

Submission ID #: 69467

Scriptwriter Name: Kathyayani Sridharan

Project Page Link: <https://review.jove.com/account/file-uploader?src=21187483>

Title: Determination of Microbial Biomass in Soil using Digital Droplet PCR (ddPCR)

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**
- 3. Filming location:** Will the filming need to take place in multiple locations? *Yes*
If **Yes**, how far apart are the locations? **A 5 min walk to a different building.**
- 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: 52

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Note: The statements have been edited for length and clarity

INTRODUCTION:

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

- 1.1. **Shayma Alathari:** Our study uses droplet digital PCR and whole genome sequencing to accurately quantify microbial biomass across various soil types.

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. **Shayma Alathari:** Conventional methods require large soil samples and harmful chemicals. ddPCR uses minimal soil for absolute gene and biomass carbon quantification.

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. **Shayma Alathari:** This protocol allows researchers to measure soil community dynamics and structure through microbial biomass and fungal to bacterial ratio.

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. **Shayma Alathari:** The same template DNA used for ddPCR can be used for DNA sequencing to enable microbial community and functional analysis.

1.4.1. 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's NOTE: Unfortunately I did not have enough time to film reusable angles and multiple takes of shots for section 2 Protocol. Shayma told me that they did not allow people in this lab after 5 pm. This meant I had to rush my shots and do one take for most shots

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

2. Microbial DNA Extraction from Soil Samples

Demonstrator: Gaby Joslin

2.1. To begin, assemble the reagents of the soil DNA (*D-N-A*) extraction kit to isolate the DNA from soil microbes at twenty degrees Celsius room temperature [1]. Spin the two-milliliter bead-beating tube briefly [2] and verify that the beads have settled at the bottom [3]. Add hundred milligrams of soil, then eight hundred microliters of Solution CD1 (*C-D-one*) [4] and vortex briefly to mix [5].

2.1.1. WIDE: Talent assembling the reagents and soil DNA extraction kit and placing it on the work bench. **Videographer's NOTE:** Shot 2.1.1 Take 3 is the best take to use from this shot

2.1.2. Talent placing the 2 ml bead beating tube in the spinner.

2.1.3. Talent checking the beads in the 2 mL bead beating tube.

Videographer's NOTE: Shot 2.1.3 Take 4 is a wide shot. This can be cut with the extra shot we added called Shot 2.1.3B or the prior takes of this shot if it works with the edit. As these shots are close ups

2.1.4. Talent adding 100 mg of soil and 800 μ L Solution CD1 to the tube.

Videographer's NOTE: Shots 2.1.4B & 2.1.4C are extra shots that can be cut with Shot 2.1.4. Take 2 of Shot 2.1.4B is the better take to use

2.1.5. Talent placing the mixture in the vortex.

Videographer's NOTE: Shots 2.1.5 & 2.2.1 were combined together

2.2. Use a bead mill homogenizer at a speed of six meters per second for forty seconds to lyse the sample thoroughly [1] and centrifuge the bead beating tube at 15000 *g* for one minute at room temperature to achieve mechanical cell lysis [2]. Transfer the supernatant that may still contain soil particles to a clean two milliliter microcentrifuge tube [3].

2.2.1. Talent homogenizing the sample using bead mill homogenizer.

2.2.2. Talent centrifuging the homogenized mixture at 15000 *g*.

2.2.3. Talent transferring the supernatant to a clean 2 ml tube.

Videographer's NOTE: Shots 2.2.3, 2.3.1 & 2.3.2 were combined together. The Author has requested in editing to show the process for one tube

2.3. Add two hundred microliters of Solution CD2 (*C-D-two*) [1], vortex for five seconds [2] and centrifuge at 15000 *g* for one minute [3]. Transfer up to seven hundred microliters of supernatant to a clean two milliliters microcentrifuge tube, without disturbing the pellet [4].

2.3.1. Talent adding 200 μ L of CD2 solution to the tube.

2.3.2. Talent placing the mixture in a vortex for 5 seconds.

2.3.3. Talent centrifuging the mixture at 15000 *g* at room temperature.

2.3.4. Talent transferring the supernatant without disturbing the pellet.

Videographer's NOTE: Shots 2.3.4, 2.4.1, 2.4.2 & 2.4.3 were combined together

2.4. Add six hundred microliters of solution CD3 (*C-D-three*) to the supernatant [1] and vortex for five seconds [2]. Load six hundred and fifty microliters of the mixture onto a spin column [3] and centrifuge at 15000 *g* for one minute [4]. Discard the flow-through and repeat the process for the remaining lysate [5].

2.4.1. Talent adding 600 μ L of solution CD3 to the supernatant.

2.4.2. Talent placing the mixture in a vortex for 5 seconds.

2.4.3. Talent loading 650 μ L of the mixture to a spin column.

2.4.4. Talent centrifuging the mixture at 15000 *g* at room temperature.

2.4.5. Talent discarding the flow-through obtained from the spin column.

Videographer's NOTE: Shots 2.4.5, 2.5.1 & 2.5.2 were combined together

2.5. Next, place the spin column into a clean two milliliter collection tube [1]. Add five hundred microliters of Solution EA (*E-A*) to the spin column [2] and centrifuge at 15000 *g* for one minute at room temperature [3].

2.5.1. Talent placing a 2 mL collection tube in the spin column.

2.5.2. Talent adding 500 μ L of Solution EA to the spin column. *Videographer: Obtain multiple reusable takes of 2.5.2 as these will be used later in the protocol*

2.5.3. Talent placing the spin column in the centrifuge and closing the lid.
Videographer: Obtain multiple reusable takes of 2.5.3 as these will be used later in the protocol

Videographer's NOTE: Shots 2.5.3 & 2.7.2 were combined together. The Authors told me they are the same shot and can be combined

- 2.6. Discard the flow-through and place the spin column back into the same collection tube [1]. Add five hundred microliters of Solution C5 (*C-five*) to the spin column and centrifuge as demonstrated previously [2].
- 2.6.1. Talent placing the spin column back in the same tube after discarding the flowthrough *Videographer: Obtain multiple reusable takes of 2.6.1 as these will be used later in the protocol*
- 2.6.2. *Reuse 2.5.2 and 2.5.3*
- 2.7. After discarding the flow-through, place the spin column into a new two milliliter collection tube [1] and centrifuge at 16000 *g* for two minutes at room temperature [2].
- 2.7.1. *Reuse 2.6.1*
- 2.7.2. Talent placing the spin column in the centrifuge and closing the lid.
Videographer: If possible, please obtain shot of display showing parameters
- 2.8. Place the spin column in new one-point milliliter elution tube [1]. Add fifty to hundred microliters of the Solution C6 (*C-six*) to the center of the filter membrane of the spin column [2]. Centrifuge at 15000 *g* for one minute [3]. Store the tube containing elute at four degrees Celsius after discarding the spin column [4]. Quantify extracted DNA using a fluorometer and a double stranded DNA Broad Range assay kit, following the manufacturer's instructions [5].
- 2.8.1. Talent placing the spin column in a 1.5 mL tube.
Videographer's NOTE: Shots 2.8.1 & 2.8.2 were combined together
- 2.8.2. Talent adding 50-100 μ L C6 solution to the center of filter membrane of the spin column.
- 2.8.3. Talent placing the spin column in the centrifuge and closing the lid.
- 2.8.4. Talent placing the 1.5 mL tube containing the elute in 4 °C refrigerator.
- 2.8.5. Representative shot of talent analyzing the values on a computer connected to the fluorometer.
Videographer's NOTE: Shot 2.8.5 - The Authors have requested that this shot starts after one tube sample has been selected and the rest have been deselected

3. Soil Microbial Quantification Using Digital Droplet PCR (ddPCR)

Demonstrator: Lucy Wierzbicki

- 3.1. For analysis of bacteria and fungi, select specific 16S rRNA (*sixteen-S-R-R-N-A*) and 18S rRNA (*eighteen-S-R-R-N-A*) primers [1]. After thawing, vortex all reagents on ice [2]. Set up the digital droplet polymerase chain reaction [3] or ddPCR (*D-D-P-C-R*) [4].

3.1.1. Talent placing vials of primers on ice.

Videographer's NOTE: Shots 3.1.1 & 3.1.2 were combined together

3.1.2. Talent placing the reagents in a vortex after thawing.

3.1.3. CU: Representative shot of talent adding DNA sample in a PCR tube.

3.1.4. TEXT ON A PLAIN BACKGROUND

Reaction Mixture:

10 μ M Forward Primer (16S rRNA or 18S rRNA) 0.1 μ L

10 μ M Reverse Primer (16S rRNA or 18S rRNA) 0.1 μ L

ddPCR Evagreen Supermix 10 μ L

DNA template 0.5 ng

Adjust the final volume to 22 μ L with nuclease-free water

Negative Control:

Reaction mixture without the DNA template

3.2. Place the droplet generator cartridge or DG8 (*D-G-eight*) into the cartridge holder [1]. Add a total of twenty microliters of the reaction mixture into each of the sample wells in the cartridge [2]. Then, add seventy microliters of Droplet Generation Oil for Evagreen into the cartridge well labelled 'oil' [3].

3.2.1. Talent placing the DG8 cartridge into the cartridge holder.

Videographer's NOTE: Shots 3.2.1 & 3.2.2 were combined together

3.2.2. Talent adding 20 μ L of reaction mixture to the wells of cartridge.

3.2.3. Talent adding 70 μ L of droplet generation oil to the well labelled oil.

3.3. Place the cartridge into the droplet generator [1] and cover it with a gasket [2]. Transfer forty microliters of the generated droplets to each well of a ninety-six-well semi-skirted plate for PCR amplification [3]. Seal the plate using a plate sealer pre-set to 180 degrees Celsius for 5 seconds [4].

3.3.1. Talent placing the cartridge into the droplet generator.

Videographer's NOTE: Shots 3.3.1 & 3.3.2 were combined together

3.3.2. Talent covering the cartridge with gasket.

3.3.3. Talent transferring 40 μ L of generated droplets to a 96-well semi-skirted plate.

3.3.4. Talent sealing the plate using plate sealer.

3.4. Configure [1] the thermal cycling profile [2] and set the heated lid to one hundred and five degrees Celsius [3].

3.4.1. Talent setting the program in the PCR unit.

Videographer's NOTE: Shots 3.4.1 & 3.4.3 were combined together

3.4.2. TEXT ON A PLAIN BACKGROUND

PCR conditions:

(a) Bacteria:

- Initial denaturation: 98 °C for 5 minutes
- Denaturation: 94 °C for 30 seconds (40 cycles)
- Annealing: 61 °C for 60 seconds (40 cycles)
- Droplet stabilization: 4 °C for 5 minutes
- Enzyme deactivation: 98 °C for 10 minutes

(b) Fungi:

- Initial denaturation: 95 °C for 5 minutes
- Denaturation: 95 °C for 30 seconds (40 cycles)
- Annealing: 55 °C for 1 minute (40 cycles)
- Extension: 72 °C for 30 seconds (40 cycles)
- Droplet stabilization: 4 °C at 5 minutes
- Enzyme deactivation: 90 °C at 5 minutes

3.4.3. Talent setting the lid temperature to 105 °C

3.5. After completion of PCR, transfer the ninety-six well plate to the plate reader [1]. Using QuantaSoft version one point two [2], adjust the threshold of the amplitude to separate negative and positive droplets [3].

3.5.1. Talent transferring the 96-well plate to a plate reader.

3.5.2. Talent opening the QuantaSoft version 1.2 software.

3.5.3. Talent adjusting the amplitude threshold values for positive and negative droplets.

Videographer's NOTE: Shots 3.5.2 & 3.5.3 were combined together. Take 2 of this shot is the better take to use

NOTE: Please add "Missing LAB Media title card for steps 3.6 and 3.7"

3.6. From each well, evaluate the parameters like number of gene copies per nanogram of DNA [1], total gene copies per nanogram of extracted DNA [2], gene copies per gram dry weight of soil processed in the extraction [3] and cell count per gram dry weight of soil [4].

3.6.1. **SCREEN:** Show the calculation for number of gene copies per ng of DNA.

3.6.2. **SCREEN:** Show the calculation for total gene copies per ng of extracted DNA.

3.6.3. **SCREEN:** Show the calculation for gene copies per gram dry weight of soil processed in the extraction.

3.6.4. **SCREEN:** Show the calculation for cell count per gram dry weight of soil.

3.7. Further, calculate the microbial biomass carbon, or MBC (*M-B-C*) corresponding to bacteria [1] and fungi per gram dry weight of soil per well [2].

3.7.1. **SCREEN:** Show the calculation for microbial biomass carbon corresponding to bacteria or 16S gene copies per gram dry weight of soil.

3.7.2. **SCREEN:** Show the calculation for microbial biomass carbon corresponding to fungi or 18S gene copies per gram dry weight of soil.

Authors: Please provide screen captures showing the calculations for the parameters specified in shots 3.6.1 to 3.6.4, 3.7.1 and 3.7.2.

Authors: Please create screen capture videos for shots labelled as SCREEN, create a screenshot summary, and upload the files to your project page as soon as possible:
<https://review.jove.com/account/file-uploader?src=21187483>

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

Results

4. Results

- 4.1. Triplicate analysis of soil DNA from five locations yielded average microbial DNA concentrations ranging from thirty-two nanograms to four thousand five hundred and ninety nanograms per gram of dry soil per sample [1]. Soil B contained the fewest 16S rRNA gene copies at an average of 8.8×10^7 [2] and 18S rRNA gene copies at an average of 4.6×10^6 per gram of dry soil [3].
 - 4.1.1. LAB MEDIA: Figure 3A: *Video Editor: Please emphasize the respective Y-axis values and the dots corresponding to the soil types "B" and "E" sequentially.*
 - 4.1.2. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the respective Y-axis value and the dot corresponding to the soil type "B".*
 - 4.1.3. LAB MEDIA: Figure 3C: *Video Editor: Please emphasize the respective Y-axis value and the dot corresponding to the soil type "B".*
- 4.2. In contrast, Soil E had the highest counts of 16S and 18S rRNA gene copies with an average of 4.4×10^9 [1] and 2.5×10^9 respectively per gram of dry soil, differing significantly from all other samples [2]. While fungal copies remained similar across soils A, B, C and D [3], bacterial counts in soil D differed significantly from both B and E [4].
 - 4.2.1. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the respective Y-axis value and the dot corresponding to the soil type "E".*
 - 4.2.2. LAB MEDIA: Figure 3C: *Video Editor: Please emphasize the respective Y-axis value and the dot corresponding to the soil type "E".*
 - 4.2.3. LAB MEDIA: Figure 3C: *Video Editor: Please emphasize the dots corresponding to soil types "A", "B", "C" and "D" sequentially.*
 - 4.2.4. LAB MEDIA: Figure 3B: *Video Editor: Please emphasize the dots corresponding to soil type "D".*
- 4.3. The highest microbial content was observed in soil E [1], while the lowest microbial content was found in samples collected from soil B, differing significantly from A, D, and E [2].
 - 4.3.1. LAB MEDIA: Figure 3D: *Video Editor: Please emphasize the respective Y-axis value and the dot corresponding to the soil type "E".*
 - 4.3.2. LAB MEDIA: Figure 3D: *Video Editor: Please emphasize the respective Y-axis value and the dot corresponding to the soil type "B".*

1. Microbial
Pronunciation link: <https://www.merriam-webster.com/dictionary/microbial>
IPA: /maɪˈkraʊ.bi.əl/
Phonetic Spelling: my·kroh·bee·uhl
2. Reagents
Pronunciation link: <https://www.merriam-webster.com/dictionary/reagent>
IPA: /riˈeɪ.dʒənt/
Phonetic Spelling: ree·ay·juhnt
3. Extraction
Pronunciation link: <https://www.merriam-webster.com/dictionary/extraction>
IPA: /ɪkˈstræk.ʃən/
Phonetic Spelling: ik·strak·shuhn
4. Celsius
Pronunciation link: <https://www.merriam-webster.com/dictionary/Celsius>
IPA: /ˈsɛl.si.əs/
Phonetic Spelling: सेल·see·uhs
5. Milliliter
Pronunciation link: <https://www.merriam-webster.com/dictionary/milliliter>
IPA: /ˈmɪl.ə.li.tər/
Phonetic Spelling: mil·uh·lee·ter
6. Bead-beating
Pronunciation link: No confirmed link found
IPA: /ˈbiːd ˌbiː.tɪŋ/
Phonetic Spelling: beed·bee·ting
7. Vortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /ˈvɔr.teks/
Phonetic Spelling: vor·teks
8. Homogenizer
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenizer>
IPA: /həˈmɑː.dʒə.naɪ.zər/
Phonetic Spelling: huh·maa·juh·ny·zer
9. Lyse
Pronunciation link: <https://www.merriam-webster.com/dictionary/lyse>
IPA: /ˈlaɪz/
Phonetic Spelling: lyze
10. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsen.trəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
11. Mechanical
Pronunciation link: <https://www.merriam-webster.com/dictionary/mechanical>
IPA: /məˈkæn.i.kəl/
Phonetic Spelling: muh·kan·ih·kuhl

12. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /,suː.pəˈnei.tənt/
Phonetic Spelling: soo·per·nay·tuhnt
13. Microcentrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/microcentrifuge>
IPA: /,maɪ.kroʊˈsen.trəˌfjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
14. Pellet
Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>
IPA: /ˈpel.ət/
Phonetic Spelling: পেলে·uht
15. Lysate
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysate>
IPA: /ˈlaɪ.seɪt/
Phonetic Spelling: ly·sayt
16. Elution
Pronunciation link: <https://www.merriam-webster.com/dictionary/elution>
IPA: /iˈluːʃən/
Phonetic Spelling: ee·loo·shuhn
17. Membrane
Pronunciation link: <https://www.merriam-webster.com/dictionary/membrane>
IPA: /ˈmem.breɪn/
Phonetic Spelling: mem·brayn
18. Fluorometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorometer>
IPA: /flʊˈrɑː.mə.təʊ/
Phonetic Spelling: floor·rom·uh·ter
19. Quantify
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantify>
IPA: /ˈkwɑːn.təˌfaɪ/
Phonetic Spelling: kwon·tuh·fy
20. Assay
Pronunciation link: <https://www.merriam-webster.com/dictionary/assay>
IPA: /ˈæ.seɪ/
Phonetic Spelling: a·say
21. Quantification
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>
IPA: /ˌkwɑːn.təˌfəˈkeɪ.ʃən/
Phonetic Spelling: kwon·tuh·fuh·kay·shuhn
22. Droplet
Pronunciation link: <https://www.merriam-webster.com/dictionary/droplet>
IPA: /ˈdrɑː.plət/
Phonetic Spelling: draa·pluht

23. Polymerase
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>
IPA: /pəˈlɪm.əˌreɪs/
Phonetic Spelling: puh·lim·uh·rays
24. Thawing
Pronunciation link: <https://www.merriam-webster.com/dictionary/thaw>
IPA: /ˈθɑː.ɪŋ/
Phonetic Spelling: thaw·ing
25. Nuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
IPA: /ˈnuː.kliˌeɪs/
Phonetic Spelling: noo·klee·ays
26. Cartridge
Pronunciation link: <https://www.merriam-webster.com/dictionary/cartridge>
IPA: /ˈkɑːr.trɪdʒ/
Phonetic Spelling: kar·trɪj
27. Evagreen
Pronunciation link: No confirmed link found
IPA: /ˈiː.vəˌɡriːn/
Phonetic Spelling: ee·vuh·green
28. Gasket
Pronunciation link: <https://www.merriam-webster.com/dictionary/gasket>
IPA: /ˈɡæs.kət/
Phonetic Spelling: gas·kuht
29. Semi-skirted
Pronunciation link: No confirmed link found
IPA: /ˌsem.iˈskɜː.tɪd/
Phonetic Spelling: sem·ee skirt·id
30. Amplification
Pronunciation link: <https://www.merriam-webster.com/dictionary/amplification>
IPA: /ˌæm.plə.fəˈkeɪ.ʃən/
Phonetic Spelling: am·pluh·fuh·kay·shuhn
31. Thermal
Pronunciation link: <https://www.merriam-webster.com/dictionary/thermal>
IPA: /ˈθɜː.məl/
Phonetic Spelling: thur·muhl
32. Denaturation
Pronunciation link: <https://www.merriam-webster.com/dictionary/denaturation>
IPA: /diːˌneɪ.tʃəˈeɪ.ʃən/
Phonetic Spelling: dee·nay·chur·ay·shuhn
33. Annealing
Pronunciation link: <https://www.merriam-webster.com/dictionary/anneal>
IPA: /əˈniː.lɪŋ/
Phonetic Spelling: uh·nee·luhng

34. Stabilization

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

IPA: /,steɪ.bə.ləˈzeɪ.ʃən/

Phonetic Spelling: stay·buh·luh·zay·shuhn

35. Deactivation

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

IPA: /diˌæk.təˈveɪ.ʃən/

Phonetic Spelling: dee·ak·tuh·vay·shuhn

36. QuantaSoft

Pronunciation link: No confirmed link found

IPA: /ˈkwɑːn.təˌsɒft/

Phonetic Spelling: kwon·tuh·soft

37. Microbial biomass carbon

Pronunciation link: No confirmed link found

IPA: /maɪˈkroʊ.bi.əl ˈbaɪ.əʊˌmæs ˈkɑːr.bən/

Phonetic Spelling: my·kroh·bee·uhl by·oh·mass kar·bun

38. Triplicate

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

IPA: /ˈtrɪp.lə.kət/

Phonetic Spelling: trip·luh·kuht

39. Nanogram

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

IPA: /ˈnæn.əʊˌgræm/

Phonetic Spelling: nan·oh·gram

40. Fungal

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

IPA: /ˈfʌŋ.gəl/

Phonetic Spelling: fung·guhl