Submission ID #: 68147
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20784883

Title: Matrix-Based DNA Extraction for Targeted Next-Generation Sequencing on Decontaminated Sputum Samples

Authors and Affiliations:

Jennifer Williams[1*], Janré Steyn[1*], Emilyn Costa Conceição[1*], Felicia Bernita Wells[1], Melanie Grobbelaar[1], Nabila Ismail[1], Yonas Ghebrekristos[1,2], Christoffel Johannes Opperman[1,2,3,4], Sarishna Singh[1,2], Jason Limberis[5], Fahd Naufal[5], Brendon Coenrad Mann[1], Rebecca E. Colman[6,7], Timothy Rodwell[6,7*], Robin Mark Warren[1*], John Z. Metcalfe[5*]

[1]Division of Molecular Biology and Human Genetics, SAMRC Centre for TB Research, DSI-NRF Centre of Excellence for Biomedical TB Research, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University
[2]National Health Laboratory Service, Green Point Tuberculosis Laboratory
[3]Division of Medical Microbiology, Department of Pathology, University of Cape Town
[4]Division of Medical Microbiology, Department of Pathology, University of Cape Town
[5]Division of Pulmonary and Critical Care Medicine, Zuckerberg San Francisco General Hospital and Trauma Centre, University of California, San Francisco
[6]Department of Medicine, University of California, San Diego, School of Medicine
[7]Foundation for Innovative New Diagnostics (FIND), Campus Biotech

*These authors contributed equally

Corresponding Authors:
Jennifer Williams williamsj@sun.ac.za
Janré Steyn janre@sun.ac.za
Emilyn Costa Conceição emilyncosta@sun.ac.za

Email Addresses for All Authors:

Jennifer Williams	williamsj@sun.ac.za
Janré Steyn	janre@sun.ac.za
Emilyn Costa Conceição	emilyncosta@sun.ac.za
Felicia Bernita Wells	fbwells@sun.ac.za
Melanie Grobbelaar	melgrob@sun.ac.za
Nabila Ismail	nabilai@sun.ac.za
Yonas Ghebrekristos	yonasg@sun.ac.za
Christoffel Johannes Opperman	14487616@sun.ac.za
Sarishna Singh	14378183@sun.ac.za
Jason Limberis	jason.limberis@ucsf.edu
Fahd Naufal	fahd.naufal@ucsf.edu
Brendon Coenrad Mann	bcmann@sun.ac.za
Rebecca Colman	rebecca.colman@finddx.org
Timothy Rodwell	trodwell@health.ucsd.edu
Robin Mark Warren	rw1@sun.ac.za
John Metcalfe	john.metcalfe@ucsf.edu

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: **05/05/2025**

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

Current Protocol Length

Number of Steps: 13
Number of Shots: 28

Introduction

1.1. **Felicia Wells:** Our research is about reducing the turn-around time of TB diagnosis and subsequent treatment initiation by incorporating NGS for personalized patient care.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll: 3.2*

What research gap are you addressing with your protocol?

1.2. **Felicia Wells:** Our protocol optimizes tNGS for routine use by addressing workflow integration, cost, and turnaround time, aiming to reduce diagnostic delays and improve timely treatment initiation **[1]**.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.3. **Janré Steyn:** This protocol allows for cost-effective, rapid and sufficient quality DNA extraction from clinical samples within hours, compared to the conventional CTAB method which takes up to 3 days **[1]**.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What new scientific questions have your results paved the way for?

1.4. **Jennifer Williams:** Our results raise key questions on tNGS integration into routine diagnostics and optimizing extraction methods for smear-negative, scanty samples to improve sensitivity and clinical utility **[1]**.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll: 3.3*

What research questions will your laboratory focus on in the future?

1.5. **Jennifer Williams:** We will explore whether remnant samples from routine diagnostics can be used for downstream sequencing, including direct whole genome sequencing of sputum, to streamline and expand TB genomic surveillance **[1]**.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by Research Ethics Committee: Biological and Environmental Safety (REC: BES) at Stellenbosch University

Protocol

2. DNA Extraction and Purification from Sputum Samples Using Magnetic Beads

Demonstrators: Janré Steyn, Jennifer Williams

2.1. To begin, obtain a heat-inactivated sputum sediment **[1]**. Centrifuge the sample at 16,800 *g* or full speed for 15 minutes **[2]**. With a 20 to 200 microliter pipette, gently aspirate and discard the supernatant, ensuring the pellet remains undisturbed **[3]**.

2.1.1. WIDE: Talent holding a heat-inactivated sputum sediment sample.

2.1.2. Talent placing the sample tubes into the centrifuge.

2.1.3. Talent using a pipette to carefully remove and discard the supernatant without disturbing the pellet.

2.2. Gently shake the matrix suspension to mix **[1]**. Then pipette 200 microliters of the well-mixed matrix to resuspend the pellet **[2]**.

2.2.1. Talent gently shaking the matrix suspension.

2.2.2. Talent pipetting 200 microliters of matrix into the tube and resuspending the pellet.

2.3. Transfer the full volume into a 1.5-milliliter screw-cap tube containing three presterilized glass beads of 2-millimeter diameter **[1]**.

2.3.1. Talent transferring the resuspended sample into a screw-cap tube with glass beads.

2.4. Now, place the samples in a heating block at 56 degrees Celsius for 15 minutes **[1]**. After incubation, homogenize the samples with a vortex for 10 seconds to disperse the cells **[2]**. Then incubate the samples in a heating block at 100 degrees Celsius for 10 minutes **[3]**.

2.4.1. Talent placing tubes into the heating block set to 56 degrees Celsius.

2.4.2. Talent vortexing the tubes for 10 seconds to homogenize the sample.

2.4.3. Talent placing tubes into the heating block set to 100 degrees Celsius.

2.5. Homogenize the samples using a high-speed homogenizer with one cycle of 60 seconds at 4.0 meters per second **[1]**. Then, centrifuge the suspension at 12,000 *g* for 5 minutes **[2]**.

2.5.1. Talent placing the tube in the homogenizer and starting the cycle.

2.5.2. Talent placing the samples into the centrifuge and setting the spin parameters.

2.6. Transfer 130 microliters of the DNA-containing supernatant to a fresh 1.5-milliliter low-binding tube without disturbing the pellet **[1]**. Discard the first tube containing the matrix and cell debris **[2]**.

2.6.1. Talent using a pipette to carefully transfer 130 microliters of the supernatant into a new tube.

2.6.2. Talent discarding the first tube containing the matrix and cell debris.

2.7. To purify the DNA using magnetic beads, first vortex the magnetic bead stock bottle or prepared aliquot thoroughly to resuspend the beads before use **[1-TXT]**. Then add 1.2 times the volume of magnetic beads to the extracted DNA **[2]**.

2.7.1. Talent vortexing the magnetic bead stock bottle. **TXT: Repeat vortexing after every 10 samples to ensure homogenous suspension**

2.7.2. Talent pipetting 156 microliters of magnetic beads into the DNA sample.

2.8. Pipette the suspension 10 times to mix the samples before incubating at room temperature for 5 minutes **[1]**. Place the samples on a 1.5-milliliter tube magnetic rack for 3 minutes or until the liquid becomes clear **[2]**. Then carefully aspirate and discard the supernatant without disturbing the beads **[3]**.

2.8.1. Talent mixing the sample by pipetting up and down and setting a timer for 5 min

2.8.2. Talent placing the tubes on the magnetic rack.

2.8.3. Talent using a pipette to carefully remove the supernatant.

2.9. With the tubes on the magnet, add 200 microliters of 80% ethanol, ensuring the beads remain undisturbed **[1]**. After a 30-second incubation, carefully aspirate and discard the ethanol without disturbing the beads **[2-TXT]**. After the second wash, remove any residual ethanol using a 1 to 10 microliter pipette **[3]**.

2.9.1. Talent pipetting ethanol into the tube while keeping the beads undisturbed.

2.9.2. Talent removing ethanol using a pipette. **TXT: Repeat ethanol wash once more**

2.9.3. Talent using a pipette to remove residual ethanol.

2.10. Leave the tubes open to air dry the beads for 10 minutes or until they have a matte appearance **[1]**.

2.10.1. Talent leaving the tubes open to allow beads to dry.

2.11. Once the beads have a matte appearance, remove the tubes from the magnet **[1]** and add 50 microliters of nuclease-free water directly onto the beads in each sample **[2]**.

2.11.1. Shot of the tubes being removed from the magnet.

2.11.2. Talent adding 50 µL of nuclease-free water directly onto the beads.

2.12. Then, mix each individual sample by pipetting 10 times **[1]**. Inspect the tube for any beads stuck inside **[2-TXT].**

2.12.1. Talent pipetting to mix the sample.

2.12.2. Talent checking for beads stuck to the tube walls. **TXT: Repeat mixing if beads remain on the tube wall**

2.13. After a 5-minute incubation at room temperature, place the tubes back on the magnetic rack for 3 minutes or until the liquid is clear **[1]**. With the tubes on the magnetic rack, transfer the DNA-containing supernatant to a clearly marked, sterile, low-binding tube **[2-TXT]**.

2.13.1. Talent placing the tubes on the magnetic rack.

2.13.2. Talent carefully transferring the DNA supernatant to a fresh tube. **TXT: Ensure that beads are not transferred to the supernatant**

Results

3. Results

3.1. DNA concentration was higher in 2-milliliter sediment samples compared to 500 microliters across all smear grades **[1]**. Greater variability in DNA yield was observed in 500-microliter sediment samples **[2]**.

3.1.1. LAB MEDIA: Figure 4.
Video Editor: Please highlight the boxes of 2 mL

3.1.2. LAB MEDIA: Figure 4.
Video Editor: Please highlight the boxes of 500 µL

3.2. Coverage depth of sequencing reads was higher in 2-milliliter sediment samples compared to 500 microliters across all smear grades **[1]**. Variability was greater in 3+ *(three-plus)* smear grade samples extracted from 500-microliter sediment **[2]**.

3.2.1. LAB MEDIA: Figure 5.
Video Editor: Please highlight the boxes of 2 mL

3.2.2. LAB MEDIA: Figure 5.
Video Editor: Please highlight the green box of 500 µL

3.3. Sequencing acceptability scores were generally higher in 2-milliliter samples compared to 500 microliters, with a greater proportion of highly acceptable results **[1]**. Smear grade 3+ samples had the highest proportion of acceptable results compared to smear grades 1+ and 2+ **[2]**. The 500-microliter samples had more cases classified as unacceptable or not determined **[3]**.

3.3.1. LAB MEDIA: Figure 6 *Video Editor: Please highlight the +++ columns of 2 mL*

3.3.2. LAB MEDIA: Figure 6 *Video Editor: Please sequentially highlight the green columns then the red and blue columns*

3.3.3. LAB MEDIA: Figure 6 *Video Editor: Please highlight the – and ND columns of 500 µL*

Pronunciation Guide:

1. Sputum
   - Pronunciation link: https://www.merriam-webster.com/dictionary/sputum
   - IPA: /ˈspjuːtəm/
   - Phonetic Spelling: spyoo-tuhmmerriam-webster.commerriam-webster.com+11merriam-webster.com+11merriam-webster.com+11

2. Centrifuge
   - Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
   - IPA: /ˈsɛntrəˌfjuːdʒ/
   - Phonetic Spelling: sen-truh-fyoojmerriam-webster.commerriam-webster.com

3. Pipette
   - Pronunciation link: https://www.merriam-webster.com/dictionary/pipette
   - IPA: /paɪˈpɛt/
   - Phonetic Spelling: pie-petmerriam-webster.com+1merriam-webster.com+1

4. Supernatant
   - Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
   - IPA: /ˌsuːpərˈneɪtənt/
   - Phonetic Spelling: soo-per-nay-tuhntmerriam-webster.com+1merriam-webster.com+1merriam-webster.com+1merriam-webster.com+1

6. Homogenize
   - Pronunciation link: https://www.merriam-webster.com/dictionary/homogenize
   - IPA: /həˈmɑːdʒəˌnaɪz/
   - Phonetic Spelling: huh-mah-juh-nize

7. Vortex
   - Pronunciation link: https://www.merriam-webster.com/dictionary/vortex
   - IPA: /ˈvɔːrtɛks/
   - Phonetic Spelling: vor-teksmerriam-webster.com+1merriam-webster.com+1merriam-webster.com

8. Homogenizer
   - Pronunciation link: https://www.merriam-webster.com/dictionary/homogenizer
   - IPA: /həˈmɑːdʒəˌnaɪzər/
   - Phonetic Spelling: huh-mah-juh-nize-ermerriam-webster.com

9. Nuclease
   - Pronunciation link: https://www.merriam-webster.com/dictionary/nuclease
   - IPA: /ˈnuːkliˌeɪs/

- Phonetic Spelling: noo-klee-acemerriam-webster.com

10. Aliquot
    - Pronunciation link: https://www.merriam-webster.com/dictionary/aliquot
    - IPA: /ˈælɪkwət/
    - Phonetic Spelling: al-ih-kwot

12. Sediment
    - Pronunciation link: https://www.merriam-webster.com/dictionary/sediment
    - IPA: /ˈsɛdəmənt/
    - Phonetic Spelling: sed-uh-muhnt

14. Debris
    - Pronunciation link: https://www.merriam-webster.com/dictionary/debris
    - IPA: /dəˈbriː/
    - Phonetic Spelling: duh-breemerriam-webster.com

15. Magnetic
    - Pronunciation link: https://www.merriam-webster.com/dictionary/magnetic
    - IPA: /mægˈnɛtɪk/
    - Phonetic Spelling: mag-net-ikmerriam-webster.com+4merriam-webster.com+4merriam-webster.com+4

18. Matte
    - Pronunciation link: https://www.merriam-webster.com/dictionary/matte
    - IPA: /mæt/
    - Phonetic Spelling: mat

19. Pellet
    - Pronunciation link: https://www.merriam-webster.com/dictionary/pellet
    - IPA: /ˈpɛlɪt/
    - Phonetic Spelling: pel-it

20. Suspension
    - Pronunciation link: https://www.merriam-webster.com/dictionary/suspension
    - IPA: /səˈspɛnʃən/
    - Phonetic Spelling: suh-spen-shun