Bead Beating in Custom Buffer Followed by XP Bead Cleanup (NGS Workflow)

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Protocol status: Working
We use this protocol and it's working

ABSTRACT

DNA extraction method for Mycobacterium tuberculosis from various sample types as described in: "**Insights into *Mycobacterium tuberculosis* DNA extraction for targeted deep sequencing using the Deeplex Myc-TB assay: Lessons for improved drug resistance diagnosis."**

MATERIALS

Equipment

FastPrep-24 Classic bead beating grinder and lysis system	NAME
Bead beater	TYPE
MPBio	BRAND
116004500	SKU

Screw cap micro tube 1.5 ml PCR Performance Tested Low DNA-binding **Sarstedt Catalog # 72.703.700**

Agencourt AmPure XP beads **Contributed by users Catalog #A63880**

0.1 mm Zirconia/Silica Beads **Bio Spec Products Inc. Catalog #11079101z**

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SAFETY WARNINGS

Work done with *Mycobacterium tuberculosis* must comply with laws, rules, and regulations.

BEFORE START INSTRUCTIONS

Prepare buffers

Prepare Buffers

1

	Component	Volume (ml)
	H2O	95.8
	NaCl (5M)	2
	Tris-HCl pH 8.3 (1M)	1
	Triton X-100	1
	EDTA (0.5M)	0.2

Custom Triton Buffer pH 8.3 . To prepare 100 mL of the **Custom Triton Buffer**, simply add each component in the specified amount then add H_2O to a final volume of 100 mL . Filter sterilize the solution before use. This will result in a final buffer concentration of [M] 100 millimolar (mM) NaCl, [M] 10 millimolar (mM) Tris-HCl; [M] 1 millimolar (mM) EDTA, [M] 1 % (v/v) Triton X-100.

	Component	Volume (ml)
	H2O	99
	Tris-HCl (1M, pH 8)	1
	EDTA (0.5M)	0.02

Low EDTA TE (1X) pH 8 . To prepare 100 mL of the **Low EDTA Tris Buffer** simply add each component in the specified amount then add H_2O to a final volume of 100 mL . The final buffer has a concentration of
[M] 10 millimolar (mM) Tris-HCl and [M] 01. millimolar (mM) EDTA.

Step 1 includes a Step case.
Sputum sample
MGIT culture
Prepare lysis tubes

Prepare Input

step case 30s

Sputum sample

2 Add four volumes of 100mM dithiothreitol to the sputum sample and vortex for > 00:00:30

3 Incubate at Room temperature for 00:15:00 15m

4 Remove and discard the supernatant, and resuspend the pellet in 350 µL of **Custom Triton Buffer**

5 Vortex sample and , 00:15:00 , max speed 15m

6 ***If it is not possible to do the above in your laboratory.***
Use BBL MycoPrepTM (BD) reagent to process the sample according to the manufacturer's instructions with the following modification: Resuspend the sediment in step 8 in the protocol in 350 µL of Custom Triton Buffer

7 If required (i.e., to remove samples for processing outside a BSL-3), decontaminate the sample

according to the standard operating procedures of your facility.

Extract DNA

2m

8 Transfer the inactivated bacterial suspension to a new well-labeled Starsted screw cap tube containing ~ 250 µL of Mini-BeadBeater Zirconia-Silicate Beads, -0.1 mm

9 Bead beat the lysate at 6.5m/s for 00:00:45 with 00:02:00 rest on ice between runs

9.1 Repeat for a total of three bead beating cycles

10 Centrifuge at max speed 10000 rcf, Room temperature, 00:02:00 and transfer the supernatant to a new well-labeled tube. 2m
Take care not to transfer beads or cell debris.

11 Add 250 µL (1.2X volume) AMPure XP beads and mix by pipetting up and down 10 times

12 Incubate at Room temperature for 00:05:00

13 Place on magnetic rack and wait for the solution to clear, ~ 00:02:00

14 Discard the supernatant and wash the beads twice with freshly prepared [M] 70 % (v/v) ethanol without disrupting the beads

15 Dry the beads briefly, ~ 00:02:00 .
Tip: Remove residual EtOH with a p10 pipette

16 Immediately after the bead pellet becomes opaque, remove the tube from magnetic rack and resuspend in 20 µL of **Low EDTA Tris Buffer**. Ensure all beads are in solution.

17 Incubate at room temperature for 00:05:00

18 Place on magnetic rack, wait for the solution to become clear ~ 00:02:00 , and transfer the eluted DNA to a new well-labeled tube