Material

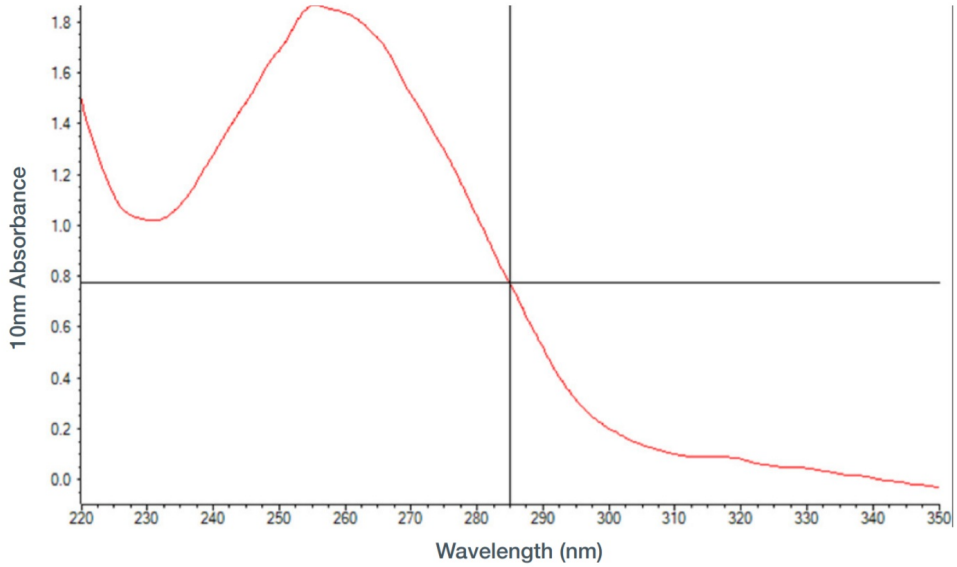
- 1 rat stool pellet
- QIAGEN MagAttract HMW DNA Kit
- QIAGEN Proteinase K
- QIAGEN RNase A
- TE buffer (1 mM EDTA, pH 8.0)
- 2 ml Eppendorf tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Eppendorf ThermoMixer
- Incubator
- Microfuge

Method

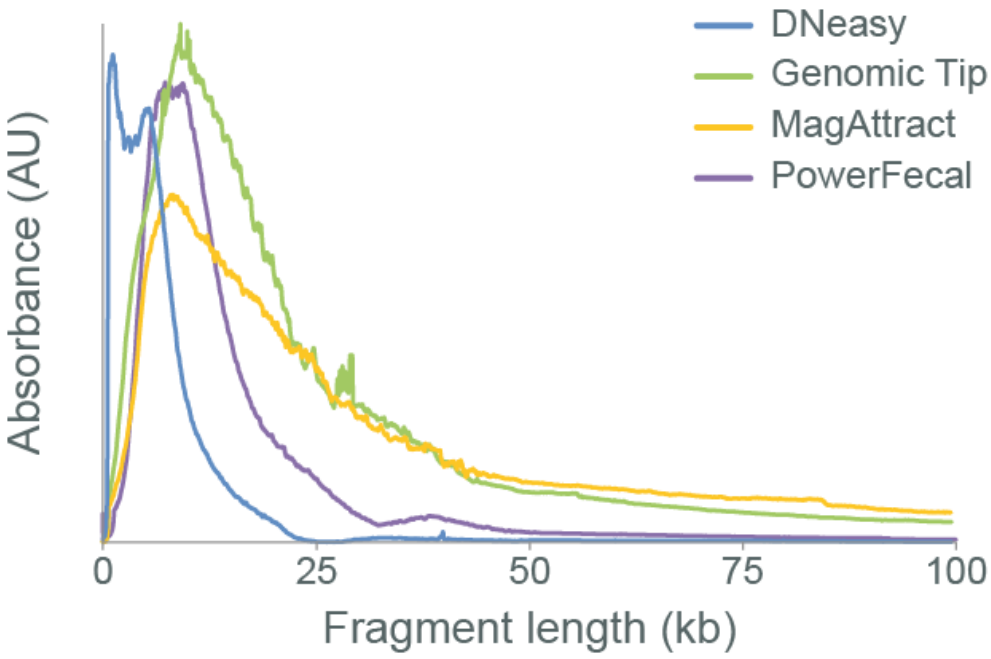
1. Transfer 1 rat stool pellet to a 2 ml Eppendorf tube and add ~500 µl of ATL (enough to cover the pellet) and 20 µl of Proteinase K, both supplied with the MagAttract HMW DNA Kit. Mix thoroughly by inversion.
2. Incubate the sample at 56° C for 1 hour, at 20 rpm.
3. Centrifuge the tube at 8000 rpm for 1 minute, and transfer the supernatant to a 2 ml Eppendorf tube. Take care to avoid transfer of solid matter. If necessary, repeat the spin step.
4. Add 4 µl of RNase A to the sample, and incubate at room temperature for 2 minutes.
5. Purify the lysate according to the MagAttract HMW DNA Kit protocol (page 24, steps 3–13).
6. 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 ThermoMixer, and incubate for a further 10 minutes at room temperature, without agitation.
7. Pellet the beads on a magnetic rack, and transfer the eluted DNA to a clean 1.5 ml Eppendorf DNA LoBind tube.
8. Take ~25 µl of eluate (corresponding to 3 µg of DNA) and perform a SPRI size selection.

Results

- **Yield:** 9–12 µg
- **OD 260/280:** 1.96 (after SPRI size selection)
- **OD 260/230:** 1.80 (after SPRI size selection)



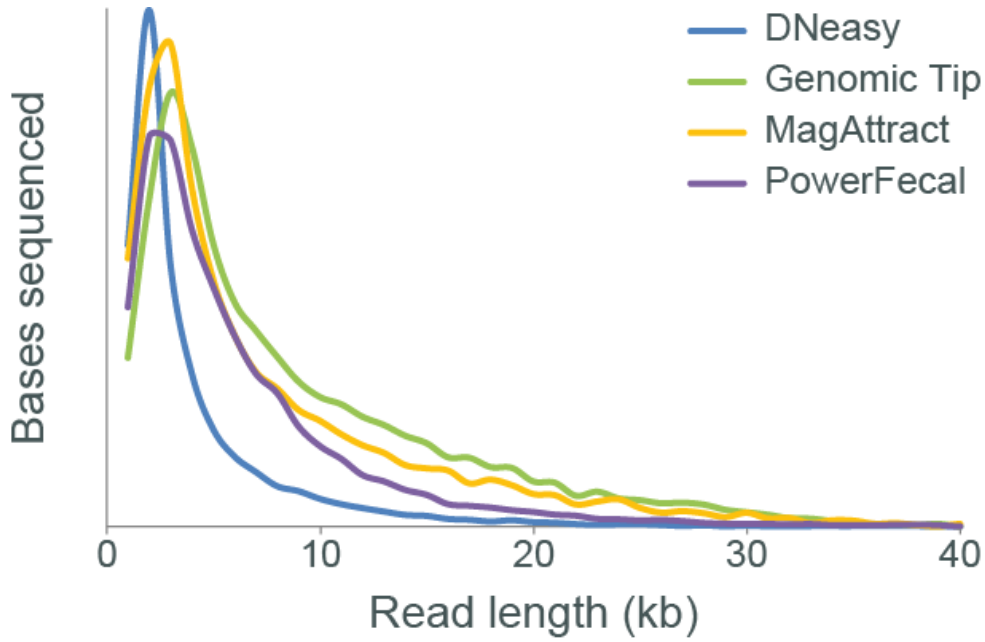
- **Fragment size (FEMTO pulse) after SPRI size selection:**



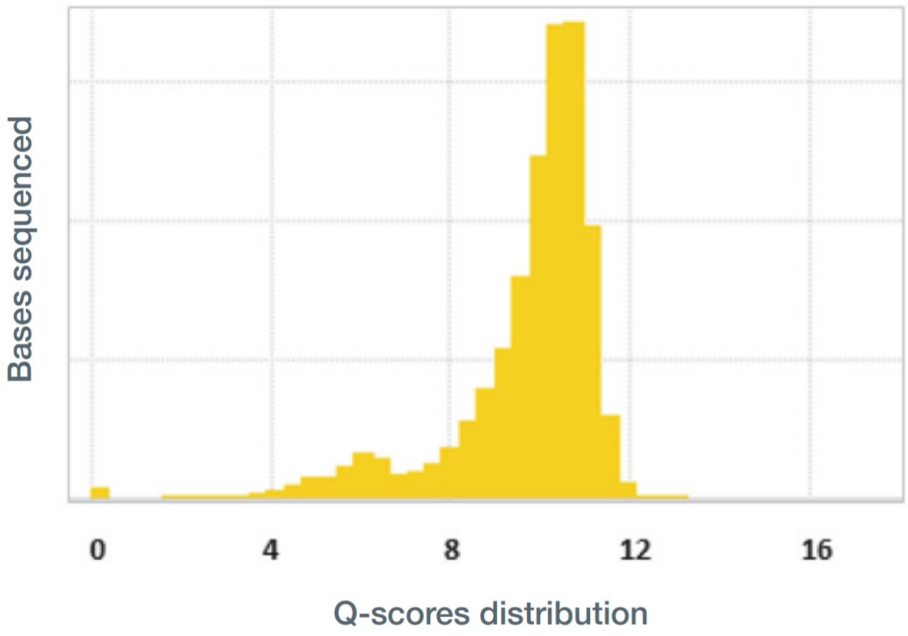
Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



- Qscore distribution:



- Alignment results: