Materials

- 2 x 10^9 bacterial cells (this corresponds to a cell pellet weighing ~3 mg)
- QIAGEN Puregene Cell Kit
- QIAGEN Lytic Enzyme Solution
- 2 ml Eppendorf tubes
- Nuclease-free water
- 70% ethanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Microfuge
- Shaker
- Magnetic rack
- Incubator or water bath

Method

1. Lyse the cells and purify the lysate according to theQIAGEN Puregene Handbood (steps 1–19, pages 62-63), incubating for 1 hour at step 10:

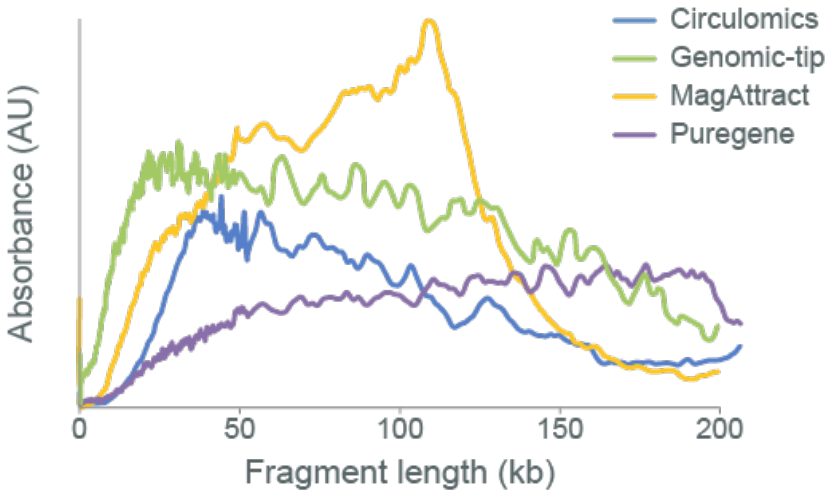
   a. Transfer 500 µl of the cell culture to a 2 ml Eppendorf tube on ice.
   b. Centrifuge for 5 seconds at 13,000 - 16,000 x g to pellet the cells.
   c. Carefully discard the supernatant by pipetting or pouring.
   d. Add 300 µl of TE buffer and mix by pipetting.
   e. Add 1.5 µl of Lytic Enzyme Solution and mix by inverting 25 times. Incubate for 30 minutes at 37°C.
   f. Centrifuge for 1 minute at 13,000 - 16,000 g to pellet the cells.
   g. Carefully discard the supernatant with a pipette.
   h. Add 300 µl of Cell Lysis Solution and mix by pipetting to lyse the cells. An incubation for 5 minutes at 80°C may be necessary to lyse the cells of some species.
   i. Add 1.5 µl of RNase A Solution and mix by inverting 25 times. Incubate for 1 hour at 37°C.
   j. Incubate for 1 minute on ice to quickly cool the sample.
   k. Add 100 µl of Protein Precipitation Solution and vortex vigorously for 20 seconds at high speed. For some species with high polysaccharide content, incubate the sample on ice for 15-60 minutes.
   l. Centrifuge for 3 minutes at 13,000 - 16,000 x g. The precipitated proteins should form a tight pellet. If the pellet is not tight, incubate on ice for 5 minutes and repeat the centrifugation.
   m. Pipette 300 µl of isopropanol into a clean 1.5 ml Eppendorf tube and add the supernatant from the previous step by pouring carefully. Be sure the protein pellet is not dislodged during the pouring.
   n. Mix by inverting gently 50 times.
   o. Centrifuge for 1 minute at 13,000 - 16,000 x g. The DNA will be visible as a small white pellet.
   p. Carefully discard the supernatant and drain the tube by inverting on a clean piece of absorbant paper, taking care that the pellet remains in the tube.
   q. Add 300 µl of 70% ethanol and invert several times to wash the DNA pellet.
   r. Centrifuge for 1 minute at 13,000 - 16,000 x g.
   s. Carefully discard the supernatant. Drain the tube on a clean piece of absorbant paper, taking care that the pelle remains in the tube. Allow to air dry for 5 mintues. The pellet might by loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

- Elute the DNA overnight in 200 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
- Take ~150 µl of eluate (pooled from several replicates, corresponding to 3 µg DNA) and perform a SPRI size selection.

Results

- **Yield:** 1-2 µg

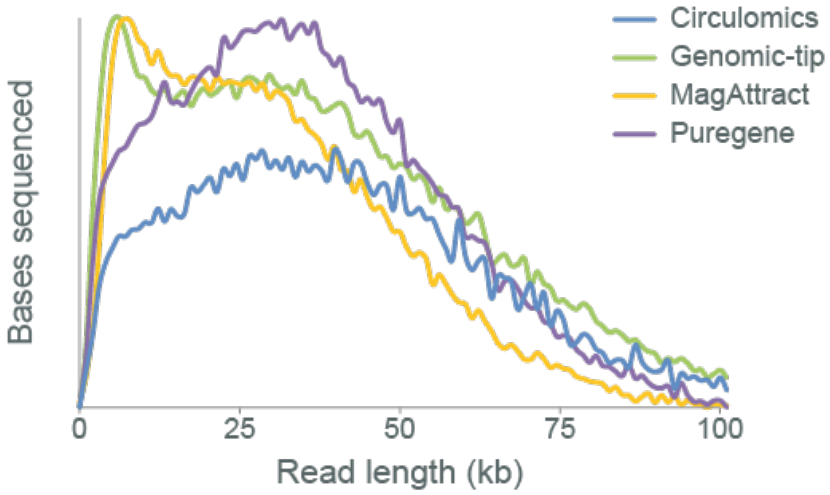
- **OD 260/280 (after SPRI size selection):** 2.15
- **OD 260/230 (after SPRI size selection):** 7.01 10mm
  (Note, the expected values for “pure” DNA are in the range of 2.0-2.2, and therefore the value obtained here is somewhat unexpected.)
- **Fragment size (FEMTO pulse, after SPRI size selection):**



Sequencing performance

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

- Read length profile:



Change log

Version	Change
v2, January 2023	Updated protocol to be aligned with the QIAGEN handbook update and updated the recommend Puregene extraction kit.
v1	Initial publication