Materials

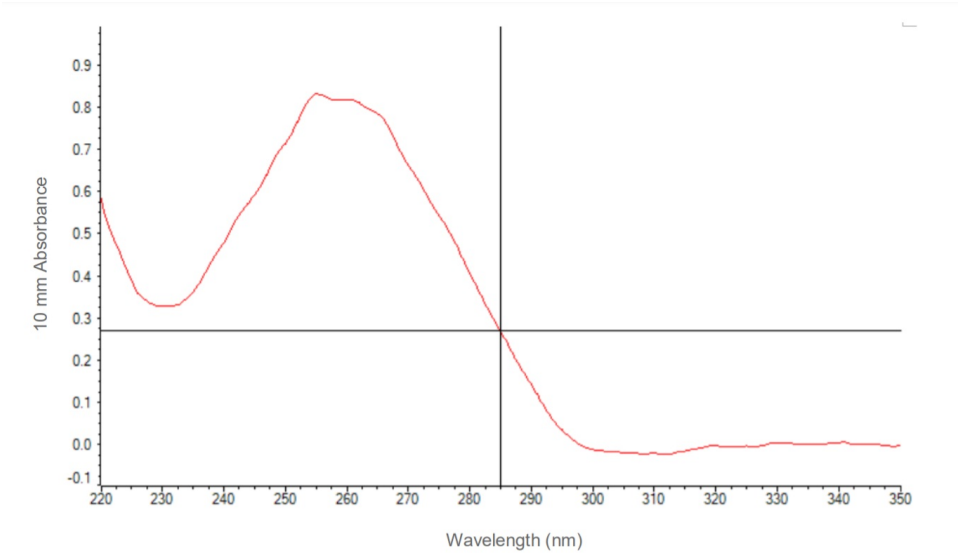
- 100 mg chicken fat
- QIAGEN Blood and Cell Culture DNA Midi kit
- QIAGEN Proteinase K
- RNase A
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Isopropanol
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Refrigerated centrifuge capable of taking 15 ml Falcon tubes
- Incubator or water bath set at 50°C
- Tweezers and scalpel for homogenising tissue

Method

1. To a 15 ml Falcon tube, add 19 µl of RNase A (100 mg/ml) and 9.5 ml of G2 buffer.
2. Grind up to 100 mg chicken fat tissue, using tweezers and scalpel, and transfer the resulting pulp to the 15 ml Falcon tube containing the G2 buffer. As an alternative, users may grind the tissues with liquid nitrogen, mortar, and pestle, or even using a sample disruption device (such as the TissueRuptor), although we have not validated these methods for these tissues.
3. Add 250 µl of Proteinase K, and vortex thoroughly. It is important that the solution is well mixed for the lysis to be efficient.
4. Incubate at 50°C overnight.
5. **Critical Step** If the lysate is not homogenous or if bits of tissue are still visible, centrifuge at 2000 x g for 10 minutes, at 4°C. If the lysate is not homogenous, it is likely that it will block the Genomic-tip column. Transfer the supernatant to the Genomic-tip column and discard the pellet.
6. Purify the lysate according to the QIAGEN handbook, Isolation of genomic DNA from blood, cultured cells, tissues, yeast, or bacteria using genomic-tips section (steps 1 to 6, pages 49-52).
7. **Critical Step** To maximize the DNA yield we recommend that the elution is performed at 50°C for two hours, with end-to-end rotation, using 150 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
8. Take 3 µg of DNA and perform a SPRI size selection.

Results

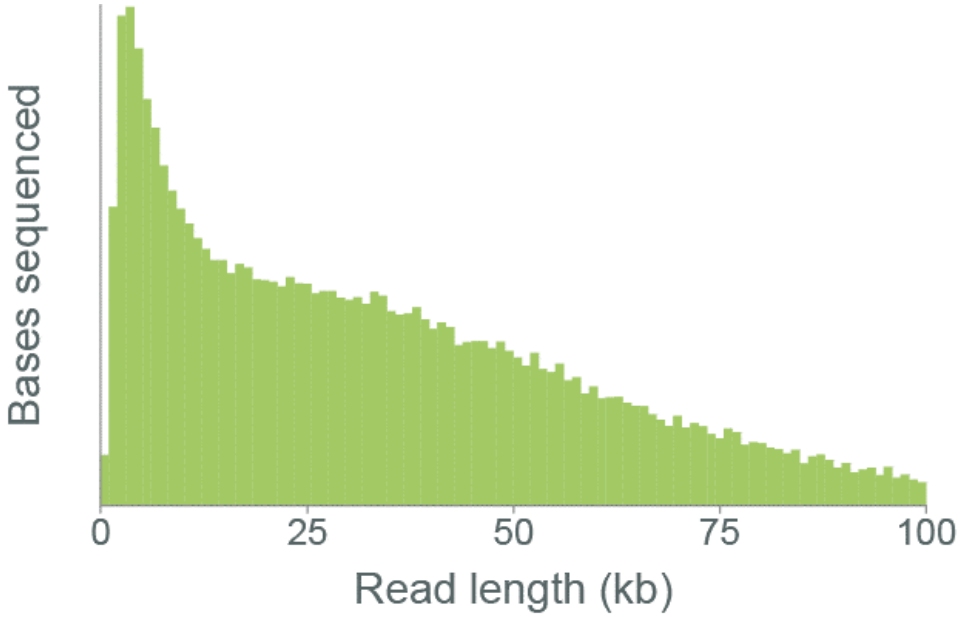
- **Yield:** ~30-50 µg
- **OD 260/280:** 2.01
- **OD 260/230:** 2.50



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Output from the flow cell may be increased by performing a flow cell wash step (at the point where the rate of data acquisition begins to deteriorate due to the accumulation of pores in the “unavailable” or “recovering” state) and then adding a new library.
- Read length profile:



Change log

Version	Change
v1, 11th February 2019	Initial protocol publication