Introduction

This is a method to extract and sequence high molecular weight genomic DNA from the Atlantic salmon (*Salmo salar*), as an example of fish blood. We found that samples extracted from blood stored in ethanol at a final concentration of 90% typically generated the highest sequencing output compared with samples extracted from tissues (Fig. 3). Further, we observed that shearing the sample using the Covaris g-TUBE TM or the Megaruptor® could also be used to increase sequencing output but at the expense of read N50. For further general data showing performance comparison of fragmented gDNA, please see 'Optional Fragmentation of gDNA'. In order to obtain maximum sequencing data from the flow cell, we recommend using the Flow Cell Wash Kit. Sequencing performance was assessed using the Ligation Sequencing Kit.

Materials

- Salmon blood with ethanol added to a final concentration of ~90% and stored at −80°C prior to gDNA extraction
- MagAttract HMW DNA kit
- Magnetic rack
- ThermoMixer™
- Vortex
- 1.5 ml and 2 ml Eppendorf tubes
- Microcentrifuge
- TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 8.0)
- PBS 1X

Method

1. Take the tube with the blood and ethanol mix from the freezer and invert 10 times to mix.
2. With a wide bore pipette tip, transfer 200 µl of the blood and ethanol mix to a new 2 ml Eppendorf tube.
3. Centrifuge at 10,000 x g for 2 minutes and discard the supernatant containing the ethanol.
4. Add 200 µl of 1X PBS and flick the tube to resuspend the sample.
5. Centrifuge at 10,000 x g for 2 minutes and discard the supernatant containing the PBS.
6. Add 200 µl of 1X PBS and pulse vortex twice to resuspend the sample.
7. Add 20 µl of proteinase K and 150 µl of AL buffer from the MagAttract HMW DNA Kit, and vortex to mix.
8. Incubate on the ThermoMixer™ at 56°C for 1 hour at 900 rpm to agitate
9. Briefly centrifuge the tube and add 4 µl of RNase A from the MagAttract HMW DNA Kit. Flick the tube to mix.
10. Incubate at room temperature for 3 minutes.
11. Add 40 µl of MagAttract Suspension G.
12. Follow the recommended protocol from step 7 to step 15 of the handbook (pages 19-20).
13. Add 250 µl of TE buffer and incubate the sample at room temperature for 30 minutes.
14. Incubate the sample on the ThermoMixer™ at 37°C for 1 minute at 1400 rpm.
15. Briefly centrifuge the tube and place it on the magnetic rack.
16. Transfer the supernatant containing the DNA to a fresh 1.5 ml Eppendorf tube.
17. Repeat steps 11-14 twice more, using fresh Eppendorf tubes each time.

Results

Sample	Yield (µg)	A260/280	A260/230
Blood	6-16	1.92	0.43
Heart	25-35	1.79	1.91
Liver	70-80	2.00	2.25
Spleen	60-70	1.81	2.00
Fin	50-60	1.95	2.37
Brain	30-40	1.55	1.02

Table 1. Genomic DNA quantification and QC data. Genomic DNA extracted from various salmon tissues, including blood, was quantified using a Qubit fluorometer and purity was assessed using a NanoDrop.

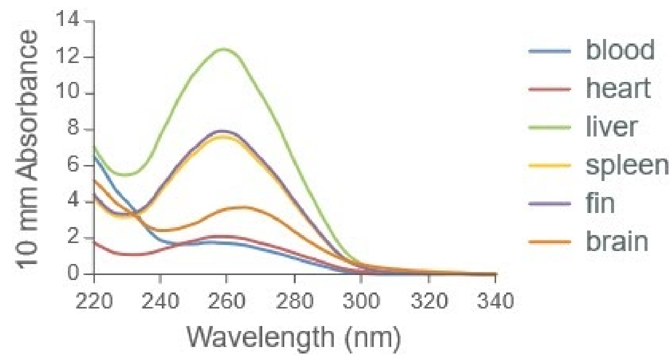

Figure 1. NanoDrop spectra for gDNA extracted from various salmon tissues, including blood.

Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

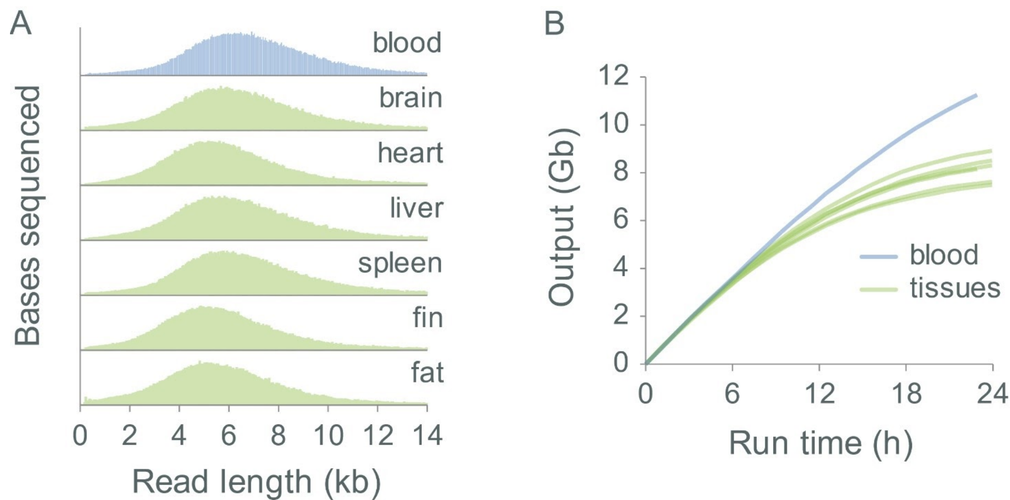

Figure 2. Sequencing performance of gDNA extracted from salmon blood. Extracted gDNA was sheared using the Megaruptor® and a library prepared using the Ligation Sequencing Kit before sequencing on the PromethION for 48 hours. The flow cell was washed using the Flow Cell Wash Kit and library re-loaded after ~16 hours and again at ~30 hours of sequencing to maximise flow cell output. **Panel A:** The read length distribution obtained from the blood sample produced a read N50 of 20-25 kb.**Panel B:** The cumulative sequencing output graph shows that 60+ Gb was obtained in around 48 hours of sequencing.

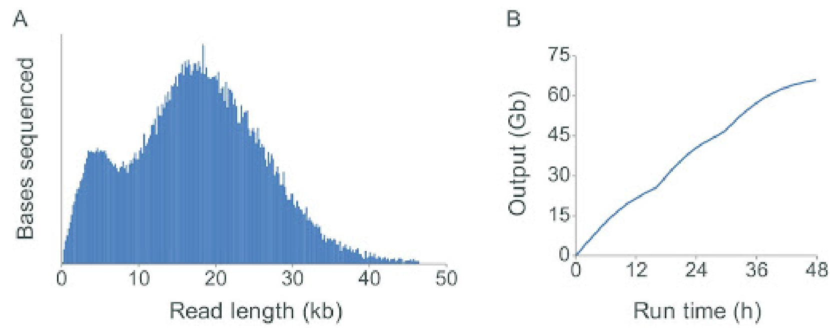

Figure 3. Sequencing performance of gDNA extracted from various salmon tissues, including blood. Extracted gDNA was sheared using the Covaris g-TUBE to normalise fragment lengths between the various tissues and libraries were prepared using the Ligation Sequencing Kit and sequenced on MinION for 24 hours. **Panel A:** The read length distributions obtained from the blood and other tissue samples show that read lengths were successfully normalised by the shearing procedure. **Panel B:** Higher sequencing outputs were obtained using gDNA extracted from blood rather than gDNA extracted from tissue samples.

Change log

Version	Change
v1, 28th July 2021	Initial protocol publication