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We use this protocol and it's working

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CTAB

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ABSTRACT

Effective extraction and purification of DNA from mycobacteria is critical for downstream molecular applications. However, mycobacteria have a complex cell wall structure that makes DNA extraction challenging. Here, we describe an optimized protocol for extracting DNA from mycobacterial cultures grown on solid or in liquid media. Mycobacterial cells were harvested and underwent heat killing to inactivate nucleases. Cells were lysed using lysozyme, SDS, and proteinase K to break down the cell wall structure and release DNA. Contaminants were removed through extraction with chloroform/isoamyl alcohol and DNA was precipitated using isopropanol. Further washes with ethanol removed residual impurities. DNA yields from 32 extractions were quantified by quibit. The mean yield was 15.3 µg and the median 14 ng/ µl, with a range of 6-49 ng/ µl. The interquartile range was 11.5 to 18 ng/ µl. One outlier of 49 µg was observed. The chloroform extraction was critical to remove contaminating proteins, cell debris, and nucleases that could reduce DNA purity and yield. This protocol allows effective extraction of DNA from diverse mycobacterial species grown in either liquid or solid media. The DNA isolated can be used for downstream applications including PCR, restriction digestion, sequencing, and other molecular biology techniques.

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GUIDELINES

1. Prevent inhalation of SDS when handling solid SDS. Use an exhaust hood and a mouth cap.
2. Be careful with chloroform. Its inhalation is unhealthy and it can damage certain plastics.
3. Always use an exhaust hood. Do not spill chloroform on plastic racks.
4. For all incubations during the DNA isolation procedure a waterbath can be used. If shaking is required use 45 movements per min. Alternatively, a thermomixer can be used. This is an apparatus that heats and shakes the tubes simultaneously (see item 15). For shaking incubations
5. use position 7, for vortexing use the highest shaking position. This apparatus can be used for the entire DNA isolation procedure, but it is important to check whether the vortexing went well after addition of CTAB/NaCl. We advise not to use this apparatus for heat-killing the bacteria and vortexing after the addition of chloroform/isoamylalcohol. These steps should preferably be done manually.
6. It is convenient to use an aspirator. Alternatively a pipette can be used.
7. If mycobacteria are isolated which grow well on solid media, such as e.g. M. tuberculosis, then use at least two loops (0.5 cm diameter) of bacteria. Alternatively, in case of e.g. M. avium complex strains, or other mycobacteria growing very smooth on solid media, take a well grown 50 mL liquid culture. Transfer the liquid culture to a suitable centrifuge tube and centrifuge for 15 min at 3000xg using aerosol-containment buckets. Discard the supernatant and add 200 µL of 1X TE buffer to the tube. Resuspend the pellet by vortexing. Transfer 200 µL resuspended pellet to a microcentrifuge tube and add 200 µL 1X TE.
8. Do not use a mycobacterial culture grown on 7H10 medium, because for unknown reasons DNA isolated from mycobacteria grown on this medium is not well digested by restriction enzymes.
9. Use microcentrifuge tubes with a safe-lock, or jam the microcentrifuge tubes in such a way that the lids cannot open spontaneously.
10. Incubation should preferably occur overnight, especially when DNA is isolated from M. bovis or M. microti strains.
11. Pre warming the CTAB/NaCl solution in a waterbath at 65°C will make the solution less viscous and therefore easier to pipette. The aim of CTAB treatment is to remove cell wall debris, denatured protein, and polysaccharides complexed to CTAB, while retaining the nucleic acids in solution. Adding salt is very important, since a CTAB-nucleic acid precipitate will form if the salt concentration drops below about 0.5 M at RT.
12. The chloroform/isoamyl alcohol extraction precipitates the CTAB-protein/polysaccharide complexes. A white interface should be visible after centrifugation.
13. Be careful not to transfer anything of the inter phase, this will result in impure DNA.

14. There is no need to add salt for precipitation of the DNA since the NaCl concentration is already sufficient.
15. While turning the tube upside down precipitated DNA may or may not become visible, depending on the amount of mycobacterial cells started with. Stop shaking when the precipitate is formed and the solution becomes clear. If there is no precipitate of nucleic acids visible, then dissolve the pellet in step 20 in 20 µL 1X TE. If there is a small precipitate visible, then dissolve the pellet in 35 µL. Medium and large precipitates require 50 and 80 µL, respectively.
16. This step is not necessary, but ensures that all DNA precipitates. The incubation time can be extended as long as is convenient, since DNA can be kept in these conditions for even years.
17. Be sure that all traces of ethanol are removed, otherwise the pellet cannot dry and the precipitated DNA can even redissolve after a while.
18. Dissolving the DNA pellet at RT may take some time. To dissolve the DNA more quickly, incubate at 37°C for 1 h. Alternatively, the DNA can be incubated overnight at 4°C. Dissolved DNA can be stored, until use, at 4°C. For longer periods of time the DNA can be stored at 20°C (for years)

SAFETY WARNINGS

Phenol itself does not directly increase DNA yield. Its primary role in DNA extraction protocols is to remove proteins and other contaminants that can interfere with downstream applications or affect the quality of the DNA. By removing these contaminants, phenol treatment can improve the purity of the DNA preparation.
However, it's worth mentioning that phenol treatment may indirectly lead to an increase in DNA yield in some cases. Contaminants such as proteins and lipids can co-precipitate with DNA during precipitation steps, resulting in lower DNA yields. By effectively removing these contaminants, phenol treatment can enhance the recovery of DNA during precipitation steps, leading to a higher apparent DNA yield.
Nevertheless, it's important to note that the primary purpose of phenol treatment in DNA extraction protocols is to purify the DNA rather than directly increase its yield. The yield of DNA will ultimately depend on various factors such as the starting material, extraction method, and efficiency of subsequent purification steps. Additionally, phenol can also help in inactivating nucleases, enzymes that can degrade DNA, thereby protecting the extracted DNA from degradation.

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2 Grow strains of interest on Löwenstein-Jensen medium at 37°C until growth becomes clearly visible

3 Transfer an appropriate number (1-3 inoculation loops) of bacterial cells into a microcentrifuge tube containing 400 µl TE-Puffer

4 Incubate for 20 min at 80° C in a water bath to kill bacteria (check temperature with thermometer as this seems to be crucial for DNA quantity and quality afterwards)

5 Centrifuge for 5 min at 13000 rcf, discard supernatant and add 400 µl TE-Puffer

6 Add 50 µl of 10mg/ml lysozym, vortex und incubate at least 1 h at 37° C or o/n (we always incubate over night)

7 Add 70 µl of 10% SDS and 10 µl proteinase, vortex shortly and incubate 1 hr at 60° C

8 Add 100 µl 5M NaCl

9 Add 100 µl CTAB/NaCl mix (prewarmed at 65°C), vortex until the liquid content becomes white and incubate 10 min at 65° C

10 Add approx. 750 µl chloroform/isoamyl alcohol mix (24:1), vortex 10 sec and centrifuge 15 min at 13000 rcf at RT

11 Transfer aqueous supernatant in a new tube

12 Add 0,6 volume (450 µl) isopropanol, mix carefully and incubate 30 min at -20°C

13 Centrifuge 15 min at 13000 g

14 Remove most of supernatant and add 500 µl of cold 70 % ethanol

15 Centrifuge for 15 min at ±11,000 xg.

16 Discard most of the supernatant; leave about 20 µL (3 mm height) above the pellet (see Note 4).

17 Add 1 mL of cold 70% ethanol (from the -20 °C freezer) and turn the tube a few times upside down to wash the DNA precipitate.

18 Centrifuge for 5 min at ±11,000 xg.

19 Discard most of the supernatant; leave about 20 µL (3 mm height) above the pellet (see Note 4).

20 Centrifuge for 1 min at ±11,000 xg.

21 Remove the remaining supernatant from above the pellet by pipetting very carefully with a 20µL pipette (see Note 15).

22 Permit the pellet to dry for ± 15 min at RT. Check whether all ethanol is evaporated. If not, then extend the drying time.

23 Dissolve the pellet in the amount of 1X TE estimated in step 10 (see Note 16).