Materials

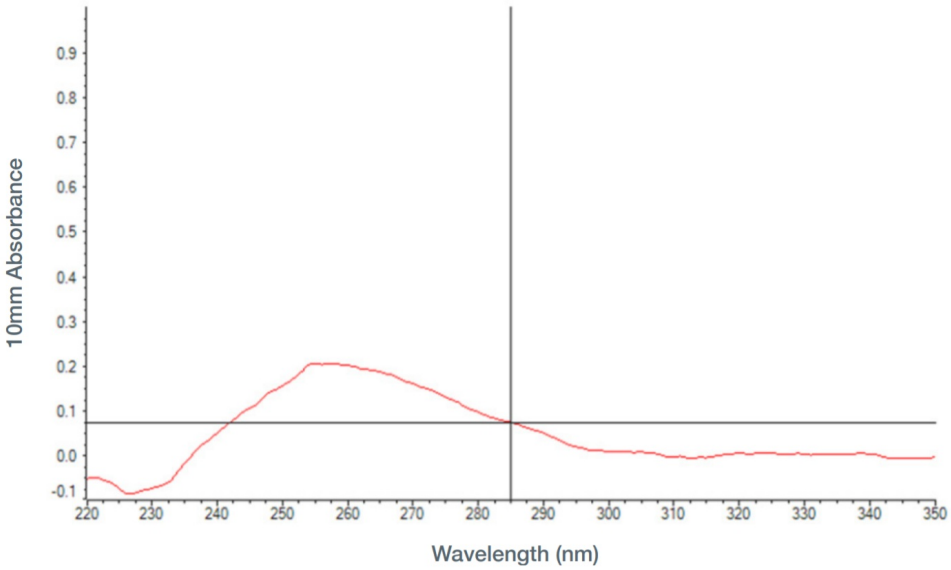
- 2 x 10^9 bacterial cells (this corresponds to a cell pellet weighing ~3 mg)
- QIAGEN MagAttract HMW DNA Kit
- 1.5 ml Eppendorf DNA LoBind tubes
- Nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Eppendorf ThermoMixer
- Magnetic rack

Method

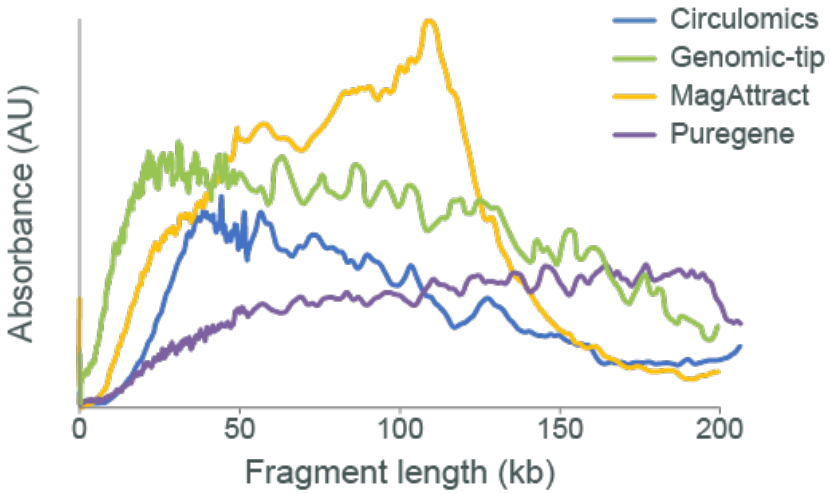
1. Lyse the cells according to the standard protocol (steps 2–6, page 30), with a 2 hour incubation in step 4.
2. Purify the lysate according to the protocol (steps 7–17, page 31).
3. To elute, remove the tube holder of the MagAttract Magnetic Rack from its magnetic base and add 150 µl of TE buffer. Place the tube holder onto the Eppendorf ThermoMixer and incubate at room temperature for 3 minutes at 1000 rpm. Remove the tube holder from the mixer and incubate for a further 10 minutes, without agitation.
4. Pellet the beads on a magnet, and transfer the supernatant to a clean 1.5 ml Eppendorf DNA LoBind tube.
5. Repeat steps 3 and 4.
6. Take ~75 µl of eluate (corresponding to 3 µg DNA) and perform a SPRI size selection.

Results

- **Yield:** 30-40 µg
- **OD 260/280 (after SPRI size selection):** 2.10
- **OD 260/230 (after SPRI size selection):** -2.58 (Note, the expected values for “pure” DNA are in the range of 2.0-2.2, and therefore the value obtained here is somewhat unexpected.)



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



Sequencing performance

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

- Read length profile: