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Isolation of bacterial genomic DNA using QuickPick™ gDNA kit

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ABSTRACT

The QuickPick™ gDNA kit is intended for use with human whole blood and blood components such as leukocytes and buffy coat, as well as human cultured cells. However, the chemistry may be applied to other starting materials with good results as well. Below is described the purification of bacterial DNA from a cell culture of *Escherichia coli.* The purification was carried out following the kit instructions and no protocol modifications or additional reagents were required.

INTRODUCTION

Purification of DNA is a central and necessary technique in molecular biology. Methods are generally based on lysis of cell material, removal of proteins and other contaminants, and finally the collection of DNA. Proteins are digested using proteinase K, and can be removed through organic extraction or salting out. Alternatively DNA is removed from the sample mixture by using different solid phase techniques; common methods are spin columns or magnetic particles to which DNA is first specifically bound and then eluted.

An ideal method for DNA purification is rapid, simple, economical and gentle on the sample. PickPen technology is based on the transfer of magnetic particles using the PickPen tool, rather than the use of external magnetic racks. This method is faster and more convenient than rack-based methods while maintaining other magnetic particle-method benefits over alternatives such as spin columns or other commonly used techniques.

PRINCIPLE OF QuickPick gDNA

DNA in the sample is released from cells using Proteinase K and Lysis Buffer. The released DNA is bound specifically to the MagaZorb™ * Magnetic Particles in the presence of Binding Buffer. PickPen 1-M is used to capture the magnetic particles with bound DNA, and to carry out subsequent washes to remove contaminants. Finally, DNA is eluted from the particles using Elution Buffer, and DNA is ready for use in downstream applications. The protocol is carried out in 20 minutes, and throughput can be further increased by using PickPen 8-M.

MATERIALS & METHODS

One *E. coli* colony was inoculated into LB medium containing tetracycline (10 µg/ml) and was grown for 15.5 h at + 37 ºC with shaking (250 rpm). 2 ml of this culture was withdrawn, centrifuged, the pellet suspended in PBS, again centrifuged, and the pellet suspended into 200 µl of PBS (this was the the 1:1 sample.) From this sample was made a dilution series in PBS (1:10, 1:100 and 1:1000). 50 µl of each dilution was used as a sample for each DNA isolation. Isolations were carried out in duplicate, following the QuickPick gDNA instructions for use.

The sample dilutions correspond to the amount of original culture as follows:

50 µl of	1:1 is equivalent to	500 µl culture
50 µl "	1:10 "	50 µl "
50 µl "	1:100 "	5 µl "
50 µl "	1:1000 "	0,5 µl "

Note that the 1:1 sample was eluted into 200 µl according to the insert instructions, while the remaining samples were eluted into 100 µl to obtain a more concentrated sample. Reducing the elution volume has been found to reduce the yield slightly, but this effect was negligible in the above experiment where the intention was to qualitatively determine isolation of DNA.

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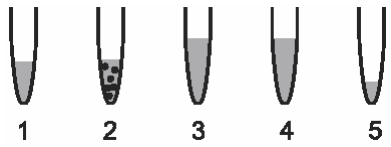
The DNA isolation protocol is as follows:

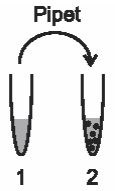
Predispense reagents and sample into tubes:



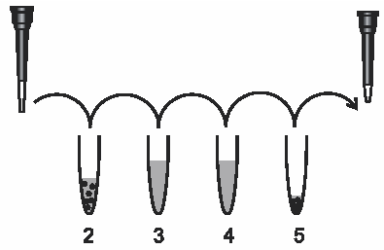
Tube 1: Lysis Buffer,sample, Proteinase K Solution
Tube 2: MagaZorb Magn. Particles, Binding Buffer
Tube 3: gDNA Wash Buffer
Tube 4: gDNA Wash Buffer
Tube 5: gDNA Elution Buffer

Incubate the sample, Proteinase K Solution and Lysis Buffer at 56 ºC for 10 minutes (tube 1).

Pipet the contents of tube 1 (sample mix) into tube 2 (containing magnetic particles) and incubate for 2 minutes at RT.



Wash the sample twice in Wash Buffer (tubes 3-4) using PickPen to carry out the transfers.



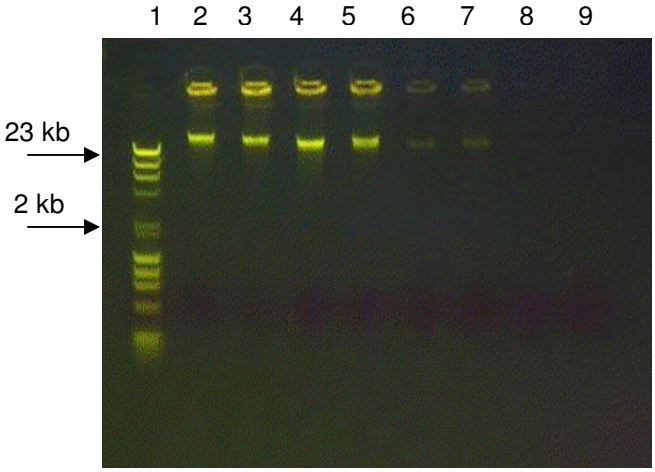
Transfer the sample into Elution Buffer (tube 5) with PickPen and elute DNA by incubating at RT for 2 minutes. Collect the magnetic particles from tube 5 and discard them. The eluate containing DNA is now ready for downstream applications.

RESULTS

The isolated DNA was loaded onto a 1% agarose gel. 5 µl of each sample was used. DNA was clearly visible both from the 1:1 and 1:10 dilutions, with a very slight band showing even at the 1:100 dilution, corresponding to 5 µl of original cell culture.

Fig 2. DNA on 1% agarose gel

Lane 1	= Marker λ-DNA-HIND III
Lanes 2-3	= 1:1 sample
Lanes 4-5	= 1:10 sample
Lanes 6-7	= 1:100 sample
Lanes 8-9	= 1:1000 sample



The QuickPick gDNA kit can be used to isolate bacterial genomic DNA from *E. coli.* No changes to the kit protocol were made. Each isolation takes 20 minutes, but in this case the 8 isolations were carried out in parallel with PickPen 1-M. The reagents were pipetted in advance, and transfers carried out so that all 8 samples were pipetted in turn and incubated at the same time for all incubations. Incubation times can be extended up to 30 minutes for the cell lysis step and up to 10 minutes for wash and elution.

* MagaZorb technology is a trademark of Cortex Biochem, Inc.