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Title: Optimized Hot Phenol–Based RNA Extraction from Mycobacteria: A Robust Approach for Reliable Gene Expression Analysis

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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? **No**
3. **Filming location:** Will the filming need to take place in multiple locations? **Yes, Labs are situated on the First and second floor**
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: 15

Number of Shots: 42

Interviews

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. **Kajal Kanchan**: Our research focuses on transcriptomic analysis of mycobacterial adaptation within host microenvironments using a reliable and efficient RNA extraction method.
 - 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. **Manoj Kumar**: This rapid, reliable, optimized protocol uses routine chemicals and minimum instrumentation, overcoming technical challenges associated with extracting high-quality RNA from mycobacterium.
 - 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. **Hritika Verma**: The protocol allows high-quality RNA extraction from both pathogenic and non-pathogenic mycobacteria for downstream genomic and molecular studies.
 - 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. **Rishav S. Kumar**: The critical challenge in preserving RNA integrity is to avoid contamination during the repeated cell disruption process.
 - 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. **Hritika Verma:** Further studies can extend this protocol to other difficult-to-lyse bacteria, providing a scalable solution in both advanced and resource-limited settings.

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. Mycobacterium Culture for RNA Isolation

Demonstrator: Rishav Kumar Singh

- 2.1. To begin, inoculate 1-milliliter glycerol stock of *Mycobacterium smegmatis* or *Mycobacterium tuberculosis* in 10 milliliters of Middlebrook 7H9 (*Seven-H-nine*) medium supplemented with 0.2 percent glycerol, 0.05 percent Tween 80, and 10 percent albumin-dextrose-catalase [1]. Incubate at 37 degrees Celsius with shaking at 150 revolutions per minute until the optical density reaches 1 to 1.5 to obtain the primary culture [2].
 - 2.1.1. WIDE: Talent inoculating the glycerol stock of Msmeg or Mtb into the medium.
***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent placing the inoculated culture on the shaker incubator for incubation.
- 2.2. To obtain the secondary culture, add 1 percent primary culture to 10 milliliters of 7H9 medium [1] and incubate until the culture reaches mid-log phase, with an optical density of 0.6 to 0.7 at 600 nanometers [2].
 - 2.2.1. Talent adding the primary culture into 7H9 medium.
 - 2.2.2. Talent checking the OD value.
- 2.3. Split the culture into 3-milliliter aliquots [1] and centrifuge at 2000 g for 5 minutes at 23 to 25 degrees Celsius followed by ribonucleic acid, or RNA (*R-N-A*) extraction [2].
 - 2.3.1. Talent aliquoting the culture into 3-milliliter portions.
 - 2.3.2. Talent placing the aliquots in the centrifuge.

3. RNA Extraction Using Hot Phenol Method

Demonstrator: Hritika Verma

- 3.1. For Hot-Phenol method, add 200 microliters of [1] TES (*T-E-S*) buffer to the cell pellet [2]. Resuspend the pellet by vortexing [3].
 - 3.1.1. Talent adding TES buffer to the cell pellet.
 - 3.1.2. TEXT ON A PLAIN BACKGROUND
TES Buffer: Volume 5 mL
100 mM Tris-HCl, pH 8.0 500 µL from 1 M stock

5 mM EDTA, pH 8.0	50 µL from 0.5 M stock
0.1 M NaCl	100 µL from 5 M stock
0.5% SDS	250 µL from 10% stock
1% β-mercaptoethanol	50 µl from 100%
RNase-free water	Upto 5 mL

- 3.1.3. Talent placing the sample on the vortex to resuspend the pellet.
- 3.2. Add approximately 0.1 grams of 0.1-millimeter silica beads to the sample [1]. Use a handheld tissue grinder to homogenize the pellet on ice for 10 to 15 seconds, processing until the sample is completely thawed [2]. Snap-freeze the sample in liquid nitrogen between each round of the 2 additional homogenization cycles [3].
 - 3.2.1. Talent adding silica beads to the sample.
 - 3.2.2. Talent grinding the sample on ice.
 - 3.2.3. Talent snap-freezing the sample in liquid nitrogen.
- 3.3. Alternatively, use a high-speed tabletop bead ruptor at a speed setting of 5 meters per second for 1 minute at room temperature for 3 cycles [1]. Place the sample on ice after each cycle to prevent heating [2].
 - 3.3.1. Talent placing the sample in the high-speed tabletop bead ruptor.
 - 3.3.2. Talent placing the sample on ice after the cycle.
- 3.4. Add an additional 200 microliters of TES buffer pre-warmed to 65 degrees Celsius to each sample to bring the volume to 400 microliters [1]. Next, mix 200 microliters of chloroform with 200 microliters of acidic phenol equilibrated with 100 millimolar citrate buffer at pH 4.5 [2]. Add the equal ratio mixture of acidic phenol and chloroform to the sample [3].
 - 3.4.1. Talent adding pre-warmed TES buffer to each sample.
 - 3.4.2. Talent mixing the chloroform and acid phenol reagents in 1:1 ratio.
 - 3.4.3. Talent adding the acidic phenol and chloroform mixture to the sample.
- 3.5. Place the sample on a heat block at 65 degrees Celsius with shaking at 900 revolutions per minute for 30 minutes [1] Then, centrifuge at 12,000 g for 15 minutes at room temperature [2]. Confirm phase separation, with a clear upper aqueous phase containing RNA, a distinct deoxyribonucleic acid, or DNA (D-N-A) interphase, and a lower organic phase containing proteins and lipids [3].
 - 3.5.1. Talent placing the sample on the heat block.
 - 3.5.2. Talent placing the sample in the centrifuge.
 - 3.5.3. Close shot of the separated phases in the sample.

- 3.6. Carefully transfer the upper aqueous phase into a new 1.5-milliliter microcentrifuge tube [1]. Add an equal volume of acidic phenol: chloroform [2]. Centrifuge at 12,000 *g* for 15 minutes at room temperature, ensuring proper phase separation [3].
 - 3.6.1. Talent transferring the aqueous phase into a new 1.5-milliliter microcentrifuge tube.
 - 3.6.2. Talent adding acidic phenol: chloroform.
 - 3.6.3. Talent placing the tube in the centrifuge and then checking phase separation.
- 3.7. After transferring the upper aqueous layer into a fresh 1.5-milliliter microcentrifuge tube, add 30 microliters of 3 molar sodium acetate buffer at pH 5.3 and 240 microliters of chilled isopropanol [1]. Incubate at minus 80 degrees Celsius for 30 minutes to 1 hour [2].
 - 3.7.1. Talent adding sodium acetate buffer and chilled isopropanol to the 1.5 mL tube.
 - 3.7.2. Talent placing the tube at minus 80 degrees Celsius.
- 3.8. Centrifuge the sample at 12,000 *g* for 1 hour at room temperature [1]. After verifying pellet formation, carefully remove the supernatant without disturbing the pellet [2].
 - 3.8.1. Talent placing the sample in the centrifuge.
 - 3.8.2. Talent carefully removing the supernatant.
- 3.9. Resuspend the pellet in 1 milliliter of 75 percent ethanol [1]. Centrifuge the sample at 12,000 *g* for 1 minute at room temperature for washing [2]. After two washes, air-dry the pellet [3] and resuspend it in 50 microliters of nuclease-free water [4].
 - 3.9.1. Talent adding 75 percent ethanol to the pellet.
 - 3.9.2. Talent placing the sample in the centrifuge.
 - 3.9.3. Talent placing the tube with pellet aside for air-drying.
 - 3.9.4. Talent adding nuclease-free water to the pellet.

4. RNA Quantification, qRT-PCR and Statistical Analysis

Demonstrator: Hritika Verma

Videographer: *Please record the computer screen for the shots labeled as SCREEN as back-up*

Note: Please use the backup shots filmed by the videographer for all shots labeled SCREEN. The author-provided SCREEN shots are unusable. Please reach out to Kathyayani Sridharan if any clarifications are required.

- 4.1. Before RNA quantification, add 1 μ L of 10 units DNase I (*D-N-ace-one*) to each RNA sample to remove genomic DNA contamination. [1]. Add 5 microliters of 1X RDD (*R-D-*

D) buffer [2] and incubate at room temperature for 30 minutes [3].

4.1.1. Talent adding DNase I to the RNA sample.

4.1.2. Talent adding RDD buffer to the sample.

4.1.3. Talent placing the sample aside for room-temperature incubation.

4.2. Use the Nanodrop instrument for spectrophotometric quantification of RNA samples [1]. Assess RNA absorbance and accept the sample if it meets the RNA purity criteria [2]. Perform agarose gel electrophoresis to assess the RNA quality [3]. Measure the fluorescence reading using Qubit 4 [4] to check RNA integrity, or RIN (*R-I-N*) value [5].

4.2.1. Talent placing the RNA samples on the Nanodrop.

4.2.2. SCREEN: Show the ideal concentration and A_{260}/A_{280} values and the evaluated RNA samples falling in that range.

4.2.3. Talent loading the samples on agarose gel and starting the electrophoresis

4.2.4. Talent placing the sample in the qubit instrument.

4.2.5. SCREEN: Show the ideal RIN value on the Qubit screen, which should be close to 10

4.3. Prepare complementary deoxyribonucleic acid, or cDNA (*C-D-N-A*), from 1 microgram of RNA sample and the cDNA Reverse Transcription kit [1]. Run the reaction in a thermal cycler according to the manufacturer's instructions [2]. Perform quantitative real-time polymerase chain reaction, or qRT-PCR (*Q-R-T-P-C-R*) according to the manufacturer's instructions [3] using 50 nanograms of cDNA and specific forward and reverse primers [3].

4.3.1. Representative shot of talent preparing the cDNA reaction mixture.

4.3.2. Talent placing the reaction in the thermal cycler.

4.3.3. Representative shot of talent performing the qRT-PCR.

~~4.3.4. SCREEN: showing the Ct values and amplification plot on qRT-PCR.~~ Note: The authors removed this shot.

Results

5. Results

- 5.1. A comparison of hot phenol method against TRIzol and commercial kit protocols revealed distinct, sharp bands corresponding to 23S (23-S) and 16S (16-S) ribosomal RNA, or rRNA (R-R-N-A), across all three approaches, indicating successful isolation of total RNA [1]. The Hot Phenol and TRIzol methods showed 3 clear bands corresponding to 23S, 16S, and 5S (5-S) rRNA, indicating intact, high-quality RNA [2]. The column-based kit method showed only the 23S and 16S rRNA bands, with the 5S band absent or below detection [3].
- 5.1.1. LAB MEDIA: figure-2-final.pdf. Figure 2A. *Video editor: Highlight the dark horizontal bands visible in all 3 sample groups labeled “Hot Phenol”, “TRIzol”, and “RNeasy”*
- 5.1.2. LAB MEDIA: figure-2-final.pdf. Figure 2A. *Video editor: Zoom in on the 3 visible horizontal bands in the lanes labeled “Hot Phenol” and “TRIzol”*
- 5.1.3. LAB MEDIA: figure-2-final.pdf. Figure 2A. *Video editor: Highlight the 2 visible bands in the “RNeasy” lanes and show the absence of the lower faint band seen in the other methods*
- 5.2. The Hot Phenol method yielded 1.5 to 2-fold more RNA than TRIzol and RNeasy [1]. Average RNA yields were approximately 60 microgram for Hot Phenol [2], approximately 45 microgram for TRIzol [3], and approximately 30 microgram for RNeasy [4].
- 5.2.1. LAB MEDIA: figure-2-final.pdf. Figure 2C. *Video editor: Highlight the taller bar labeled “Hot Phenol” compared with the shorter “TRIzol” and “RNeasy” bars*
- 5.2.2. LAB MEDIA: figure-2-final.pdf. Figure 2C. *Video editor: Highlight the height of the bar labeled “Hot Phenol” and the corresponding Y-axis value 60 on the vertical axis*
- 5.2.3. LAB MEDIA: figure-2-final.pdf. Figure 2C. *Video editor: Highlight the height of the bar labeled “TRIzol” and the corresponding Y-axis value 45 on the vertical axis*
- 5.2.4. LAB MEDIA: figure-2-final.pdf. Figure 2C. *Video editor: Highlight the height of the bar labeled “RNeasy” and the corresponding Y-axis value 30 on the vertical axis*
- 5.3. All extraction methods resulted in good RNA purity with acceptable A260/A280 (A-two-sixty over A-two-eighty) ratios around 2.0 [1] RNA integrity values were comparable

across methods ranging from 7.5 to 10 [2].

5.3.1. LAB MEDIA: figure-2-final.pdf. Figure 2D. *Video editor: Highlight the bars for “Hot Phenol”, “TRIzol”, and “RNeasy”, which are all close to 2.0*

5.3.2. LAB MEDIA: figure-2-final.pdf. Figure 2E. *Video editor: Highlight the bars for “Hot Phenol”, “TRIzol”, and “RNeasy”, which are all at similar heights*

5.4. RNA extracted using Hot Phenol [1] and TRIzol gave comparable cycle threshold values of approximately 22 to 25 for *mysA* (*MISS-ay*), *sigE* (*SIG-ee*), and *sigH* (*SIG-aitch*) genes [2]. The kit method showed slightly higher cycle threshold values of approximately 27 to 30, indicating lower levels of detectable transcript [3]. Quantitative polymerase chain reaction analysis using *M.tuberculosis* genes yielded acceptable cycle threshold values of approximately 20 to 25, confirming suitability for downstream gene expression studies [4].

5.4.1. LAB MEDIA: figure-2-final.pdf. Figure 2I. *Video editor: Highlight the “Hot Phenol” and “TRIzol” bars for *mysA*, *sigE*, and *sigH* that appear at similar heights*

5.4.2. LAB MEDIA: figure-2-final.pdf. Figure 2I. *Video editor: Zoom in on the bars between approximately 22 and 25 on the vertical axis*

5.4.3. LAB MEDIA: figure-2-final.pdf. Figure 2I. *Video editor: Highlight the taller “RNeasy” bars for *mysA*, *sigE*, and *sigH* compared with the other methods*

5.4.4. LAB MEDIA: Figure 3E. *Video editor: Highlight the bars labeled “*sigA*”, “16sRNA”, and “hsp70” between approximately 20 and 25 on the vertical axis*

Pronunciation Guide:

1. Mycobacterium

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

IPA: /ˌmaɪkoʊˈbækˈtɪriəm/

Phonetic Spelling: my·koh·bak·TEER·ee·um

2. smegmatis

Pronunciation link: No confirmed link found

IPA: /smegˈmætɪs/

Phonetic Spelling: smeg·MAT·is

3. tuberculosis

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

IPA: /tuːˌbɜːkjəˈloʊsɪs/

Phonetic Spelling: too·bur·kyuh·LOH·sis

4. Middlebrook

Pronunciation link: No confirmed link found

IPA: /ˈmɪdəlˌbrʊk/

Phonetic Spelling: MID·uhl·brook

5. albumin
Pronunciation link: <https://www.merriam-webster.com/dictionary/albumin>
IPA: /æl'bjʊ:mən/
Phonetic Spelling: al·BYOO·min
6. dextrose
Pronunciation link: <https://www.merriam-webster.com/dictionary/dextrose>
IPA: /'dɛk,stroʊs/
Phonetic Spelling: DEK·strohs
7. catalase
Pronunciation link: <https://www.merriam-webster.com/dictionary/catalase>
IPA: /'kætə,leɪs/
Phonetic Spelling: KAT·uh·lays
8. inoculate
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculate>
IPA: /ɪ'nɑ:kjə,leɪt/
Phonetic Spelling: ih·NAA·kyuh·layt
9. aliquots
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /'ælɪ,kwɑ:t/
Phonetic Spelling: AL·ih·kwaat
10. centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrə,fju:dʒ/
Phonetic Spelling: SEN·truh·fyooj
11. ribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic>
IPA: /,raɪboʊnu:'kleɪk/
Phonetic Spelling: rye·boh·noo·KLAY·ik
12. phenol
Pronunciation link: <https://www.merriam-webster.com/dictionary/phenol>
IPA: /'fi:,nɑ:l/
Phonetic Spelling: FEE·naal
13. microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkroʊ,li:tər/
Phonetic Spelling: MY·kroh·lee·ter
14. resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /,ri:sə'spend/
Phonetic Spelling: ree·suh·SPEND
15. vortexing
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>

IPA: /'vɔːrˌtɛksɪŋ/

Phonetic Spelling: VOR·tek·sing

16. millimolar

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

IPA: /ˌmɪləˈmoʊlər/

Phonetic Spelling: mil·uh·MOH·ler

17. β-mercaptoethanol

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

IPA: /ˌbɛtə mæˌkæptəʊˈɛθəˌnoːl/

Phonetic Spelling: BAY·tuh mer·kap·toh·ETH·uh·naal

18. RNase

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

IPA: /ɑːrˈɛnˌeɪs/

Phonetic Spelling: ar·EN·ays

19. silica

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

IPA: /ˈsɪlɪkə/

Phonetic Spelling: SIL·ih·kuh

20. homogenize

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

IPA: /həˈmɑːdʒəˌnaɪz/

Phonetic Spelling: huh·MAA·juh·nyze

21. homogenization

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

IPA: /həˌmɑːdʒənəˈzeɪʃən/

Phonetic Spelling: huh·maa·juh·nuh·ZAY·shun

22. ruptor

Pronunciation link: No confirmed link found

IPA: /'rʌptər/

Phonetic Spelling: RUP·ter

23. chloroform

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

IPA: /'klɔːrəˌfɔːrm/

Phonetic Spelling: KLOR·uh·form

24. acidic

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

IPA: /əˈsɪdɪk/

Phonetic Spelling: uh·SID·ik

25. equilibrated

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

IPA: /ˌiːkwəˈlaɪˌbreɪtɪd/

Phonetic Spelling: ee·kwuh·LY·bray·tid

26. citrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/citrate>
IPA: /ˈsɪˌtreɪt/
Phonetic Spelling: SIT-rayt
27. aqueous
Pronunciation link: <https://www.merriam-webster.com/dictionary/aqueous>
IPA: /ˈeɪkwɪəs/
Phonetic Spelling: AY-kwee-us
28. deoxyribonucleic
Pronunciation link: <https://www.merriam-webster.com/dictionary/deoxyribonucleic>
IPA: /diˌɑːksɪraɪboʊnuːˈkleɪɪk/
Phonetic Spelling: dee-aak-see-rye-boh-noo-KLAY-ik
29. interphase
Pronunciation link: <https://www.merriam-webster.com/dictionary/interphase>
IPA: /ˈɪntərˌfeɪz/
Phonetic Spelling: IN-ter-fayz
30. microcentrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>
IPA: /ˌmaɪkroʊˈsentrəˌfjuːdʒ/
Phonetic Spelling: my-kroh-SEN-truh-fyooj
31. acetate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acetate>
IPA: /ˈæsəˌteɪt/
Phonetic Spelling: AS-uh-tayt
32. isopropanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropanol>
IPA: /ˌaɪsoʊˈprɒpəˌnɔːl/
Phonetic Spelling: eye-soh-PROH-puh-naal
33. supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpərˈneɪtənt/
Phonetic Spelling: soo-per-NAY-tunt
34. nuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
IPA: /ˈnuːkliˌeɪs/
Phonetic Spelling: NOO-klee-ays
35. quantification
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>
IPA: /ˌkwɑːntəfəˈkeɪʃən/
Phonetic Spelling: kwaan-tuh-fuh-KAY-shun
36. DNase
Pronunciation link: <https://www.merriam-webster.com/dictionary/DNase>

IPA: /ˌdiːˈɛnˌeɪs/

Phonetic Spelling: dee·EN·ays

37. genomic

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

IPA: /dʒəˈnoʊmɪk/

Phonetic Spelling: juh·NOH·mik

38. spectrophotometric

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

IPA: /ˌspektrəʊˈfəʊtəˈmetrɪk/

Phonetic Spelling: spek·troh·foh·tuh·MET·rik

39. agarose

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

IPA: /ˈæɡəˌroʊs/

Phonetic Spelling: AG·uh·rohs

40. electrophoresis

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

IPA: /ɪˌlektroʊfəˈriːsɪs/

Phonetic Spelling: ih·lek·troh·fuh·REE·sis

41. fluorescence

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

IPA: /flʊˈresəns/

Phonetic Spelling: floo·RES·uns

42. integrity

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

IPA: /ɪnˈtɛɡrəti/

Phonetic Spelling: in·TEG·ruh·tee

43. complementary

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

IPA: /ˌkɑːmpləˈmentəri/

Phonetic Spelling: kaam·pluh·MEN·tuh·ree

44. transcription

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

IPA: /trænˈskrɪpʃən/

Phonetic Spelling: tran·SKRIP·shun

45. polymerase

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

IPA: /pəˈlɪməˌreɪs/

Phonetic Spelling: puh·LIM·uh·rays

46. primers

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

IPA: /ˈpraɪmər/

Phonetic Spelling: PRY·mer

47. amplification

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

IPA: /,æmpləfə'keɪʃən/

Phonetic Spelling: am·pluh·fuh·KAY·shun