Materials

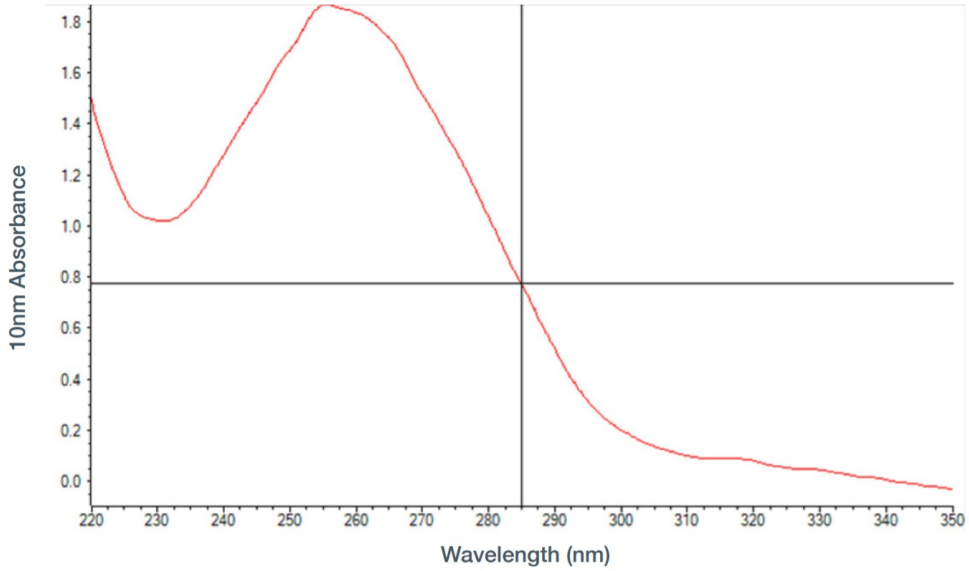
- 3 rat stool pellets
- QIAGEN Genomic DNA Buffer Set
- QIAGEN Genomic-tip 20/G
- QIAGEN Proteinase K
- QIAGEN RNase A
- TE buffer (1 mM EDTA, pH 8.0)
- Lysis buffer (100 mM Tris-HCl, 100 mM EDTA, 10 mM NaCl, 1% N-lauroyl-sarcosine, pH 7.5) – as described by Maudet et al.
- 20 ml scintillation tubes
- 2 ml Eppendorf tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- HulaMixer™ Sample Mixer
- Isopropanol
- Incubator

Method

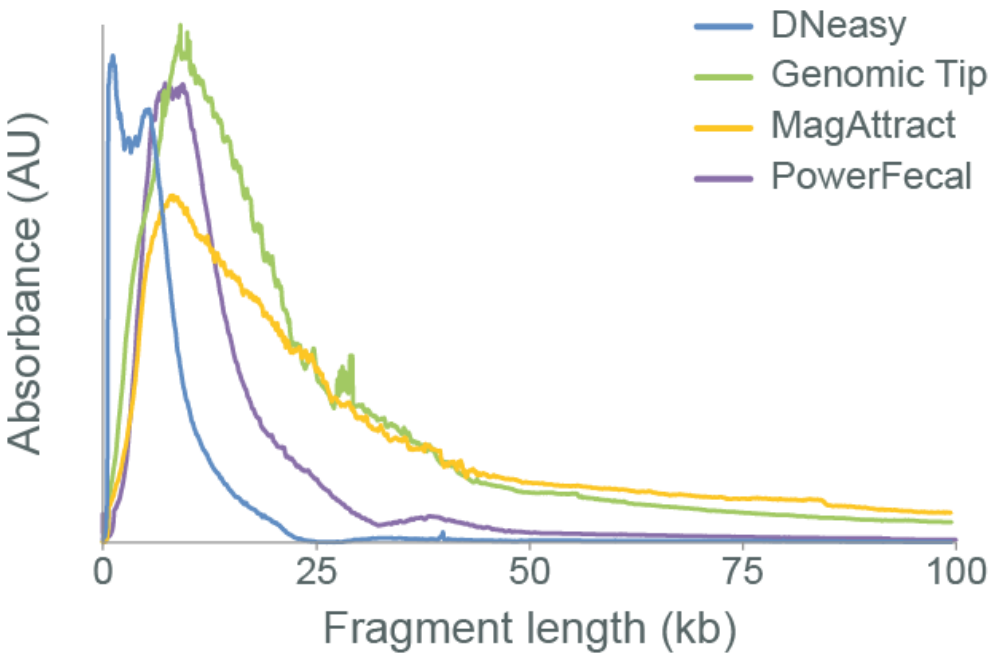
1. Transfer 3 rat stool pellets to a 20 ml scintillation tube and add 500 µl of lysis buffer.
2. Using a HulaMixer™ Sample Mixer, rotate the tubes at 20 rpm for 15 minutes at room temperature. If a HulaMixer™ Sample Mixer is not available, gently invert the tubes every minute for 15 minutes. Depending on the sample condition (e.g. moisture content and preservation state), it is possible that the pellets start to absorb the lysis buffer – if this happens, more lysis buffer should be added.
3. Transfer the supernatant, which should be ~200-300 µl, to a 2 ml Eppendorf tube. Take care to avoid transfer of solid matter.
4. Add 1000 µl of buffer G2 from the Genomic-tip 20/G kit, 4 µl of RNase A (100 mg/ml), and 25 µl of Proteinase K to the tube with the supernatant, and mix by inversion.
5. Incubate the sample for 90 minutes at 56°C.
6. Purify the lysate according to the QIAGEN protocol for blood samples (page 49, steps 1-6).
7. Elute the DNA pellet overnight in TE buffer.
8. Take ~50 µl of eluate (corresponding to 3 µg of DNA) and perform a SPRI size selection.

Results

- **Yield:** 8–13 µg
- **OD 260/280:** 1.78 (after SPRI size selection)
- **OD 260/230:** 1.95 (after SPRI size selection)



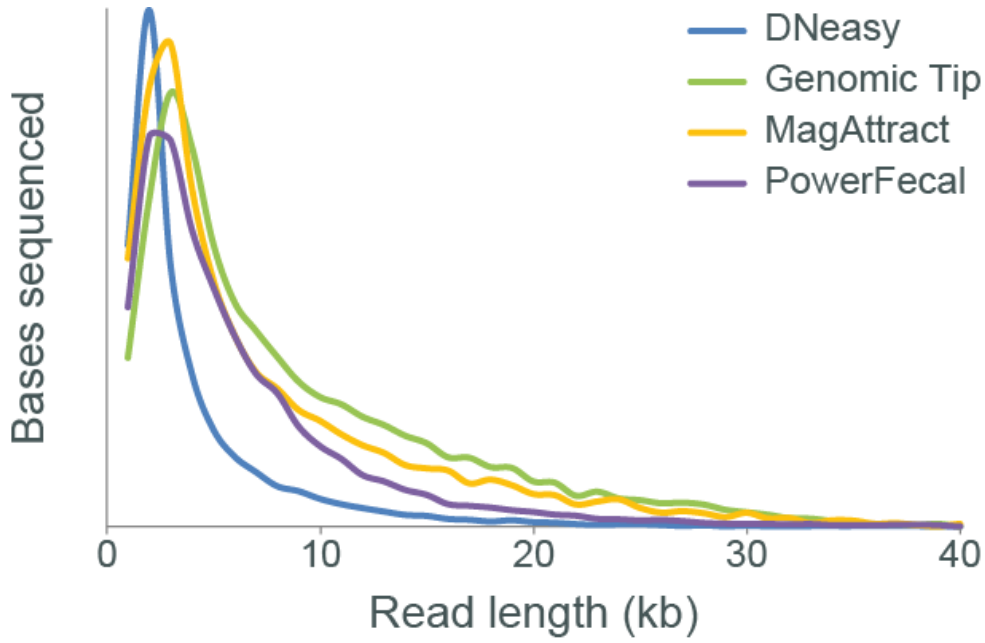
Fragment size (FEMTO pulse) after SPRI size selection:



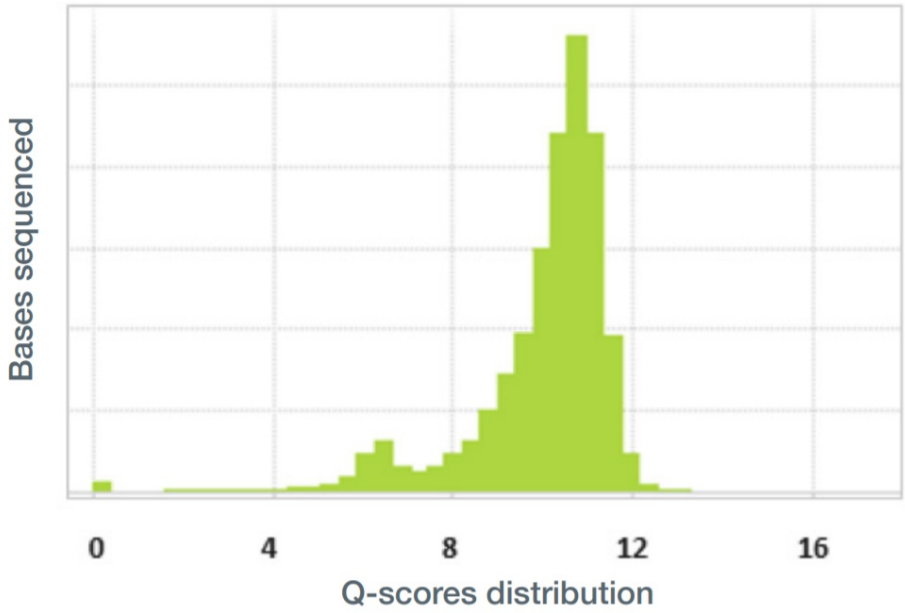
Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



- Qscore distribution



- Alignment results: