SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

Since 1991, all culture-positive isolates from TB patients in Denmark and Greenland have been stored as frozen glycerol stocks at -80°C at the International Reference Laboratory of Mycobacteriology at Statens Serum Institut (SSI) in Copenhagen. Before freezing, glycerol stocks are prepared by adding 700 µl of bacterial growth from Dubos to 300 µl of a 50% glycerol solution.

DNA Isolation

Cell lysis of freeze-isolates

First, 100 µl of frozen glycerol stocks were transferred to a tube containing 100 µl of DNA/RNA-free water and glass-dust. Additional 300 µl of DNA/RNA-free water were added before samples were centrifuged for 15 min. at 15,700 G. The supernatant was discarded and the cell-pellet resuspended in 200 µl of DNA/RNA-free water. Hereafter, the cells were heat-inactivated at 95°C for 30 minutes. Tubes were vortexed for 30 seconds and put in an ultrasonic bath (Becton Dickinson, 2510E-DTH) at 60°C for 15 minutes. Lastly, the tubes were centrifuged for 1 minute at 15,700 G and the supernatant (approximately 200 µl) carefully transferred to clean tubes.

DNA concentration

Samples were placed in Centrifugal Filter Units (Microcon®, Merck KGaA, Darmstadt, Germany) and centrifuged for 8 minutes at 500 G until a volume of approximately 50 µl was reached.

Purification

Purification was performed with EtOH precipitation and bead clean up (AMPure XP bead, Beckman Coulter, Krefeld, Germany) (1). Samples were centrifuged for 10 minutes at maximum speed (20,817 G) and 40 µL supernatant transferred to new tubes. Hereafter, 4 µL (1/10 of volume) of sodium acetate and 80 µL (2 x volume) of ice-cold 96% EtOH were added before storing for 1 hour at -20°C. Samples were centrifuged again for 15

minutes at maximum speed. The supernatant was removed, the precipitate washed with 1 mL 70% EtOH, and samples dried at room temperature for 10-15 minutes. Then samples were re-suspended in 50 µL depc water by heating at 55°C for 10 minutes before transferring 45 µL of the supernatant to a well plate. For AMPure XP beads clean-up, 81 µL of beads were added to the wells with subsequent vortexing for 1 min and incubation at room temperature for 10 minutes. The plate was placed on a magnetic stand for 3 minutes and the supernantant removed. While still on the magnetic stand, samples were washed 2 times with 80% EtOH and then dried for 10-15 minutes before thorough re-suspension in 25 µL of depc water. After that, plates were put on the magnetic stand for 3 min and finally 25 µL of the supernantant without beads were transferred to new tubes.

DNA extraction with the traditional CTAB procedure

After approximately 10 weeks of growth on LJ slants, all visible growth from the samples was transferred to micro centrifuge tubes containing 400 µL of 1X TE buffer. After this, the protocol for the CTAB procedure for isolation of high molecular weight genomic DNA was followed without further modifications (2).

DNA concentration measurements

Measurements of DNA concentration were performed with the Qubit® dsDNA HS Assay Kit (0.2–100 ng) with the Qubit® 2.0 Fluorometer according to the manufacturer's instructions (Life Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA).

References

1. Votintseva AA, Pankhurst LJ, Anson LW, Morgan MR, Gascoyne-Binzi D, Walker TM, Quan TP, Wyllie DH, Del Ojo Elias C, Wilcox M, Walker AS, Peto TEA, Crook DW. 2015. Mycobacterial DNA extraction for whole-genome sequencing from early positive liquid (MGIT) cultures. J. Clin. Microbiol.
2. Van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. 1991. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J. Clin. Microbiol. 29:2578–2586.