Introduction

This protocol describes a method to extract high molecular weight genomic DNA from rat tail, as an example of mammal tail. The tail was collected and stored at -80°C before gDNA extraction. The extraction method was performed using QIAGEN MagAttract HMW DNA Kit. Sequencing performance was determined using the MinION, using the Ligation Sequencing Kit to generate sequencing libraries.

Materials

- 25 mg rat tail
- QIAGEN MagAttract HMW DNA Kit
- TE Buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0)
- Magnetic rack
- ThermoMixer™
- Vortex Mixer
- 1.5 ml Eppendorf DNA LoBind tubes
- 2 ml Eppendorf DNA LoBind tubes
- Microcentrifuge
- Ice bucket with ice
- Weighing boats
- Tweezers and scalpel
- P20, P100, P200 and P1000 pipettes, tips and wide-bore pipette tips

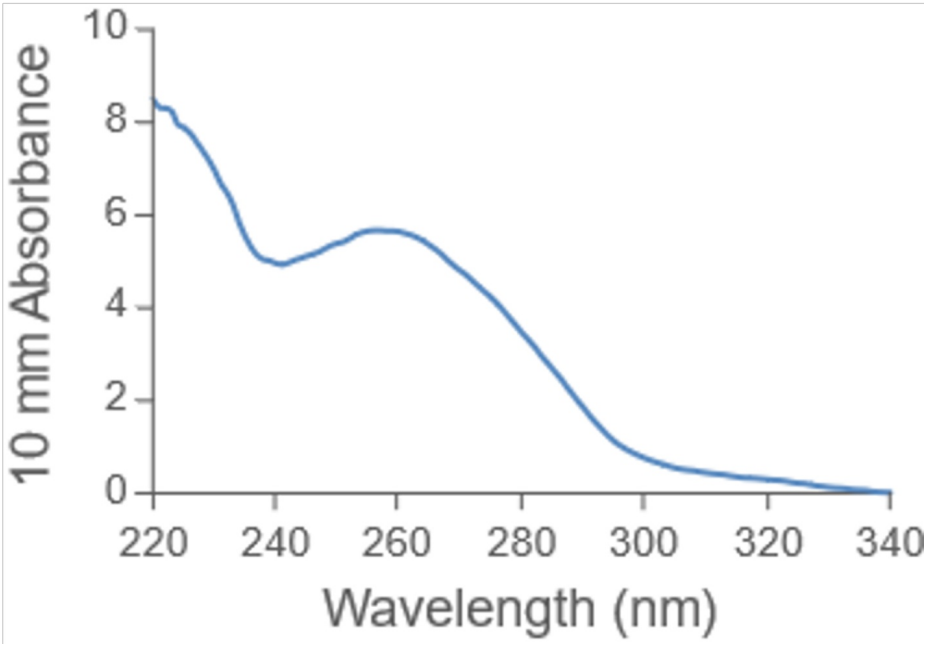
Method

1. Use up to 25 mg of tail tissue and cut the sample into small pieces before mashing it as much as possible using the tweezers and scalpel. We recommend processing the tissue in a weighing boat placed on ice to keep the tissue cold.
2. Transfer the tissue to a 2 ml Eppendorf DNA LoBind tube containing 220 µl of ATL buffer as soon as you finish processing it.
3. Add 20 µl of proteinase K and vortex to mix.
4. Incubate on the ThermoMixer™ at 56°C for 3 hours at 900 rpm. The lysate should be homogeneous at this point. If there are still chunks of tissue present, centrifuge the tube at 20,000 x g for 1 minute and transfer the supernatant to a new tube. Avoid carrying any tissue that has not been lysed with the supernatant.
5. Add 4 µl of RNase A and flick the tube to mix. Incubate at room temperature for 3 minutes.
6. Add 150 µl of AL buffer and mix by pipetting. In this step, precipitation can occur so we recommend using wide-bore tips.
7. Follow the protocol in the QIAGEN MagAttract HMW DNA Handbook from step 4 to 13 on pages 20-21.
8. Add 150 µl of TE buffer and incubate the sample at room temperature for 30 minutes.
9. Incubate the sample on the ThermoMixer™ at 37°C for 1 minute at 1,400 rpm.
10. Briefly centrifuge the tube and place it on the magnetic rack.
11. Transfer the supernatant containing the DNA to a fresh 1.5 ml Eppendorf DNA LoBind tube.
12. Repeat steps 8-11 two more times, using different elution tubes each time. You should end up with three separate tubes, each with 150 µl and different concentrations of DNA.

Results

- **Yield:** 15-30 µg
- **A260/280:** 1.63

- **A260/230:** 0.81



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Output from the cell can be increased by washing the flow cell using the Flow Cell Wash Kit (EXP-WSH004) and re-loading the library after ~20 hours and again at ~40 hours of sequencing.
- Read length profile: