

Oct 28, 2024

Version 1

🌐 **Mycobacterium tuberculosis DNA Extraction Using InstaGene Matrix and High Speed Homogenizer from Clinical Primary Culture V.1**

📖 [The Lancet. Microbe](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvr8rwzlmk/v1>

Emilyn Conceição¹, Felicia Wells¹, Jennifer Williams¹, Nabila Ismail¹, john.metcalfe², Annelies van Rie³, Robin Mark Warren¹

¹South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa; ²Division of Pulmonary and Critical Care Medicine, University of California, San Francisco; ³University Antwerp

Emilyn Conceição: Conception, design, evaluation and validation of the protocol; acquisition of data; protocol writing and graphic visualization edition.;

Felicia Wells: Validation, acquisition of data, protocol edition and final revision.;

Jennifer Williams: Acquisition of data, protocol edition and final revision.

Nabila Ismail: Protocol edition and final revision.

john.metcalfe : Supervision, resources (reagents, materials, and equipment) used in the protocol, and approval of the submitted version.

Annelies van Rie: Design, supervision, resources (reagents, materials, and equipment) used in the protocol, and approval of the submitted version.

Robin Mark Warren: Design, supervision, resources (reagents, materials, and equipment) used in the protocol, and approval of the submitted version.



Emilyn C Conceição

Stellenbosch University



Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account



DOI: <https://dx.doi.org/10.17504/protocols.io.6qpvr8rwzlmk/v1>

External link: <https://doi.org/10.1016/j.lanmic.2025.101293>

Protocol Citation: Emilyn Conceição, Felicia Wells, Jennifer Williams, Nabila Ismail, John Metcalfe, Annelies van Rie, Robin Mark Warren 2024. Mycobacterium tuberculosis DNA Extraction Using InstaGene Matrix and High Speed Homogenizer from Clinical Primary Culture. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.6qpvr8rwzlmk/v1>

Manuscript citation:

Steyn J, Williams J, Naufal F, Limberis J, Grobbelaar M, Ismail N, Ghebrekristos Y, Opperman CJ, Nalyvayko A, Singh S, Colman RE, Rodwell TC, Theron G, Warren RM, Metcalfe JZ (2026) Bedaquiline resistance in patients with Xpert MTB/RIF Ultra-tested rifampicin-resistant tuberculosis in the Western Cape, South Africa: a prospective study. *The Lancet. Microbe* 7(3). doi: [10.1016/j.lanmic.2025.101293](https://doi.org/10.1016/j.lanmic.2025.101293)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: July 25, 2024

Last Modified: October 28, 2024

Protocol Integer ID: 104082

Keywords: Tuberculosis, Mycobacterium tuberculosis, Mycobacteria, DNA extraction, DNA isolation, Whole genome sequencing, Next generation sequencing, Liquid culture, MGIT, Mycobacteria Growth Indicator Tube, Instagene matrix, Fastprep, DNA yield, Sequencing, Molecular Biology, Clinical Primary Culture, High speed homogenizer, extracting mycobacterium tuberculosis dna, mycobacterium tuberculosis dna, dna extraction, alternative approach for dna extraction, high speed homogenizer from clinical primary culture, dna yield, mycobacteria growth indicator tube, sequencing analysis, solid culture sample use, genomic dna, chelating resin, dna, clinical primary culture, generation sequencing, using instagene matrix, genome sequencing, extraction, use of igm, high speed homogenizer, instagene matrix, liquid culture, target genome, routine diagnostic laboratory workflow

Funders Acknowledgements:

FWO TBM (Applied Biomedical Research with a Primary Social finality)

Grant ID: T001018N

FWO Odysseus

Grant ID: G0F8316N

Global Health EDCTP3 Joint Undertaking and its members as well as Bill & Melinda Gates Foundation - GenPath Africa

Grant ID: GA N° 101103171

NIH (TS Eliot)

Grant ID: R01AI153213



Disclaimer

This work was independently developed and is not affiliated with BD (MGIT), Bio-Rad (InstaGne Matrix), MP Biomedicals (FastPrep) or Benchmark Scientific (BeadBlaster 24). The methods are based on a comprehensive literature review, comparative analysis, and optimization developed at the TB Genomics research group at Stellenbosch University, Cape Town, South Africa as part of the TORCH, GenPath and TS Eliot consortiums.

Abstract

The conventional method for extracting *Mycobacterium tuberculosis* DNA, which involves the use of Cetyltrimethylammonium-bromide (CTAB) (Van Embden, 1993), is both labor-intensive and challenging to implement in routine diagnostic laboratory workflows. Shea et al. (2017) introduced an alternative approach for DNA extraction from clinical primary cultures (CPC) intended for Whole-Genome Sequencing (WGS). This alternative method utilizes the InstaGene Matrix (IGM) (Bio-Rad Laboratories, USA) which was modified and included the use of high-speed homogenization (HSH) through bead-beating such as FastPrep (FP) to improve efficiency and DNA yield.

IGM is an optimized Chelex-100 resin designed to effectively bind metal ions such as Mg^{2+} , which serves as essential cofactors in enzymatic reactions. IGM comprises 6% w/v Chelex chelating resin, incorporated during the 'pre-incubation' phase (at 56 °C for 30 minutes) to aid in the gentle disruption of plasma membranes and the disintegration of cell clumps, a task that cannot be accomplished solely with Chelex-100 chelating resin (Kang et al., 2018).

In the context of Next-Generation Sequencing (NGS) techniques such as WGS or targeted NGS (tNGS), it is essential to incorporate a pre-treatment step before DNA extraction when working with CPC samples. This pre-treatment step is necessary to remove any potential contaminants that might be present in CPC samples that could adversely affect downstream sequencing analyses. For example, sequencing more of the contaminants' genomes than the target genome (WGS) or genes (tNGS) of *M. tuberculosis*. Initially, the IGM was primarily designed for solid culture sample use; thus, genomic DNA could be obtained from *M. tuberculosis* cultured on solid media such as 7H11 or Löwenstein-Jensen (LJ), as well as from liquid media such as 7H9, which includes the Mycobacteria Growth Indicator Tube (MGIT) (Becton Dickinson, Sparks, MD) media. This protocol describes the use of IGM/HSH for solid or liquid cultures and for WGS and/or tNGS applications as per Figure 1.

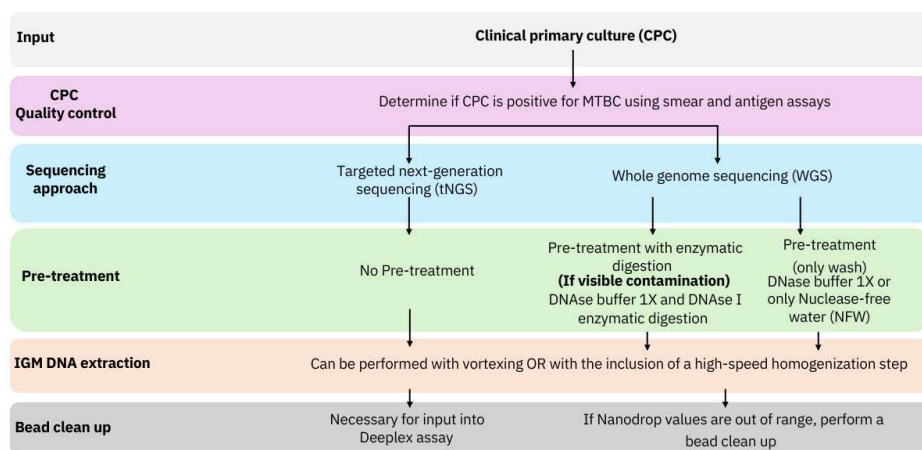


Figure 1. Workflow for DNA extraction from clinical primary cultures (CPC) for targeted Next-Generation Sequencing (tNGS) or Whole-Genome Sequencing (WGS).

Image Attribution

Mycobacterium tuberculosis clinical primary-MGIT culture (CPC) image captured by Emilyn Costa Conceição within the Stellenbosch University Biosafety-level 3 (BSL3) facility, Tygerberg Campus, Cape Town.

Guidelines

Scope

This protocol describes the workflow and procedures to be followed when extracting genomic DNA from *Mycobacterium tuberculosis* cultured in liquid media or on solid media using the InstaGeneTM Matrix (IGM) commercial kit method in combination with bead beating using a High-Speed Homogenizer (HSH).

Risk Management

Risks involved when performing the procedure described in this protocol include working with infectious *Mycobacterium tuberculosis* complex (MTBC) bacteria and working with harmful chemicals. To avoid or reduce these risks, in the BSL3 facility, correct personal protective equipment (PPE) must be donned. This includes gloves, shoe covers, oversleeves, a lab coat, and a powered air-purifying respirator (PAPR) mask. Furthermore, cultures are processed in a Level II laminar flow hood, which is serviced bi-annually, to ensure the safety of the person performing the extraction and the laboratory as a whole, while preventing contamination of the samples. Before the MTBC samples are removed from the BSL3, cultures on solid samples are heat-inactivated at 80°C for 2 h and cultures in liquid media for 1 h at 80°C. Outside the BSL3, all disposable materials used during the DNA extraction are disposed of in a hazardous waste drum. Laboratory coats, surgical gloves and closed shoes are to be always worn in the laboratory. The IGM product contains no substances that are health hazardous at their given concentrations.

Materials

1) IF PERFORMING DNA EXTRACTION FOR WGS AND tNGS

1.1) Equipments

- 1. Class II Biological Safety Cabinet (BSC).
- 2. Incubator (heating block) raging from

🌡️ 65 °C

 to

🌡️ 100 °C

 .
- 3. Incubator

🌡️ 80 °C

 (ideally an oven incubator inside BSL3).
- 4. Centrifuge (for 15 mL tubes).
- 5. Microcentrifuge (for 1.5 or 2 mL tubes).
- 6. Vortex.
- 7. Pipettes (P1000, P200, P100 and P10).
- 8. Qubit Fluorometer instrument.
- 9. Nanodrop spectrophotometer instrument.
- 10. High-Speed Homogenizer (FastPep or BeadBlaster).
- 11. Magnetic bead separation rack (DynaMag-2 Magnet).

Equipment	
FastPrep-24™ Classic bead beating grinder and lysis system	NAME
High Speed Homogenizer	TYPE
FastPrep Instruments Models	BRAND
116004500	SKU
https://www.mpbio.com/us/fastprep-24-classic-instrument-1-each	LINK
The FastPrep-24™ Classic Instrument is a high-speed benchtop homogenizer offering the ultimate in speed and performance for the lysis of biological samples. Simultaneous homogenization of up to 24 samples takes place within 40 seconds.	SPECIFICATIONS
 	

Equipment

BeadBlaster 24

NAME

High-speed homogenizer

TYPE

Benchmark

BRAND

D2400

SKU

<https://www.benchmarkscientific.com/product/d2400/>

LINK

The BeadBlaster™ 24 completely lyses, grinds and homogenizes a broad variety of biological samples. Even the most difficult and resistant samples are fully homogenized, often in 35 seconds or less.

SPECIFICATION



1.2) Consumables

1. *M. tuberculosis* cultures on 7H11 plates, LJ or 7H9 media (including MGIT media).
2. Rapid immunochromatographic assay for MTBC detection (MPT64 TB antigen detection - Capilia TB Neo SKU: CATB0870/CATB0871).
3. 15 mL polypropylene tubes.
4. 1.5 or 2 mL screw cap tubes (low-binding).
5. 1.5 or 2 mL tubes (low-binding).
6. 2 or 3 mL Disposable Pasteur pipette.
7. Tips (1000 µL, 200 µL, 100 µL and 1-10 µL).
8. 1 or 2 mm glass beads (ideally 1 mm). **NOTE: If using 1mm beads, then should use 12 units, instead of 3 units.**
9. Inoculation loops size 10 µL (if using solid media).
10. Nuclease-free water (NFW) (Merk, LSKNF0500).
11. InstaGene Matrix (Bio-rad, product number: 7326030).
12. AMPure XP Bead-Based Reagent (Beckman Coulter, product number: A63881).
13. Qubit dsDNA HS Assay Kit (Invitrogen, product number: Q32851). Alternatively, Qubit 1X dsDNA HS Assay Kit (Invitrogen, product number: Q33230). The 1X formulation provides a ready-to-use working solution that enables simple dsDNA sample quantification on Qubit Fluorometers.
14. Qubit Assay Tubes (Invitrogen, product number: Q32856) or Qubit Flex Assay Tube Strips (product number: Q33252).
15. Kim wipe tissue (to clean Nanodrop pedestal).
16. Ethanol Molecular Grade (Merk, E7023-500ML).



2) IF PERFORMING DNA EXTRACTION FOR WGS ONLY (ADDITIONAL MATERIAL IF VISIBLE CONTAMINATION)

Note

*If an MGIT culture is positive for *M. tuberculosis* and shows signs of contamination (such as media turbidity). Treating the contaminated MGIT culture with **1X DNase Buffer** and the **DNase I enzyme** is advisable.*

2.1) Additional Equipment

1.  37 °C incubator.

2.2) Additional Consumables

1. DNase I enzyme 150 units/sample (10 µL/sample) (Merk, D5025 15-K).
2.  1 Mass Percent Tris-HCL pH 7.5 (to be prepared).
3.  1 Mass Percent Calcium Chloride - CaCl₂.
4.  1 Mass Percent Magnesium Chloride Solution - MgCl₂.
5. Double distilled water (ddH₂O) (MilliQ water).
6. Filter: ABLUO Cellulose Acetate Syringe Filters, 0.2µm, 25mm, Sterile.
7. Plastic syringe 50ml to use the filter.

2.3) Reagent Preparation

2.3.1) 1X DNase Buffer (Saline Solution)

- Firstly, prepare  100 mL of 10x DNase buffer (100 mM Tris-HCL pH 7.5, 25 mM MgCl₂, 5 mM CaCl₂). To a glass bottle, add:
1.  10 mL 1M Tris-HCL pH 7.5 (autoclaved or filtered).

**Note**

1) To prepare a stock of 200 mL 1M Tris-HCL pH 7.5 , first, calculate the molarity. For the current product number 93363-50G:

$g = \text{moles} \times \text{molecular weight (MW)}$

$g = 1 \text{ mol} \times 157.60 \text{ (g/mol)}$

$g = 157.60$

2) In a 200 ml glass bottle, add 157.6 g of Tris-HCL and 100 mL of ddH₂O. Mix until dissolved. Top up the final volume to 200 mL while adjusting the pH 7.5 (This solution will be 1M Tris-HCL pH 7.5).

3) Autoclave or filter the solution.

2. 2.5 mL 1M MgCl₂ (autoclaved or filtered) (Bioultra, 63069-100ML).

3. 0.5 mL 1M CaCl₂ (autoclaved or filtered) (Bioultra, 21115-100ML).

4. 87 mL ddH₂O.

- Autoclave and filter.
- Dilute from 10x DNase buffer to 1x DNase buffer.
- To have a final volume of 100 mL 1x DNase buffer, for example:

Note

Use the following calculation:

$$C1 \times C2 = V1 \times V2$$

$$10X \times 1X = V1 \text{ mL} \times 100 \text{ mL}$$

$$V1 = 10 \text{ mL}$$

Interpretation: Add 10 mL of 10x DNase buffer in 90 mL of ddH₂O to have a final dilution of 1x DNase buffer.

2.3.2) DNase I enzyme elution and dilution

- The initial lyophilized DNase 1 powder is 15,000 Units (U) or 15K U.
- To prepare a final concentration of 15U/μL, resuspend the lyophilized DNase 1 powder with 1000 μL of ddH₂O (MilliQ water).
- Dissolv by vortexing (00:00:05) to ensure DNase 1 powder is properly dissolved.
- Make aliquots of 150 μL in 0.5 μL PCR tubes and store them at - 20 °C .



Note

Aliquots are made to minimize the number of freezing and thawing cycles of the DNase I enzyme which reduces the enzyme activity.

Safety warnings

- ❗ To prevent contamination during the DNA extraction, exercise caution when handling the InstaGene Matrix reagent. It is recommended to aliquot the required amount of reagent for each sample batch to minimize the risk of cross-contamination.

Before start

Preparation and Quality Control

Before initiating the experiment, verify that the CPC has undergone successful quality control testing (smear microscopy or antigen testing kit). Inspect all consumables and equipment for proper function. Prepare necessary reagents and clearly label sample tubes with appropriate identifiers.

Critical Procedure Steps

- **Bead Preparation:** Introduce three 1 or 2 mm beads into each 1.5 or 2 mL **low-binding screw-cap tube** and sterilize through autoclaving.
- **Sample Handling:** Remove the IGM reagent from refrigeration ( 4 °C) and allow it to reach  Room temperature for atleast  00:30:00 . Subsequently, homogenize the sample using a magnetic mixer as per the manufacturer's instructions.
- **Reagent Preparation:** If utilizing 1X DNase Buffer, prepare the reagent in advance.

METHODS

- 1 Prepare cultured samples, reagents, and review the equipment and consumable list before beginning the DNA extraction process.

Note

The method includes the following five sections:

- 1) Mycobacterial culture preparation and heat-inactivation (In the BSL3).
- 2) Pre-treatment of the heat-inactivated culture before DNA extraction.
- 3) DNA extraction using InstaGene matrix and bead-beating.
- 4) DNA quality control.
- 5) Purification with AMPure XP Bead-Based Reagent (Beckman Coulter) (Optional for WGS).

1. Mycobacterial culture preparation and heat-inactivation (In the BSL3)

2 IF WORKING WITH SOLID CULTURE

Note

This workflow is visually represented in Figure 1 (if using solid culture).

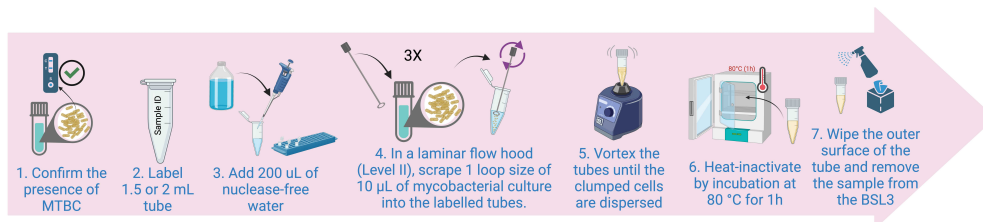


Figure 1. Workflow to process solid culture for DNA extraction.

- 2.1 In a BSL3 facility, prepare the CPC Mycobacterial culture sample by performing the quality control (QC) using the rapid immunochromatographic assay (antigen test, Capilia TB-Neo assay) for MTBC detection.
- 2.2 Label the 1.5 or 2 mL tubes (containing 3 sterile beads of 2 mm) with the corresponding sample identification on the culture tubes.

- 2.3 Add  200 μL of nuclease-free water (NFW) to the labelled tubes.

Note

This step can be done in a BSL2 laboratory.

- 2.4 In a laminar flow hood (Level II), scrape 1 loop size of 10 μL of mycobacterial culture into the labelled tubes.

- 2.5 Vortex the tubes until the clumped cells are dispersed.

- 2.6 Heat-inactivate the bacterial samples at  80 $^{\circ}\text{C}$ for  01:00:00 before being removed from the BSL3.

1h

- 2.7 Wipe the outer surface of the tube and remove the heat-inactivated solid culture samples from the BSL3 to a BSL2 laboratory.

3 IF WORKING WITH LIQUID CULTURE (MGIT)

Note

This workflow is visually represented in Figure 2 (if using liquid culture).

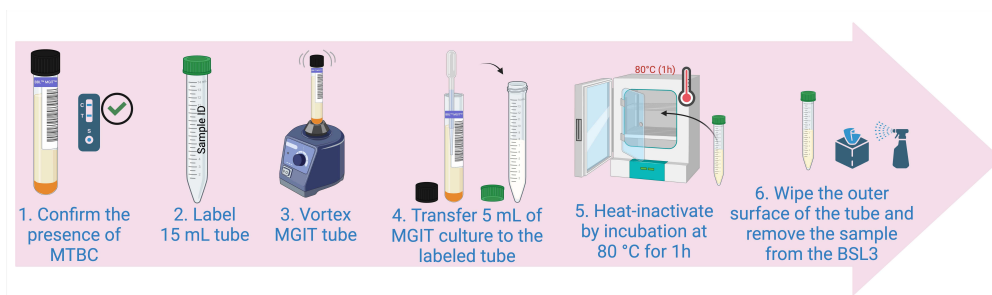


Figure 2. Workflow to process liquid culture for DNA extraction.

- 3.1 Label each 15 mL polypropylene tube with the sample identification.

- 3.2 Vortex the MGIT tube (liquid culture in 7H9 MGIT media) previously confirmed as positive for *M. tuberculosis* until the bacterial biomass from the bottom is homogenized and the clumped cells are dispersed (about 00:00:05).
- 3.3 Transfer 5 mL of the BBL MGIT content (liquid culture) into 15 mL a polypropylene tube using a 2 or 3 mL Pasteur pipette and discard the pipette.
- 3.4 Place the aliquoted culture (5 mL) into the pre-heated oven at 80 °C and allow it to inactivate for 01:00:00 at 80 °C before being removed from the BSL3 laboratory.
- 3.5 Wipe the outer surface of each tube and transfer the heat-inactivated liquid culture samples to a BSL2 laboratory.

5s

1h

2. Pre-treatment of the heat-inactivated culture before DNA extraction

- 4 This workflow is visually represented in Figure 3 (if using liquid culture).

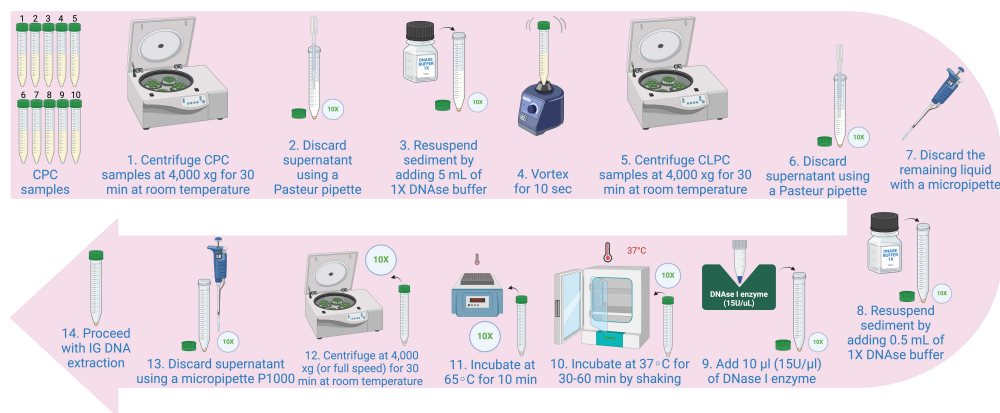


Figure 3. Pre-treatment workflow for DNA extraction from heat-inactivated liquid culture.

**Note**

When dealing with CPC samples, host (human) DNA and other contaminants persist (bacterial, fungi and viral), even after sputum decontamination and using the BD MGIT PANTA antibiotic mixture within the BD MGIT BBL tube. To address this issue, it is advised to use a saline wash as a pre-treatment step in conjunction with DNase I enzyme digestion to remove contaminating extracellular DNA. This combined approach eliminates non-mycobacterial DNA that can interfere with subsequent WGS processes. In the context of tNGS, a saline wash as pre-treatment is not required because non-mycobacterial DNA does not amplify and thereby affects the analysis, given that the NGS is a targeted PCR approach.

Figure 2. Pre-treatment workflow for DNA extraction from heat-inactivated solid culture.

- 5 In the general BSL2 lab, centrifuge the 15 mL tubes at 4.000 x g for 00:30:00 at Room temperature . 30m
- 6 Using a Pasteur pipette carefully aspirate the supernatant, without disturbing the pellet, and discard it.
- 7 Resuspend the pellet by adding 5 mL of 1X DNase buffer. *
- 8 Homogenize using a vortex for 00:00:10 to disperse cells. 10s
*
- 9 Centrifuge the 15 mL tubes at 4.000 x g for 00:30:00 at Room temperature . 30m
*
- 10 Using a Pasteur pipette carefully aspirate the supernatant, without disturbing the pellet, and discard it. *
- 11 Remove the remaining supernatant with a P1000 pipette. *
- 12 Resuspend the pellet in 500 µL of 1X DNase buffer within the 15 mL tube. *

**Note**

To better obtain the mycobacterial cells:

- Resuspend the pellet in  350 μL of 1x DNase buffer and mix gently.
- Transfer to a 1.5 or 2 mL low-binding screw cap tube.
- Add  150 μL of 1x DNase buffer to the remaining pellet within the 15 mL tube.
- Pipette up and down to clean the tips/tube (collect as many cells as possible).
- Transfer the cells to the same low-binding screw cap tube to have a final volume of  500 μL .

- 13 Add  10 μL (15 U/ μL , totalling 150 U per sample) of DNase I enzyme. 
- 14 Incubate at  37 $^{\circ}\text{C}$ for  00:30:00 with gentle shaking to digest extracellular DNA (host and commensal) in suspension from the host or commensal organisms.  
- 15 Incubate at  65 $^{\circ}\text{C}$ for  00:10:00 to deactivate the DNase I enzyme.  
- 16 Centrifuge at  21000 rcf (full speed) for  00:20:00 to pellet the mycobacterial cells.  
- 17 Discard the supernatant carefully using a P200 or P100 pipette without disturbing the pellet. 
- 18 Proceed with IGM/FP DNA extraction.

3. DNA extraction using InstaGene matrix and bead-beating

- 19 Before the DNA extraction (Illustrated in Figure 4), proceed as follows:

Note

- Prepare a batch of tubes as follows: Add three 2 mm beads to each 1.5 or 2 mL low-binding screw-cap tube and autoclave.
- Remove the IGM from refrigeration (4 °C) and allow it to thaw to equilibrate Room temperature at least 00:30:00 . Subsequently, homogenize the IGM using a magnetic mixer according to the manufacturer's guidelines.

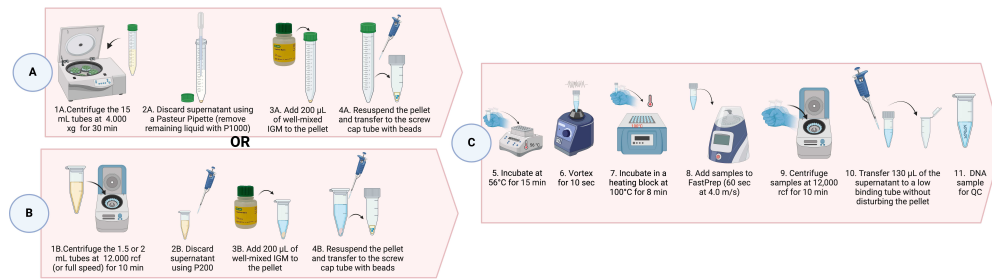


Figure 4. Schematic representation of DNA extraction using InstaGene™ matrix kit with a high-speed homogenizer (e.g.: FastPrep). **A)** Steps if using liquid culture (from 1-A to 4A); **B)** Steps if using solid culture (from 1-B to 4B); **C)** Common procedures for both types of culture.

- 20 In the general BSL2 lab, centrifuge the 15 mL tubes at 4.000 x g (if using liquid culture) for a duration of 00:30:00 or at 12000 x g (if using solid culture) or at Room temperature

30m

Note

Skip this step if the pre-treatment of the liquid heat-inactivated culture was performed.

- 21 Add 200 µL of well-mixed IGM to the 15 mL tube containing the pellet of the heat-inactivated culture.

Note

If using solid culture, centrifuge the 1.5 or 2 mL tubes with the samples at 12.000 rcf for 00:01:00 and remove the supernatant without resuspending the pellet.



- 22 Gently resuspend the cell pellet with a P200 pipette and transfer the entire suspension to the pre-labelled tube containing the three beads. 

Note

Recover all possible cells from the tube wall.

- 23 Incubate at  56 °C for  00:15:00 . 15m
- 24 Homogenize using a vortex for  00:00:10 to disperse cells. 10s
- 25 Incubate the tubes in a heating block set at  100 °C for  00:08:00 . 8m
- 26 Homogenize the samples using an HSH FastPrep or BeadBlaster 24 (program:  00:01:00 at 4.0 m/s, 1 cycle). 1m
- 27 Centrifuge the tubes at  12.000 rcf for  00:15:00 . 15m
- 28 Transfer  130 µL of the supernatant with DNA to a 1.5 or 2 mL tube without disturbing the pellet (matrix with cell debris). 
- 29 Proceed with quality control (QC).

4. DNA Quality control

- 30 Perform quality control of the genomic DNA to check the quantity and purity. The DNA quantity should measure the double strand (ds) DNA using the high-sensitivity (HS) Qubit kit (following the manufacturer's instructions).
- 31 The DNA purity can be measured using the Nanodrop spectrophotometer (following the manufacturer's instructions).

Note

For the DNA concentration, the 260/230 and 260/280 ratios are essential indicators. The reference values are for DNA samples: for the 260/280 ratio, it should be 1.8. Values between 1.7 and 2.0 indicate some degree of contamination and may influence reactions depending on the downstream procedure. A minimum DNA concentration of 1.0 ng/μL is required for sequencing with the Illumina platform. The 260/230 ratio should be between 1.8 and 2.2. In general, if the ratio is remarkably different than the provided range, it may indicate the presence of residual phenol, guanidine, magnetic beads, carbohydrates, or proteins. A low 260/230 ratio may indicate an issue with the buffer used for the blank measurement.

5. Purification with AMPure beads (Optional - If needed)

- 32 If samples exhibit high levels of salt or protein contamination, DNA purification can be performed using AMPure XP beads (Figure 5).

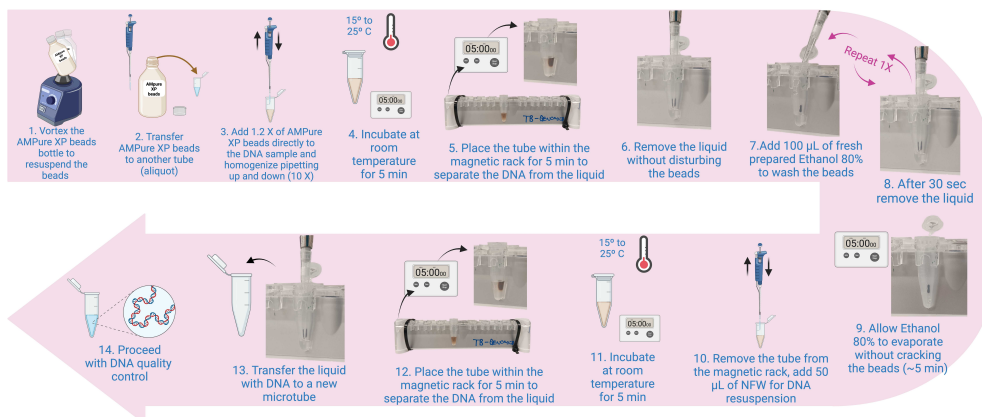


Figure 5. Workflow to process genomic DNA purification using AMPure beads.

- 33 Begin by thoroughly vortexing the cold ( 4 °C) AMPure XP beads to resuspend the magnetic beads that may have settled.
- 34 Subsequently, aliquot the desired amount into a low-binding tube(s) based on the number of samples being purified.

**Note**

Allow the aliquot to thaw to Room temperature for 00:30:00 before use.

- 35 Based on the sample volume, add a 1.2 X volume ratio of AMPure XP beads to the 1.5 mL or 2 mL low-binding tube containing the DNA sample.

Note

For example: If only 50 μL of the DNA sample is purified, use 60 μL of AMPure XP beads to get a ratio of 1.2x (AMPure XP beads to DNA sample volume).

- 36 Homogenize by pipetting up and down 10 X (times) slowly to avoid creating bubbles.

Note

If bubbles are introduced, this will reduce the final volume of the purified DNA.

- 37 Incubate at Room temperature for 00:05:00 .

5m

- 38 Place the tube into the magnetic rack for 00:05:00 until the supernatant is clear.

5m

- 39 With the tube on the magnetic rack, carefully remove the supernatant without resuspending or touching the beads.

**Note**

Avoid touching the beads while aspirating the liquid. Instead, position the pipette tip on the opposite side of the beads inside the tube.



- 40 Add  100 μL of freshly prepared 80% ethanol on the opposite side of the beads, while the tubes are still on the magnetic rack.

Note

1. Do not add the 80% ethanol directly onto the beads.
2. When cleaning up larger volumes of DNA and beads, increase the drying time. A standard  00:05:00 drying time is sufficient for cleaning up  50 μL of DNA using  100 μL of ethanol. For larger DNA volumes, proportionally increase the ethanol volume. For example, when cleaning up  130 μL of DNA using  156 μL of beads, use  200 μL of ethanol.

- 41 Incubate for  00:00:30 and remove by aspiration the 80% ethanol.

30s

- 42  41-42 Repeat steps 41 and 42. Use a P10 pipette to remove any residual 80% ethanol at the bottom of the tube on after the second wash.

- 43 Allow the 80% ethanol to evaporate without allowing the beads to crack (for about  00:05:00).

5m

Note

1. After the removal of the residual 80% ethanol, the beads have a shiny finish. When the 80% ethanol has evaporated, the beads will have a matte finish and immediately resuspend the beads in NFW to prevent over-drying and cracking. When more than 2 tubes have matte beads, close the tubes and add NFW to each tube and resuspend. This will also prevent over-drying of beads when working with a larger batch of samples.
2. When processing increased amounts of DNA and beads, double the standard drying time (about  00:10:00).

- 44 After the drying step, remove the tubes from the magnetic rack.

- 45 Immediately add  50 μL of NFW and resuspend the beads to release the DNA in the NFW.



Note

To obtain a more concentrated DNA sample, use  25 μL of NFW instead of  50 μL .

46 Incubate at  Room temperature for  00:05:00 .

5m

47 Place the tubes back on the magnetic rack and incubate for  00:05:00 .

5m

48 Transfer the supernatant (containing eluted DNA) of each sample to a newly labelled tube.

49 Proceed with DNA QC.

Abbreviations

50 CTAB: Cetyltrimethylammonium-bromide

CPC: clinical primary culture

ddH₂O: Double distilled water

BMRI: Biomedical Research Institute

BSC: Biological Safety Cabinet

BSL3: Biosafety level 3 laboratory

CaCl₂: Calcium Chloride

DNA: deoxyribonucleic acid

FP: Fast Prep



HSH: High-Speed Homogenizer

IGM: InstaGene Matrix

LJ: Löwenstein–Jensen

MgCl₂: Magnesium Chloride Solution

MGIT: mycobacteria growth indicator tube

MTBC: *Mycobacterium tuberculosis* complex

NFW: Nuclease-free water

NGS: Next-generation sequencing

PANTA: Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim, Azlocillin

PAPR: powered air-purifying respirator

PPE: personal protective equipment

tNGS: target next-generation sequencing

Tris-HCL: Tris-hydrochloride

QC: Quality Control

RCF: Relative centrifugal force

SOP: Standard Operating Procedure

WGS: Whole-genome sequencing

Acknowledgement

- 51 We acknowledge the TORCH consortium team for their valuable contributions to the development of this protocol. We thank MSc. Janré Steyn for providing user-based feedback. We are grateful to the insightful revisions offered by Dr. Anzaan Dippenaar and Dr. Fahd Naufal.



Protocol references

Van Embden JD et al. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J Clin Microbiol 1993;31: 406–9.

Kang M et al. Comparison of DNA extraction methods for drug susceptibility testing by allele-specific primer extension on a microsphere-based platform: Chelex-100 (in-house and commercialized) and MagPurix TB DNA Extraction Kit. J Microbiol Methods. 2018 Sep;152:105-108. doi: 10.1016/j.mimet.2018.07.019. Epub 2018 Jul 31. PMID: 30075237.

Shea J et al. Comprehensive whole-genome sequencing and reporting of drug resistance profiles on clinical cases of *Mycobacterium tuberculosis* in New York State. Journal of Clinical Microbiology, 2017, 55(6), 1871–1882. <https://doi.org/10.1128/JCM.00298-17>.