Introduction

This protocol describes a method for DNA extraction from *E. coli*, as an example of a Gram-negative bacteria. We recommend growing a culture to OD ~0.7, and extracting the DNA using the QIAGEN Genomic-tip 500/G columns. These are easy to use, do not require the use of phenol or chloroform, and give a consistently high yield. We have found that incubating the culture with chloramphenicol for 1 hour before collection resulted in a 4.5X increase in yield (from 7.4 µg to 33.9 µg). Addition of this antibiotic stops cell division, but still enables completion of DNA replication.

Materials

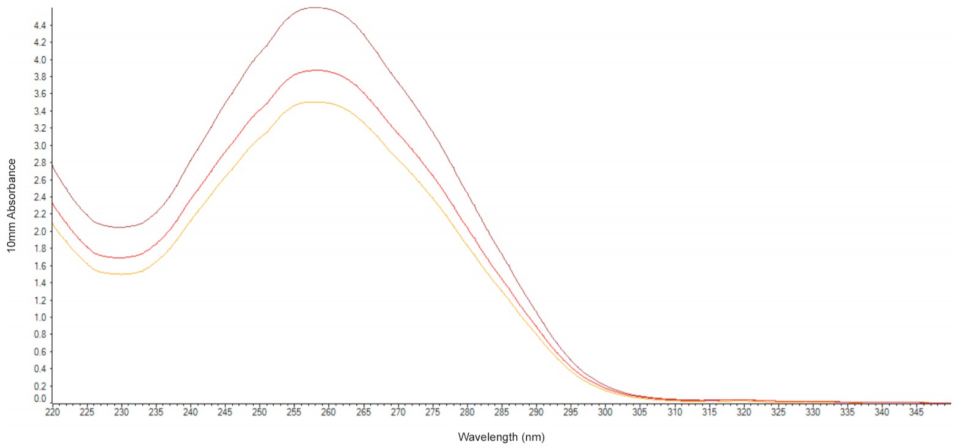
- QIAGEN Genomic-tip 500/G
- Chloramphenicol
- 10 ml renewed *E. coli* culture

Method

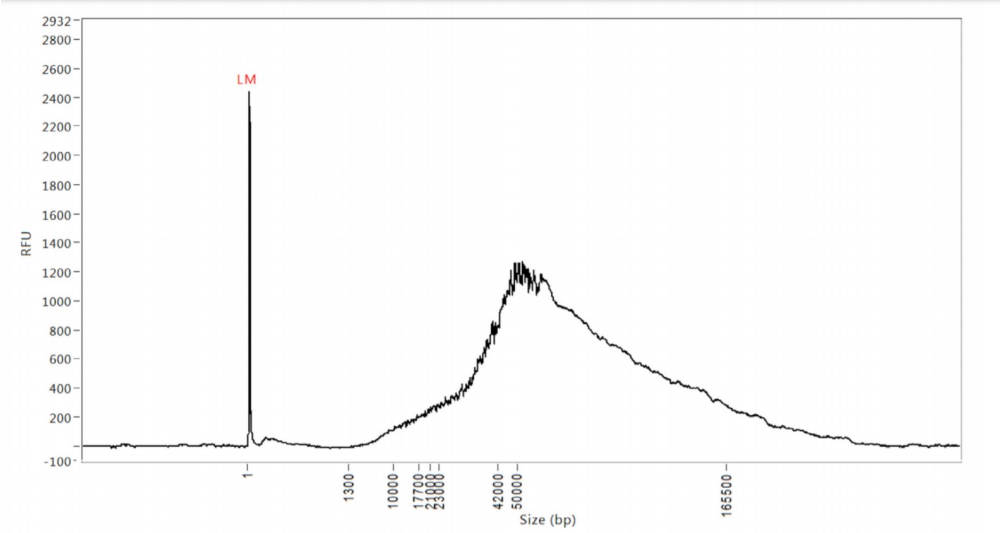
1. Culture the *E. coli* cells until the OD reaches 0.5.
2. Add chloramphenicol to the culture to a final concentration of 180 µg/ml. Incubate the culture for another hour (at the end, the OD should be ~0.7). Remove 10 ml of culture for extraction.
3. Follow the QIAGEN Genomic-tip 500/G protocol. Elute the purified DNA into 200 µl TE buffer, pH 8, and leave at room temperature until all DNA has dissolved.
4. **Critical Step:** If after several hours some precipitation is still visible in the sample, this could indicate protein contamination. In this case, spin down the sample in a microfuge for 1 min at 10,000 g, and carefully remove the supernatant.

Results

- **Yield:** 33.9 µg
- **OD 260/280:** 1.89
- **OD 260/230:** 2.29



- **Fragment size: 1.3 - 165kb (FEMTO pulse)**



Sequencing performance

Libraries for Nanopore sequencing were prepared using the Ligation Sequencing Kit, with g-TUBE fragmentation.

- Read length profile after fragmentation and sequencing: