Materials

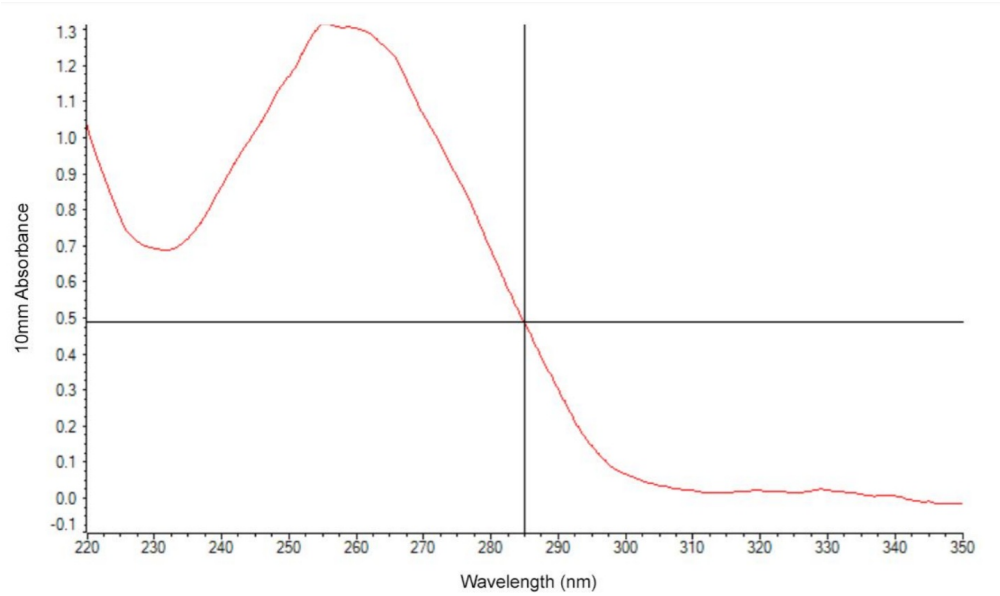
- Up to 100 mg rabbit brain tissue
- QIAGEN Blood and Cell Culture DNA Midi Kit
- QIAGEN proteinase K
- QIAGEN ATL buffer
- RNase A
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Isopropanol
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Centrifuge and rotor for 15 ml Falcon tubes
- Incubator or water bath set at 50°C
- Tweezers and scalpel

Method

1. Add 19 µl of RNase A (100 mg/ml) to a 15 ml Falcon tube, then add 1 ml of ATL buffer.
2. Grind up to 100 mg brain tissue using tweezers and a scalpel, and transfer the resulting pulp to the Falcon tube containing the ATL buffer. Alternatively, users may grind the tissue with liquid nitrogen, mortar, and pestle, or using a sample disruption device (such as the TissueRuptor), however we have not validated these methods for these tissues.
3. Add 50 µl of proteinase K, and vortex the Falcon tube thoroughly. It is important that the suspension is well mixed for the lysis to be efficient.
4. Incubate the tissue suspension overnight at 50°C.
5. Pulse-vortex the lysate and add 9 ml of G2 buffer, and a further 50 µl of proteinase K.
6. Vortex the lysate, and incubate at 50°C for 30 minutes.
7. **Critical step:** If after the incubation the lysate is not homogenous, or if pieces of tissue are still visible, centrifuge the tube at 2000x g for 10 minutes at 4°C. Discard the pellet and retain the homogenous supernatant. If the lysate is not homogenous at this stage, it is likely to block the Genomic-tip column.
8. Purify the lysate according to the QIAGEN Genomic-tip protocol (steps 1–6, pages 49–52).
9. **Critical step:** To maximize the DNA yield we recommend that the elution is performed for 2 hours at 50°C, using 150 µl TE (1 mM EDTA, pH 8.0), occasionally mixing the tube contents by gentle inversion.
10. Take 3 µg of eluate and perform a SPRI size selection.

Results

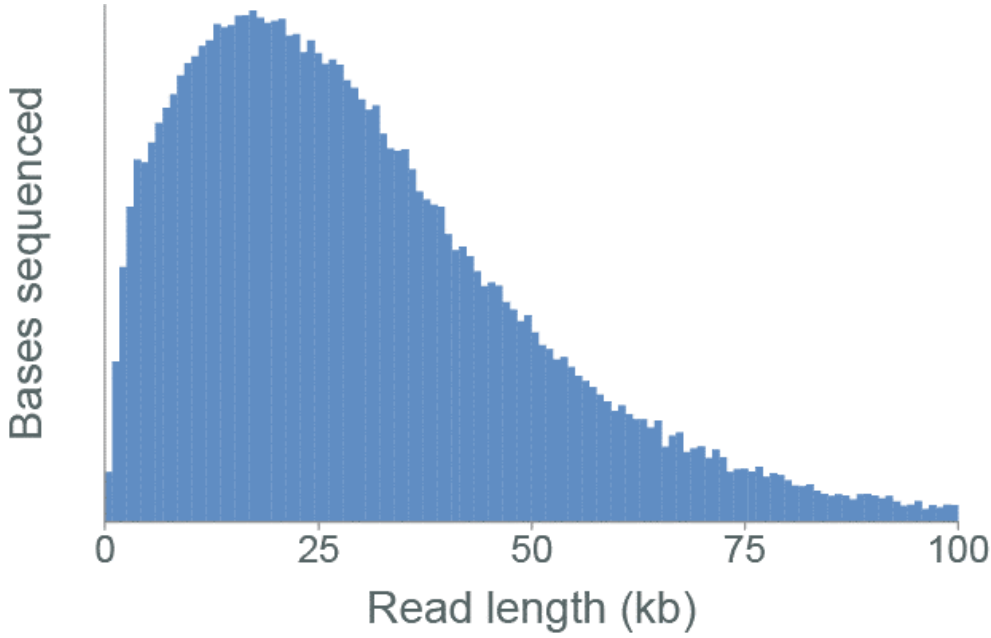
- **Yield:** 60-100 µg
- **OD 260/280:** 1.89
- **OD 260/230:** 1.89



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
v2, 14th August 2023	Updated URL link