Protocol: MTB DNA Isolation from MTB-Infected Lung Tissue and Sputa (Co-Extraction Method)

MTB DNA can be co-extracted along with MTB RNA by a slight modification of the RNA isolation protocol. MTB DNA isolated from this co-extraction is further purified by the protocol below.

1. Isolation of DNA interphase layer

To extract DNA, proceed with the standard RNA extraction protocol using Trizol but with a modification.

- **DO NOT** use Heavy-Phase Lock gel. Instead, transfer the Trizol supernatant to a new tube containing 200 µl of chloroform. Vortex tubes for 20 seconds and incubate at room temperature for 2 minutes. Centrifuge tube for 10 min. at maximum speed.

- Carefully remove the RNA-containing aqueous phase to a new tube. The interphase and organic phase contains the DNA. Transfer the remaining interphase and organic phase to a new 1.5ml eppendorf tube. To decontaminate, wipe down tubes with Staphene. The rest of the protocol can be performed in a BL2.

2. Purification of Co-Extracted MTB DNA

A. DNA precipitation:

1. Centrifuge tubes containing the interphase and organic phase at 12,000 x g for 5 min. at 4ºc. Carefully remove any remaining aqueous phase. At this point, samples can be stored at 4°c for days to weeks.
2. Add 500 µl of the BEB (back extraction buffer – see below) per 1 ml of TRIZOL used for the RNA extraction to each tube. Mix extensively for 10 min. by inversion.
3. Centrifuge tubes at 12,000 x g for 30 min. at room temperature.
4. Transfer the upper aqueous phase (contains the DNA) to a new elution tube.
5. Add 10 µl of Glycoblue coprecipitant to each tube. Glycoblue helps to visualize DNA pellet after centrifugation. Mix tube by inversion.
6. Add 400 µl of isopropanol per 1 ml TRIZOL used for the original RNA isolation. Mix and incubate for 10 min. at room temperature.
7. Centrifuge samples at 12,000 x g for 15 min. at 4ºc.
8. A blue pellet containing the DNA should be visible at this point. Carefully remove supernatant.

B. DNA purification:

1. Add 500 µl of 70% ethanol per 1 ml TRIZOL used for the original RNA isolation. Wash pellet by inversion.
2. Centrifuge samples at 12,000 x g for 15 min. at 4°c.
3. Repeat 70% ethanol wash for a total of two washes.
4. Remove the ethanol. Let tubes air-dry for ~ 5 min.
5. Dissolve pellet in ~ 30 µl of nuclease-free water. The actual amount of water added will depend on the original tissue size and amount of DNA isolated.
6. DNA can be stored at 4ºc or -80ºc for long term storage.

BACK EXTRACTION BUFFER (BEB):
This buffer can be stored at room temperature for up to 6 months.

For 250 ml: take 150 ml of nuclease-free water and dissolve

- 118.2 g guanidine thiocyanate (FW 118.2) = 4M
- 3.68 g sodium citrate (FW 294.1) = 50mM
- 30.29 g Tris (free base) (FW 121.14) = 1M
- add additional nuclease-free water to 250 ml volume
- filter sterilize solution