 - 4.5.1. Talent placing the tube into a heating device.
 - 4.5.2. Talent transferring the tube onto ice.
- 4.6. In a 200-microliter PCR tube, combine in vitro transcribed RNA carrying either top or bottom sequence, 10 times RNA refolding buffer, and nuclease-free water [1].
 - 4.6.1. Talent pipetting ribonucleic acid sample, refolding buffer, and nuclease-free water into a polymerase chain reaction tube.
- 4.7. Incubate the reaction at room temperature for 30 minutes protected from light [1].
 - 4.7.1. Talent placing the tube on the bench covered to protect from light during incubation.
- 4.8. Now, combine the RNA samples containing the top and bottom sequences into a single PCR tube and mix thoroughly by pipetting up and down [1]. Centrifuge at 2000 *g* for 2 seconds in a benchtop minicentrifuge [2]. Incubate at room temperature for 30 minutes [3].
 - 4.8.1. Talent transferring and combining ribonucleic acid samples into a single PCR tube and pipetting it.
 - 4.8.2. Talent placing the tube into a benchtop minicentrifuge.
 - 4.8.3. Talent leaving the tube on the bench for incubation.
- 4.9. For both conditions, add 6 microliters of a 1 to 2 premix of 100 millimolar spermine and 50 percent polyethylene glycol 8000 [1]. After mixing the content, centrifuge at 2000 *g* for 2 seconds in a benchtop minicentrifuge [2].

- 4.9.1. Talent adding the spermine and polyethylene glycol premix into the tube.
- 4.9.2. Talent placing the tube into a benchtop minicentrifuge.
- 4.10. Now, mix 2 microliters of 1 millimolar DFHBI-1T (*D-F-H-B-I-One-T*) to the reaction and centrifuge again [2].
 - 4.10.1. Talent adding DFHBI-1T into the reaction tube.
- 4.11. Load the reaction into a glass chambered coverglass [1]. Incubate the chamber at room temperature for 4 hours in the dark with the cover on to minimize evaporation [2].
 - 4.11.1. Talent pipetting the reaction into a glass chambered coverglass.
 - 4.11.2. Talent placing the covered chamber in a dark location for incubation.
- 4.12. Load the glass chambered coverglass onto the microscope stage [1]. Turn on the laser, then use the eyepieces to locate and focus on the sample [2]. NOTE: This step is edited and the VO is edited for the additional shot
 - 4.12.1. Talent loads the glass chambered coverglass onto the microscope stage.
 - 4.12.2. Added shot: Talent turns on the laser and locates the sample through the eyepiece.
- 4.13. Configure the microscope to image each region of interest with the cyanine 5 channel first at each z-plane [1]. Then, image the same region of interest using the green fluorescent protein channel [2].
 - 4.13.1. SCREEN: 70886_4.13.1-4.13.2.MP4: 00:08-00:16
 - 4.13.2. SCREEN: 70886_4.13.1-4.13.2.MP4: 00:16-00:23
- 4.14. Set the exposure time to 500 milliseconds for all channels [1]. Then, set the laser power to 80% for the green fluorescent protein channel and 40% for the cyanine 5 channel [2].
 - 4.14.1. SCREEN: 70886_4.14.1.MP4: 00:04-00:12
 - 4.14.2. SCREEN: 70886_4.14.2.MP4: 00:06-00:16
- 4.15. Image a coverslip region outside the reaction droplet at room temperature using a z-step size of 150 nanometers [1]. Afterwards, quantify intermolecular base pairing using image analysis software [2]

4.15.1. SCREEN: 70886_4.15.1_t1.MP4: 00:05-00:11, 70886_4.15.1_t2.MP4: 00:03-00:07

4.15.2. SCREEN: 70886_4.15.2.MP4: 00:01-00:03, 01:05-01:09

Results

5. Results

- 5.1. Upon induction of RNA clustering, both RNAs formed clusters either individually or together [1].
 - 5.1.1. LAB MEDIA: Figure 1D–Eii.
- 5.2. DFHBI-1T fluorescence within RNA clusters was substantially lower for top or bottom RNAs [1] alone compared to the co-folding condition [2]. In the separate folding condition, the normalized DFHBI-1T signal was 0.031, comparable to baseline levels observed in top or bottom alone [3].
 - 5.2.1. LAB MEDIA: Figure 1F. *Video editor: Highlight the bar labeled “top” and “bottom”*
 - 5.2.2. LAB MEDIA: Figure 1F. *Video editor: Highlight the taller bar labeled “Co-folding”*
 - 5.2.3. LAB MEDIA: Figure 1F. *Video editor: Highlight the bar labeled “Separate folding”*
- 5.3. Shu-top, shu-bottom, Shu^{anti}-top (*Shu-Anti-Top*), and Shu^{anti}-bottom individually exhibited minimal DFHBI-1T fluorescence [1]. Co-folding of Shu-top with Shu-bottom or Shu^{anti}-top with Shu^{anti}-bottom produced a higher DFHBI-1T signal than separate folding [2].
 - 5.3.1. LAB MEDIA: Figure 2A.
 - 5.3.2. LAB MEDIA: Figure 2Ci and 2 Cii *Video editor: Highlight the brighter green clusters in the co-folding panels and dimmer green clusters in the separate folding panels*
- 5.4. Co-folding of shu-top and shu-bottom resulted in a 5.63-fold increase in normalized DFHBI-1T fluorescence [1]. Separate folding of shu-top and shu-bottom showed only a 1.04-fold increase, comparable to baseline levels [2].
 - 5.4.1. LAB MEDIA: Figure 2Di. *Video editor: Highlight the bar corresponding to shu-top plus shu-bottom under +co-folding showing increased height*
 - 5.4.2. LAB MEDIA: Figure 2Dii. *Video editor: Highlight the bar for shu-top plus shu-bottom under separate folding near baseline*
- 5.5. Shu-top mixed with Shu^{anti}-bottom and Shu^{anti}-top mixed with Shu-bottom exhibited higher DFHBI-1T fluorescence than same-orientation pairs under both folding conditions [1].
 - 5.5.1. LAB MEDIA: Figure 2Ci and Cii. *Video editor: Highlight the panels showing shu-*

top with shuanti-bottom with brighter signal

- 5.6. Co-folding of Shu-top with Shu^{anti}-bottom and Shu^{anti}-top with Shu-bottom resulted in 61.86- [1] and 82.60-fold increases in DFHBI-1T fluorescence, respectively [2].
 - 5.6.1. LAB MEDIA: Figure 2Di. *Video editor: Highlight the bar for shu-top plus shuanti-bottom with very high value*
 - 5.6.2. LAB MEDIA: Figure 2Di. *Video editor: Highlight the bar for shuanti-top plus shu-bottom with the highest value*
- 5.7. Separate folding of these mixed pairs yielded smaller increases of 2.69- and 4.94-fold, respectively [1].
 - 5.7.1. LAB MEDIA: Figure 2Dii. *Video editor: Highlight the bar for shu-top plus shuanti-bottom and shuanti-top plus shu-bottom under separate folding*

1. Ribonuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonuclease>
IPA: /ˌraɪ.boʊˈnuː.kliː.ɛs/
Phonetic Spelling: rye·boh·noo·klee·ays
2. Decontamination
Pronunciation link: <https://www.merriam-webster.com/dictionary/decontamination>
IPA: /diː.kənˌtæm.əˈneɪ.ʃən/
Phonetic Spelling: dee·kuhn·tam·uh·nay·shuhn
3. Uridine
Pronunciation link: <https://www.merriam-webster.com/dictionary/uridine>
IPA: /ˈjʊr.əˌdiːn/
Phonetic Spelling: yoor·uh·deen
4. Triphosphate
Pronunciation link: <https://www.merriam-webster.com/dictionary/triphosphate>
IPA: /traɪˈfɑːs.feɪt/
Phonetic Spelling: try·fahs·fayt
5. Cyanine
Pronunciation link: <https://www.merriam-webster.com/dictionary/cyanine>
IPA: /ˈsaɪ.əˌniːn/
Phonetic Spelling: sigh·uh·neen
6. Thymine
Pronunciation link: <https://www.merriam-webster.com/dictionary/thymine>
IPA: /ˈθaɪ.miːn/
Phonetic Spelling: thigh·meen
7. Transcription
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcription>
IPA: /trænˈskrɪp.ʃən/
Phonetic Spelling: tran·skrip·shuhn

8. Deoxyribonuclease
 Pronunciation link: <https://www.merriam-webster.com/dictionary/deoxyribonuclease>
 IPA: /diˌɑːk.si.raɪ.boʊˈnuː.kliː.əs/
 Phonetic Spelling: dee·ok·see·rye·boh·noo·klee·ays
9. Ammonium
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ammonium>
 IPA: /əˈmoʊ.ni.əm/
 Phonetic Spelling: uh·moh·nee·uhm
10. Phenol-chloroform
 Pronunciation link: <https://www.merriam-webster.com/dictionary/phenol>
 IPA: /ˈfiː.nɑːl ˈklɔːr.ə.fɔːrm/
 Phonetic Spelling: fee·nawl klor·uh·form
11. Aqueous
 Pronunciation link: <https://www.merriam-webster.com/dictionary/aqueous>
 IPA: /ˈeɪ.kwi.əs/
 Phonetic Spelling: ay·kwee·uhs
12. Chloroform
 Pronunciation link: <https://www.merriam-webster.com/dictionary/chloroform>
 IPA: /ˈklɔːr.ə.fɔːrm/
 Phonetic Spelling: klor·uh·form
13. Isopropanol
 Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropanol>
 IPA: /,aɪ.səˈproʊ.pəˌnɑːl/
 Phonetic Spelling: eye·suh·proh·puh·nawl
14. Aliquoting
 Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
 IPA: /ˈæl.ɪ.kwɑːt/
 Phonetic Spelling: al·ih·kwaht
15. Ethanol
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
 IPA: /ˈeθ.əˌnɑːl/
 Phonetic Spelling: eth·uh·nawl
16. Spectrophotometer
 Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
 IPA: /,spek.troʊ.fəˈtɑː.mə.tə/
 Phonetic Spelling: spek·troh·fuh·tom·uh·ter
17. Absorbance
 Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>
 IPA: /əbˈzɔːr.bəns/
 Phonetic Spelling: ub·zor·buhns
18. Polyethylene
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polyethylene>
 IPA: /,pɑː.liˈeθ.əˌliːn/
 Phonetic Spelling: pah·lee·eth·uh·leen

19. Refolding
 Pronunciation link: <https://www.merriam-webster.com/dictionary/refold>
 IPA: /riːˈfoʊld/
 Phonetic Spelling: ree·fohd
20. Potassium
 Pronunciation link: <https://www.merriam-webster.com/dictionary/potassium>
 IPA: /pəˈtæs.i.əm/
 Phonetic Spelling: puh·tas·ee·uhm
21. Magnesium
 Pronunciation link: <https://www.merriam-webster.com/dictionary/magnesium>
 IPA: /mægˈniː.zi.əm/
 Phonetic Spelling: mag·nee·zee·uhm
22. Polymerase
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>
 IPA: /pəˈlɪm.əˌreɪs/
 Phonetic Spelling: puh·lim·uh·rays
23. Incubate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
 IPA: /ˈɪŋ.kjəˌbeɪt/
 Phonetic Spelling: ing·kyuh·bayt
24. Spermine
 Pronunciation link: <https://www.merriam-webster.com/dictionary/spermine>
 IPA: /ˈspɜː.miːn/
 Phonetic Spelling: spur·meen
25. DFHBI-1T
 Pronunciation link: No confirmed link found
 IPA: /ˌdiːˌefˌeɪtʃˌbiːˌaɪˌwʌnˌtiː/
 Phonetic Spelling: dee·ef·aitch·bee·eye one tee
26. Fluorescence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
 IPA: /flʊˈres.əns/, /flɔːˈres.əns/
 Phonetic Spelling: floor·ess·uhns
27. Intermolecular
 Pronunciation link: <https://www.merriam-webster.com/dictionary/intermolecular>
 IPA: /ˌɪn.təˌmɑːˈlek.jə.lə/
 Phonetic Spelling: in·ter·muh·lek·yuh·ler
28. Cyanine 5
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cyanine>
 IPA: /ˈsaɪ.əˌniːn faɪv/
 Phonetic Spelling: sigh·uh·neen five
29. Nanometers
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
 IPA: /ˈnæn.əˌmiː.tə/
 Phonetic Spelling: nan·uh·mee·ter

30. Co-folding

Pronunciation link: No confirmed link found

IPA: /koʊ ˈfoʊl.dɪŋ/

Phonetic Spelling: koh fohl·ding

31. Shuanti

Pronunciation link: No confirmed link found

IPA: /ʃuːˈæn.ti/

Phonetic Spelling: shoo·an·tee

32. Microliter

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

IPA: /ˈmaɪ.kroʊ.liː.tər/

Phonetic Spelling: my·kroh·lee·ter

33. Ribonucleic

Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic%20acid>

IPA: /ˌraɪ.boʊ.nuːˈkliː.ɪk/

Phonetic Spelling: rye·boh·noo·klee·ik