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A rapid and gentle method for the isolation of genomic DNA from mycobacteria

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Isolation of genomic DNA from mycobacteria is a time consuming and tedious process. The pathogenic mycobacteria are slow growing in culture and the cells are difficult to lyse. The tough mycobacterial cell walls contain thick layers of lipopolysaccharide protein complexes which make them resistant to the standard protocols (1, 2). Due to the resurgence of tubercular infections the molecular fingerprinting of mycobacterial isolates by RFLP analysis (3, 4) is gaining importance in mycobacterial research and epidemiology. This involves handling of a large number of strains at the same time. The time consuming step in the isolation of DNA from mycobacteria is to disrupt the thick and lipopolysasscharide rich cell wall without causing damage to the genomic DNA. We report a rapid and gentle method to extract sufficient quantity of unsheared genomic DNA from mycobacterial cells. We found that treating mycobacterial cells with cesium chloride greatly facilitates the susceptibility of these cells to further digestion and extraction procedures.

Mycobacterial cells cultured in liquid broth were harvested at the early exponential phase. Cells (100 mg wet weight) were washed twice with TE (Tris–HCl pH 8.0, 10 mM; EDTA 1 mM) and an equal volume of saturated cesium chloride solution containing 1% Triton-X was added to it. The mixture was then rocked manually but gently for 10 min to prepare a thorough suspension. Thirty volumes of sterile distilled water was added to the cell suspension to generate osmotic shock and burst the tough mycobacterial cell wall. At this stage 99% of the cells lost their acid-fast character (data presented but not shown). Theses pheroplastic cells were then centrifuged, mixed with 0.5 ml of fresh lysozyme (1 mg/ml) in TE buffer and kept on ice for 1 h. SDS (final conc. 0.5%) and proteinase K (100 μg/ml) were added into the reaction mixture before placing it in a 55°C water bath for 4 h. Subsequently 1 ml of 4 M GIT Cocktail (0.5 g/ml guanidium thiocyanate 0.053 M Tris–HCl, pH 7.5/12 mM EDTA, pH8.0/0.2 M NaCl/2.12% N-lauroyl sarcosine/0.15 M 2-mercaptoethanol) and 1% CTAB (Cetyl trimethyl ammonium

Table 1. A comparison of our rapid lysis protocol with the published protocols for isolation of genomic DNA from Mycobacteria

	Yield of DNA in mg/g wet wt. of cells	Purity checked by O.D. 260/280 nm	Total time in hrs for isolation of DNA from cell pellet	Preincubation of culture with cell well modifying agent necessary Yes/No	Mechanical/ physical method of disruption employed e.g. French pressure cell or bead beater
Method being reported	1.2–2.0 mg	1.8–2.0	< 7 h	No	No
Mizuguchi & Tokunaga, (1979), *J. Bacteriol.* **104**, 1020–1021.	0.67 mg	1.92	NA	Yes	No
Baess,I. (1974) *Acta Pathol. Microbiol. Scand. (B)* **82**, 780–784.	1 mg	1.92–1.94	>24 h	No	Yes
Clark-Curtiss *et al.* (1985), *J. Bacteriol.* **161**, 1093–1102.	1–2 mg	NA	>24 h	Yes	Yes
Patel,R. *et al.*, (1986), *J. Gen. Microbiol.* **132**, 541–551.	0.3–2.0 mg	NA	>24 h	Yes	No
Katoch,V.M. & Cox,R.A. (1986), *Int. J. Lepr.* **54**, 409–415.	1.1–1.6 mg	1.95–1.98	> 24 h	No	Yes

NA — Data not available.

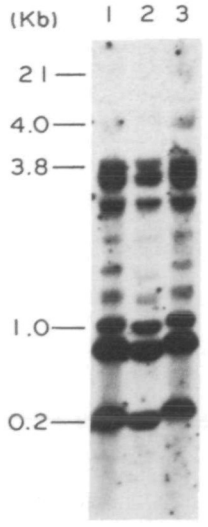

Figure 1. Southern blot hybridization of *Eco*RI digested genomic DNA (run on 1.0% agarose gel) with ribosomal RNA labelled with [γ^{32}P]ATP using T4 polynucleotide kinase. Lanes 1, 2 and 3 contain different clinical isolates of M.tb.

bromide) were added to the above mixture, and it was kept at 65°C for 30 min in a slow speed shaker water bath so as to remove the bound polysaccharides. Further purification was performed by the usual phenol–chloroform extraction and DNA was spooled out after adding 0.5 Vol. of 5 M ammonium acetate and 0.75 Vol. of isopropanol; it was washed with alcohol, partially dried and suspended in TE buffer. It was then treated with RNase A (50 μg/ml) for 30 min. at 37°C, re-extracted with chloroform–isoamyl alcohol, precipitated and finally resuspended in TE buffer. The recovered chromosomal DNA was unsheared (data presented but not shown) and the yield was 1.2 to 2.0 mg/g wet weight of packed cells. This procedure could be completed in less than seven hours and may be scaled up if required. Table 1 shows a comparison of this method with some of the published protocols for isolation of genomic DNA from mycobacteria. The DNA isolated by this method was pure enough for restriction analysis and Southern blotting. Autoradiographs of Southern blot of different mycobacterial strains are shown in Figure 1. Genomic DNA was extracted as described in the text, restriction digested with *Eco*RI and hybridized with a ribosomal RNA probe 5′ labelled using T4 polynucleotide kinase (5, 6).

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