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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	To adjust the final volume to 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: The authors specified that the shots labelled SCREEN were not filmed by the videographer as back-up. The author-provided material for these shots is unusable. So, we removed these shots and merged the VO in a concise manner. 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 to measure absorbance 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 [4].~~ **Note: The narration for the unfilmed shots has been concisely merged with the existing VO**

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.~~ **Note: This shot was not filmed**

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.~~ **Note: This shot was not filmed**

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: This shot was not filmed**

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: /ˈklɔːrəˌfɔːrm/

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: /ˌspektrəʊˈfotəˈmetrɪk/

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