

Extraction of Bacterial DNA from Gram-Positive and Gram-Negative Species

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Introduction

Extracting DNA from Gram-positive and Gram-negative bacteria is an essential preliminary step in species identification, using techniques such as PCR, restriction digestion, pulsed-field gel electrophoresis (PFGE), and optical mapping. The QuickExtract™ Bacterial DNA Extraction Kit provides a simple, scalable, single-tube method for extracting bacterial DNA. This method can be used to process one to hundreds of samples, with no sample loss or toxic organic solvents. The QuickExtract Bacterial DNA Extraction Kit has been tested on a range of Gram-positive and Gram-negative bacteria.

Methods and Results

Overview

An overview of the procedure is presented in Fig. 1. Bacterial cells can be obtained directly from a plate or from bacterial suspension cultures. We recommend using approximately 10^8 bacterial cells for each extraction. For suspension cultures, use approximately 0.5 ml of suspension, and pellet the cells by centrifugation. After washing the cell pellet once with water, recentrifuge and carefully remove and discard the supernatant. Add 100 μ l of QuickExtract Bacterial DNA Extraction Solution to the cell pellet (or directly to the bacterial stab, if plates are used). Add 1 μ l of Ready-Lyse Lysozyme Solution (provided) to each tube and mix gently by inversion. Incubate the suspension at room temperature for 15 minutes. If the solution is not clearing, wait an additional hour at room temperature. Lysis is extended to several hours if necessary. Optional: If it is important to kill any remaining viable bacteria, the sample may be heated at 80°C for 2 minutes. The DNA is now ready for PCR, restriction digests, PFGE, or optical mapping. If the DNA is to be used for PCR, it may be necessary to dilute the DNA in TE buffer.

Bacterial species tested

The kit has been tested with a range of Gram-positive and Gram-negative species (Table 1). The time required for lysis will vary from 15 minutes to several hours at room temperature. We recommend testing a range of incubation times at room temperature for lysis of each organism.

We isolated DNA from a variety of bacteria and used the DNA in PCR with universal primers designed to amplify the 16S ribosomal RNA gene (Fig. 2). All species tested yielded the expected amplification products.

Optical mapping

Optical mapping is a technology that generates high-resolution, ordered, whole-genome restriction maps from single DNA molecules.¹ During optical mapping, genomic DNA molecules, extracted either from PFGE agarose gel plugs or from direct liquid lysis of cells, are mounted on a silane-derivatized glass surface, using a microfluidic device. After applying a polyacrylamide overlay, restriction digestion is performed on the glass surface. The digested DNA molecules are then stained

Table 1. Species of bacteria that have been tested with the QuickExtract™ Bacterial DNA Extraction Kit.

Gram-Positive Species	Gram-Negative Species
<i>Bacillus subtilis</i>	<i>E. coli</i>
<i>Bifidobacterium spp.</i>	<i>Salmonella typhimurium</i>
<i>Brevibacterium linens</i>	<i>Vibrio gazogenes</i>
<i>Lactobacillus plantarum</i>	
<i>Listeria monocytogenes</i>	
<i>Staphylococcus equorum</i>	
<i>Streptococcus agalactiae</i>	
<i>Streptococcus pyogenes</i>	
<i>Streptococcus thermophilus</i>	

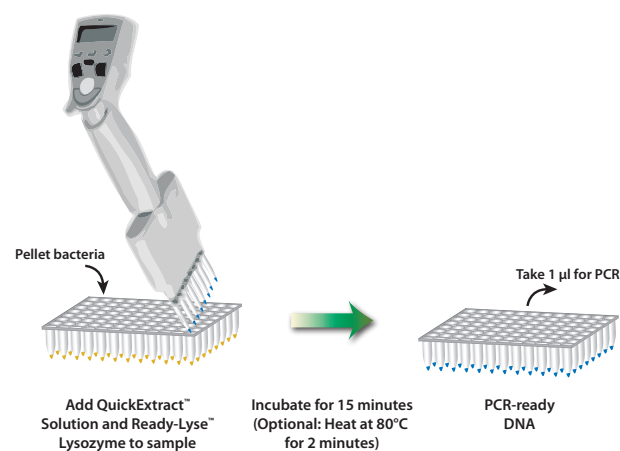


Figure 1. Overview of the QuickExtract™ Bacterial DNA Extraction Kit procedure.

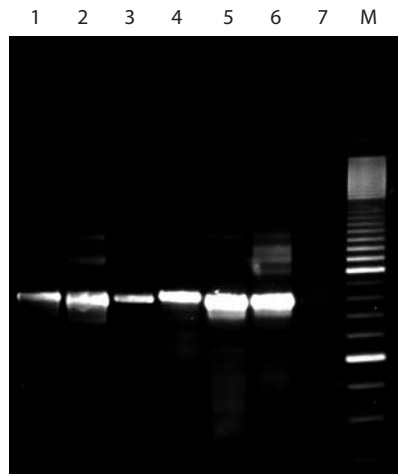


Figure 2. PCR amplification of DNA extracted with the QuickExtract™ Bacterial DNA Extraction Kit. Various Gram-positive and Gram-negative bacteria were grown on LB medium plates at 30°C or 37°C. Colony material was processed using the standard QuickExtract Bacterial DNA Kit protocol. A 1- μ l aliquot of undiluted DNA extract was used in PCR (30 cycles) with the universal bacterial rRNA gene primers 8F and 805R. The products were visualized after electrophoresis in a 2% agarose gel. Lane 1, *Bacillus subtilis*; lane 2, *Brevibacterium linens*; lane 3, *Listeria monocytogenes*; lane 4, *Lactobacillus plantarum*; lane 5, *Salmonella typhimurium*; lane 6, *E. coli*; lane 7, no-DNA control; lane M, 100-bp DNA ladder.

and imaged using fluorescence microscopy. Whole-genome optical maps are constructed by assembling single-DNA-molecule optical maps generated from image analysis using specialized software. Optical maps are very useful as scaffolds for whole-genome shotgun sequence assembly, and they have become a high-value resource for assembling whole-genome sequences from short reads of next-generation sequencing technologies. They can also be used for whole-genome analysis to identify alterations such as insertions, deletions, inversions, duplications, or other genome rearrangements.² The QuickExtract Bacterial DNA Extraction Kit has been used to extract DNA for optical mapping from *E. coli*. Fig. 3 shows that the long DNA molecules extracted using this kit were immobilized on a optical-mapping surface (many molecules were 400-500 kb in length).

Conclusions

The QuickExtract Bacterial DNA Extraction Kit provides a simple method for extracting DNA from Gram-positive and Gram-negative bacteria. The kit incorporates Ready-Lyze Lysozyme, which has 200-fold higher specific activity than

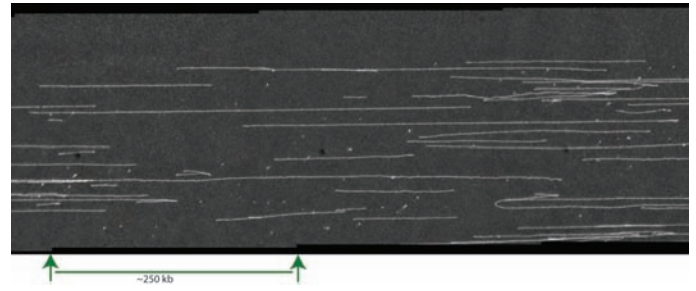


Figure 3. Long DNA strands of *E. coli* extracted using the QuickExtract™ Bacterial DNA Extraction Kit. Many strands were 400-500 kb.

egg-white lysozyme. After a 15-minute lysis step at room temperature, the DNA obtained is ready for PCR or other downstream applications such as restriction digests, PFGE, and optical mapping. The kit has been tested on a range of bacteria and has been shown to produce very long DNA strands, due to the gentleness of the extraction method. The single-tube system does not use toxic organic solvents and is amenable to high-throughput applications.

References

1. Zhou, S. *et al.* (2003) *Genome Res.* **13**, 2142
2. Zhou, S. *et al.* (2004) *J. Bacteriol.* **186**, 7773.

Cat. #	Quantity
QuickExtract™ Bacterial DNA Extraction Kit	
QEB0905T	5 ml (50 extractions)
QEB09050	50 ml (500 extractions)