Materials

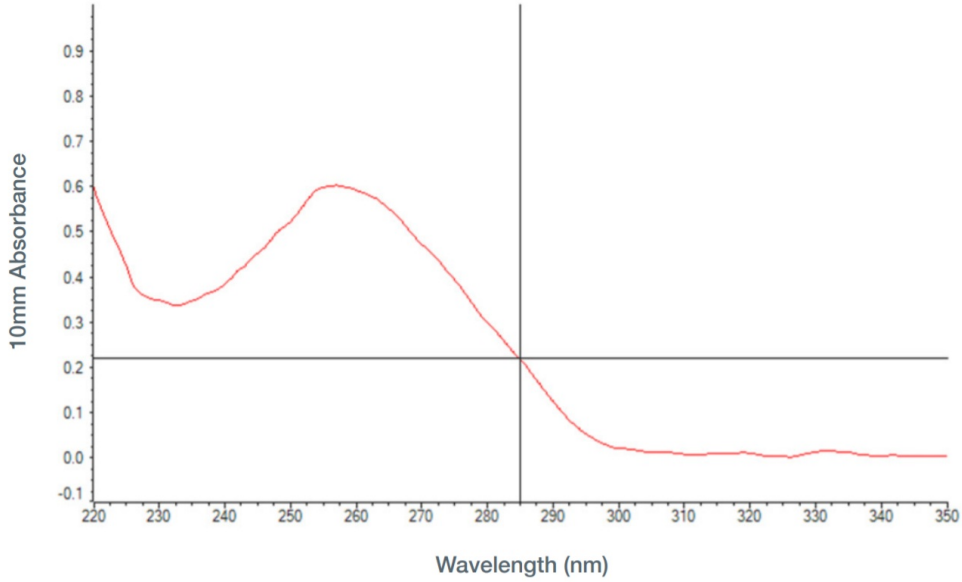
- 1 x 10^{11} bacterial cells (this corresponds to a cell pellet weighing ~450 mg)
- QIAGEN Blood and Cell Culture DNA Midi kit
- RNase A
- Lysozyme
- Isopropanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 50 ml Falcon tubes
- Centrifuge capable of taking 50 ml Falcon tubes
- Incubator or water bath

Method

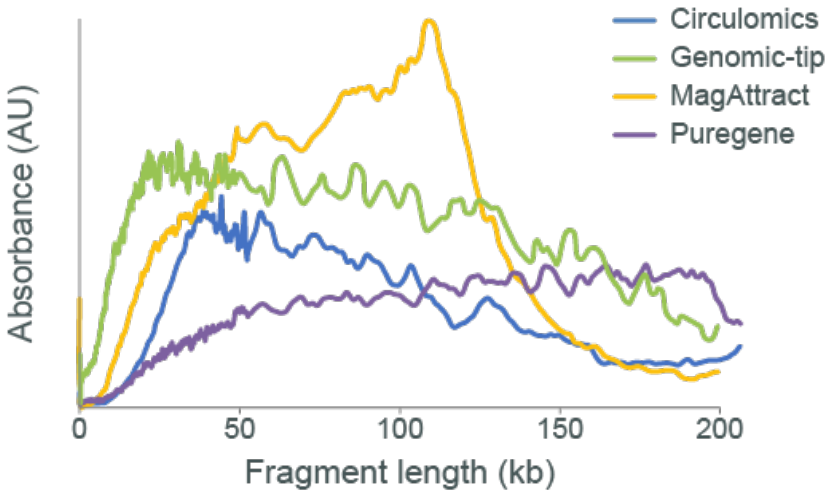
1. Lyse the cells according to the standard protocol, increasing the lysis time to 1 hour instead of 30 minutes for both steps 5 and 6 (steps 4–7, page 47).
2. Purify the lysate according to the protocol (steps 1–6, page 49).
3. **Critical Step:** To avoid DNA fragmentation, we recommend spooling the DNA (e.g. using one arm of disposable tweezers) instead of centrifugation in steps 5A and 6A, page 51.
4. To maximize the DNA yield, we recommend eluting the DNA overnight in 750 µl TE (10 mM TrisHCl, 1 mM EDTA, pH 8.0).
5. Take ~20 µl of eluate (corresponding to 3 µg DNA) and perform a SPRI size selection.

Results

- **Yield:** 125–140 µg
- **OD 260/280 (after SPRI size selection):** 1.98
- **OD 260/230 (after SPRI size selection):** 1.70



- **Fragment size (FEMTO pulse) after size selection:**



Sequencing performance

Libraries for Nanopore sequencing were prepared using the Ligation Sequencing Kit.

- Read length profile: