Introduction

This protocol describes a method to extract high molecular weight genomic DNA from western clawed frog (*Xenopus tropicalis*) muscle, as an example of amphibian tissue. The extraction was performed using the QIAGEN Blood and Cell Culture DNA Midi Kit and part of the genomic DNA was size-selected using our Size selection of HMW DNA by semi-selective DNA precipitationprotocol. Sequencing performance was assessed using the PromethION.

Materials

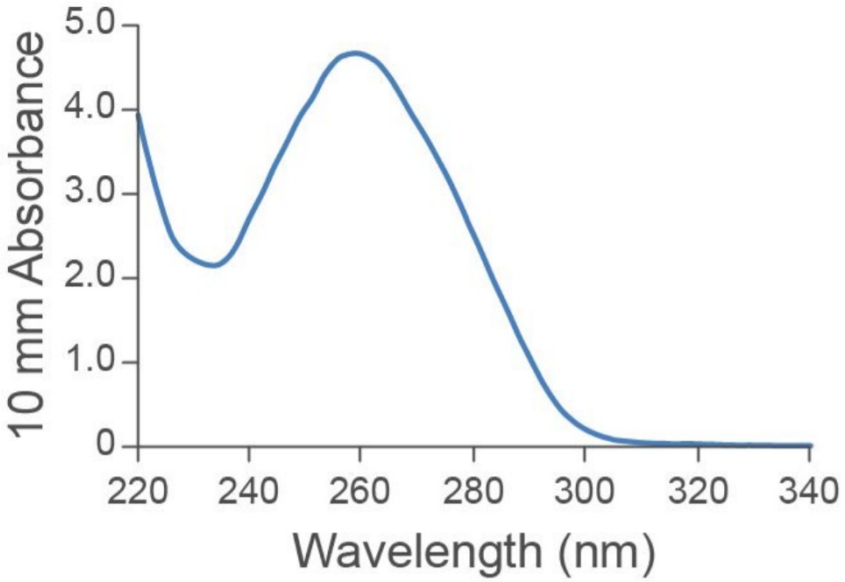
- 100 mg of frog muscle tissue, stored at –80°C until extraction
- Qiagen Blood and Cell Culture DNA Midi Kit
- QIAGEN TissueRuptor II and probes
- QIAGEN RNase A
- Proteinase K
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- Isopropano
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- Vortex mixer
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Refrigerated centrifuge and rotor for 15 ml tubes
- Vortex mixer
- Incubator or water bath with capacity for 55°C and agitation capability

Method

1. Add up to 100 mg of frozen frog muscle tissue to a 15 ml Falcon tube containing 5 ml of buffer G2. Do not allow the tissue to thaw before being placed in the lysis solution.
2. Homogenise the sample using TissueRuptor II with 2 x 15 second pulses on speed 2. If intact tissue pieces are still visible, repeat until the lysate is homogeneous.
3. Add 4.5 ml of Buffer G2 and 19 µl of RNase A and mix by inverting the tube.
4. Incubate the tube at 55°C for 2 hours, with agitation at 150 rpm.
5. Equilibrate a QIAGEN Genomic-tip 100/G column with 4 ml of Buffer QBT.
6. Pour the lysate through the column.
7. Purify the lysate according to the standard protocol (steps 3–6, pages 50–52).
8. To maximize DNA yield, we recommend that the elution is performed overnight at room temperature in 200 µl TE buffer.
9. Take the extracted DNA and perform a size selection using theSize selection of HMW DNA by semi-selective DNA precipitation protocol. The expected DNA recovery after size selection is ~25–50%.

Results

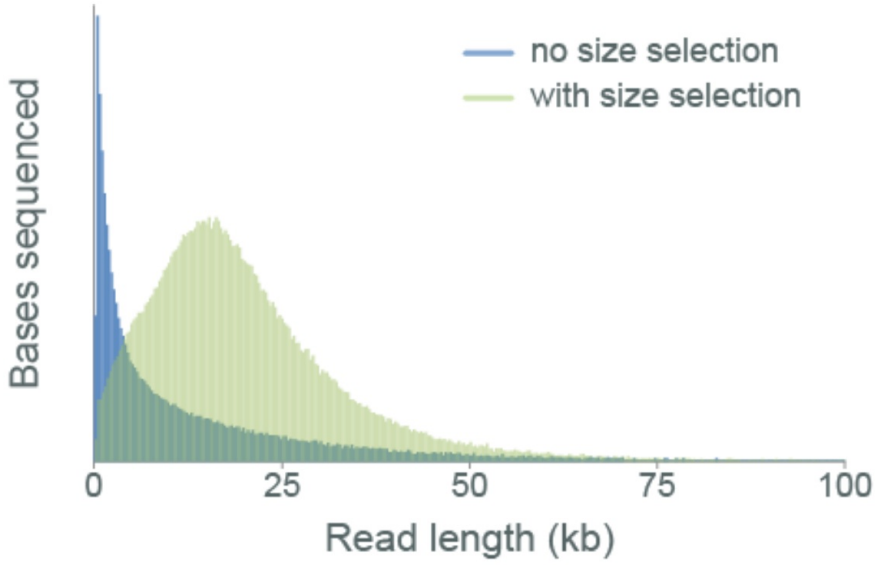
- **Yield:** 50-60 µg
- **OD 260/280** 1.98
- **OD 260/230** 2.24



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
v2, September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol
v1, 19th August 2019	Initial protocol publication