



Mechanical disruption of mycobacterial cell walls

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CONTEXT

Genomic and proteomic research on mycobacterial diseases requires high quality and quantity preparation of DNA or proteins. Effective lysis of mycobacterial cells for DNA or protein extraction is demanding due to the mycobacteria's robust and waxy cell wall features. Also, many mycobacteria are slow growers, often resulting in small amounts of starting material from a culture. Commercially available kits are not usually applicable to research methods on mycobacterial genomics and proteomics [1] [2].

MATERIAL

- Precellys®24 homogenizer.
- Beads in 2mL tube: 0.1mm zirconia beads.
- Samples: 20 mg (wet wt) pellets of Mycobacteria obtained from cultures re-suspended in phosphate-buffered saline (PBS; pH 7.4), and heat inactivated at 95°C for 60 min.

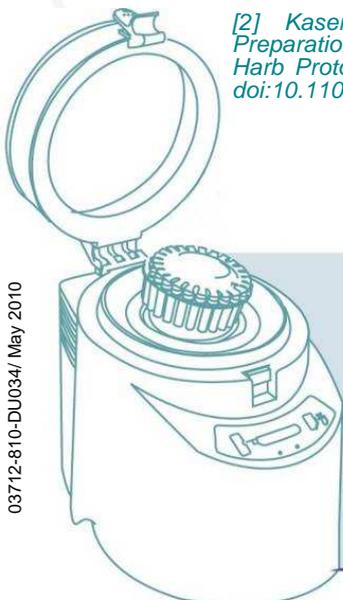
PROTOCOL

Note that pathogenic mycobacteria must be processed under appropriate biosafety containment.

- Precellys®24: 6800 rpm, 3x30 sec
- DNA extraction: phenol-chloroform extraction and chloroform purification.

[1] Kaser, et al. 2009. Optimized method for preparation of DNA from pathogenic and environmental mycobacteria. *Appl. Environ. Microbiol.* 75, no. 2 (January): 414-418.

[2] Kaser, et al. 2010. Optimized DNA Preparation from Mycobacteria. *Cold Spring Harb Protoc* 2010, no. 4 (April) :pdb.prot5408. doi:10.1101/pdb.prot5408.



RESULTS

Several elements of published DNA extraction protocols were combined and tested to improve DNA yield. Mechanical disruption of the mycobacterial cell wall, after incubation with 4% SDS (final concentration), was found to be crucial for sufficient and satisfactory yields.

DNA yield achieved when testing both combinations of DNA extraction procedures (chemical lysis only vs chemical and mechanical disruption) are shown in the Figure 1.

	A	B
SDS treatment ¹	Yes	Yes
Mechanical disruption ²	No	Yes
Precipitation/purification	EtOH	EtOH

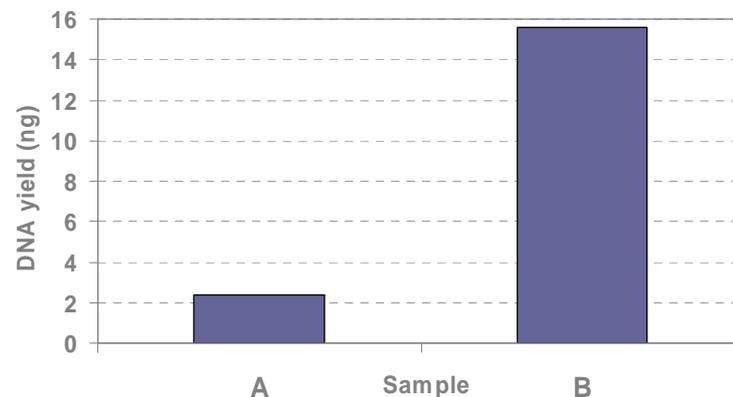


Figure 1. Twenty-milligram (wet weight) pellets of *M. ulcerans* strain IFIK1066089 were used. (Superscript 1) SDS was applied to a final concentration of 4%. (Superscript 2) Mechanical disruption performed with Precellys 24.

CONCLUSION

Small scale homogenization of samples containing mycobacteria with the Precellys®24 allows to obtain large amounts of pure genomic DNA as compared to protocols with chemical lysis only.

The additional steps of mechanical disruption and handling are worth it to increase significantly DNA yield.

For more details, please contact precellys@bertin.fr

Problem

Solution

