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Title: Whole-Genome Deoxyribonucleic Acid Extraction from *Mycobacterium* Species via the Cetyltrimethylammonium Bromide Technique

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: 08/25/2025

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 21
Number of Shots: 37

Introduction

NOTE: 1.1. has been modified

1.1. **Shatha Omar:** We investigate pathogenic mycobacteria, focusing on genetic characterization, speciation, virulence genes, and drug resistance-linked SNPs to better understand disease complexity, inform infection mechanisms, and guide therapeutic strategies.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **Shatha Omar:** Due to the thick cell wall, lipid-rich, and hydrophobic cell walls of mycobacteria, DNA extraction has been challenging and requires specialized lysis techniques to break them down and efficiently release the genomic DNA.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What research gap are you addressing with your protocol?

1.3. **Brendon Mann:** In our protocol, we are addressing DNA extraction from mycobacteria species using the CTAB method as a robust and effective method to produce high-quality DNA that is suitable for whole-genome sequencing and other molecular techniques.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1*

Protocol

2. Cell Wall Digestion and DNA Extraction

Demonstrator: Shatha Omar

2.1. To begin, heat kill the liquid culture taken in a 15-milliliter tube at 80 degrees Celsius for 1 hour **[1]**. Then, place the 15-milliliter tube in the centrifuge and spin at 3,220 *g* for 15 to 30 minutes at room temperature **[2]**. Using a sterile pipette, discard the clear supernatant, ensuring that all the media is removed **[3]**.

2.1.1. WIDE: Talent placing the 15 milliliter tube with culture in a heat- block or incubator.

2.1.2. Talent placing the tube into the centrifuge and starting the spin.

2.1.3. Talent removing the tube from the centrifuge and carefully discarding the clear supernatant using a sterile pipette or a sterile Pasteur pipette.

2.2. Resuspend the culture pellet thoroughly in 300 microliters of Tris-EDTA buffer **[1]** and transfer the resuspended pellet into a 2-milliliter tube **[1]**.

2.2.1. Talent pipetting the Tris-EDTA buffer into the tube and resuspending the pellet by pipetting up and down.

2.2.2. Talent transferring the resuspended pellet into a clean 2 milliliter tube.

2.3. Now, add 100 microliters of lysozyme solution at a concentration of 10 milligrams per milliliter to the tube **[1]** and mix by pipetting up and down 5 times **[2]**. Tap the tube gently to ensure uniform dispersion **[3]** and place the tube in a rotary incubator at 37 degrees Celsius and incubate overnight **[4]**.

2.3.1. Talent adding lysozyme solution to the sample tube.

2.3.2. Talent pipetting the mixture up and down 5 times.

2.3.3. Talent tapping the tube gently to mix.

2.3.4. Talent placing the tube into a rotary incubator set to 37 degrees Celsius.

2.4. The following day, prepare a mixture of 5 microliters of proteinase K at a concentration of 10 milligrams per milliliter and 70 microliters of 10% SDS **[1]**. Add 75 microliters of this mixture to each sample **[2]**.

2.4.1. Talent mixing the proteinase K and sodium dodecyl sulfate solution in a small tube by inverting.

2.4.2. Talent adding the prepared mixture to the sample tube.

2.5. Mix the sample by tapping the tube **[1]** and incubate at 65 degrees Celsius for 10 minutes, intermittently inverting or tapping the tube to mix **[2]**.

2.5.1. Talent tapping the sample tube to mix.

2.5.2. Talent placing the tube into an incubator or a heating block set to 65 degrees Celsius.

2.6. Next, add 100 microliters of 5 molar sodium chloride to the sample **[1]**, followed by 100 microliters of preheated CTAB-sodium chloride solution preheated to a temperature of 65 degrees Celsius to the sample **[2-TXT]**.

2.6.1. Talent adding sodium chloride solution to the sample tube.

2.6.2. Add Talent adding preheated CTAB/sodium chloride solution to the sample tube. **TXT: CTAB: Cetyltrimethylammonium Bromide**

2.7. Mix the sample by tapping until the solution becomes milky **[1]**.

2.7.1. Talent tapping the tube and showing the solution turning milky.

2.8. Place the tube in an incubator at 65 degrees Celsius for 10 minutes **[1]**, intermittently inverting or tapping the tube to mix **[2]**.

2.8.1. Talent placing the tube into an incubator or a heating block set to 65 degrees Celsius.

2.8.2. Talent inverting or tapping the tube in the incubator.

2.9. Now, add an equal volume, approximately 675 microliters, of chloroform-isoamyl alcohol solution in a ratio of 24 to 1 to the sample **[1]** and mix by tapping **[2]**.

2.9.1. Talent adding chloroform/isoamyl alcohol solution to the sample tube.

2.9.2. Talent tapping the tube to mix the contents.

2.10. Place the sample in a centrifuge and spin at 12,000 *g* for 10 minutes at room temperature **[1]**.

2.10.1. Talent placing the sample tube into the centrifuge and starting the spin.

2.11. Then, using a pipette, carefully aspirate between 550 to 600 microliters of the aqueous top phase into sterile 1.5 milliliter tubes **[1]** and label them appropriately **[2]**.

2.11.1. Talent aspirating the top aqueous phase into sterile 1.5 milliliter tubes.

2.11.2. Talent labelling the tubes.

3. DNA Precipitation and Elution

3.1. Add 550 to 600 microliters of ice-cold isopropanol to each tube **[1]** and mix the contents by inverting the tube several times **[2]**.

3.1.1. Talent adding ice-cold isopropanol to the sample tube.

3.1.2. Talent inverting the tube multiple times to mix the contents.

3.2. Place the tube in a freezer set to minus 20 degrees Celsius and incubate for 30 minutes to 1 hour **[1]**.

3.2.1. Talent placing the tube into a minus 20 degrees Celsius freezer.

3.3. Next, centrifuge the tube at the highest speed, approximately 21,130 *g*, for 30 minutes at room temperature to pellet the insoluble DNA **[1]**.

3.3.1. Talent placing the tube into the centrifuge and starting the spin.

3.4. Using a pipette, aspirate the supernatant from the front side of the tube without disturbing the DNA pellet **[1-TXT]**.

3.4.1. Talent carefully aspirating the supernatant from the front side of the tube with a pipette. **TXT: Alternatively, decant the supernatant**

3.5. Now, add 1,000 microliters of ice-cold 75 percent ethanol to the pellet **[1]** and mix the sample by inverting the tube several times **[2]**.

3.5.1. Talent adding ice-cold 75 percent ethanol to the tube containing the DNA pellet.

3.5.2. Talent inverting the tube repeatedly to mix the pellet with ethanol.

3.6. Centrifuge the sample at 12,000 *g* for 30 minutes in the same orientation as previously used **[1]**.

3.6.1. Talent placing the tubes into the centrifuge in the same orientation and starting the spin.

3.7. After centrifugation, aspirate or decant all ethanol without disturbing the pellet **[1-TXT]**.

3.7.1. Talent carefully aspirating the supernatant from the front side of the tube. **TXT: Alternatively, decant the supernatant quickly**

3.8. Allow the tube to air dry at room temperature overnight or for at least 30 minutes **[1]**.

3.8.1. Talent placing the open tubes on the bench to air dry.

3.9. Then, add between 25 to 50 microliters of Tris-EDTA buffer at pH 8 to resuspend the DNA **[1]** and place the tube at 4 degrees Celsius overnight **[1]**.

3.9.1. Talent adding Tris-EDTA buffer to the dried DNA pellet.

3.9.2. Talent placing the tube into a refrigerator set to 4 degrees Celsius.

3.10. After quality control assessment **[1]**, place the tube in a freezer set to minus 80 degrees Celsius for long-term storage **[2]**.

3.10.1. Talent loading the sample into a spectrophotometer.

3.10.2. Talent placing the tubes into a minus 80 degrees Celsius freezer.

Results

4. Results

4.1. DNA yield obtained using the CTAB extraction method ranged between 190 nanograms per microliter and 600 nanograms per microliter **[1]**.

4.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the nucleic acid column*.

4.2. The 260 by 280 absorbance ratio ranged between 1.9 and 2.0 **[1]**, and the 260 by 230 ratio ranged between 1.8 and 2.2, indicating high-purity DNA **[2]**.

4.2.1. LAB MEDIA: Table 1. *Video editor: Highlight the "260/280" column*.

4.2.2. LAB MEDIA: Table 1. *Video editor: Highlight the "260/230" column*.

4.3. A single absorbance peak was detected at 260 nanometers **[1]**.

4.3.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the tall peak exactly at "260" on the x-axis*.

4.4. Agarose gel electrophoresis of high-quality samples showed a distinct, intact, high molecular weight DNA band with minimal degradation **[1]**.

4.4.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the bright, thick band near the top of the gel lane*.

4.5. In some non-tuberculosis mycobacterium species, DNA yield was less than 50 nanograms per microliter **[1]** and purity ratios fell below the ideal range, indicating contamination **[2]**.

4.5.1. LAB MEDIA: Table 2. *Video editor: Highlight the nucleic acid column*.

4.5.2. LAB MEDIA: Table 2. *Video editor: Highlight the purity 260/280 column*.

1. **centrifuge**
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
   IPA: /ˈsɛntrəˌfjuːdʒ/
   Phonetic Spelling: SEN-truh-fyoog
2. **supernatant**
   Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
   IPA: /ˌsuːpərˈneɪtənt/
   Phonetic Spelling: soo-per-NAY-tunt
3. **resuspend / resuspension**
   – **resuspend**: Pronunciation link: https://www.merriam-webster.com/dictionary/resuspend
   IPA: /ˌriːsəˈspɛnd/
   Phonetic Spelling: ree-suh-SPEND
   – **resuspension**: /ˌriːsəˈspɛnʃən/ — ree-suh-PEN-shun
4. **Tris-EDTA**
   – **Tris**: /traɪs/ (TRYS)
   – **EDTA**: pronounced /iːˌdiːˈtiːə/ (ee-dee-TEE-uh)
   (Often "Tris-EDT-uh")
5. **lysozyme**
   Pronunciation link: https://www.howtopronounce.com/lysozyme (howtopronounce.com)
   IPA: /ˈlaɪsəzaɪm/
   Phonetic Spelling: LY-soh-zime
6. **proteinase K**
   – **proteinase**: /ˌproʊtiˈneɪs/ (proh-tee-NAYS)
   – **K**: /keɪ/ (kay)
7. **sodium dodecyl sulfate (SDS)**
   – **dodecyl**: /ˈdoʊdəsɪl/ (doh-duh-sil)
   – **sulfate**: /ˈsʌlfeɪt/ (SUL-fate)
8. **CTAB** (Cetyltrimethylammonium bromide)
   Pronunciation link: https://www.howtopronounce.com/ctab (howtopronounce.com)
   Also see Wiktionary: /ˈsiːtæb/ (Wiktionary)
   IPA: /ˈsiːtæb/
   Phonetic Spelling: SEE-tab
9. **chloroform-isoamyl alcohol**
   – **chloroform**: /ˈklɔːroʊˌfɔrm/ (KLOR-oh-form)
   – **isoamyl**: /aɪˈsoʊˌæməl/ (eye-SOH-am-ul)
   – **alcohol**: /ˈælkəˌhɔl/ (AL-kuh-hol)
10. **isopropanol**

Pronunciation link: https://www.merriam-webster.com/dictionary/isopropanol
IPA: /ˌaɪsoʊˈprɑpənɒl/
Phonetic Spelling: eye-SOH-pro-puh-nol

11. **agarose**
    Pronunciation link: https://www.merriam-webster.com/dictionary/agarose
    IPA: /əˈgeɪroʊs/
    Phonetic Spelling: uh-GAY-ros
12. **nanogram**
    Pronunciation link: https://www.merriam-webster.com/dictionary/nanogram
    IPA: /ˈnænəˌgræm/
    Phonetic Spelling: NAN-oh-gram
13. **absorbance**
    Pronunciation link: https://www.merriam-webster.com/dictionary/absorbance
    IPA: /əbˈzɔːrbəns/
    Phonetic Spelling: ab-ZOR-buns
14. **electrophoresis**
    Pronunciation link: https://www.merriam-webster.com/dictionary/electrophoresis
    IPA: /ɪˌlɛktroʊfəˈriːsɪs/
    Phonetic Spelling: ih-LEK-troh-foh-REEsis
