High molecular weight gDNA extraction from Western clawed frog (*Xenopus tropicalis*) muscle

19th August 2019

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 protocol "Size selection of HMW DNA by semi-selective DNA precipitation". 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
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 70% ethanol in nuclease-free water
- Isopropanol
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8)
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Refrigerated centrifuge with capacity for 15 ml Falcon tubes
- Vortex mixer
- Incubator or water bath with capacity for 55°C and agitation capability

Method

Step 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.

Step 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.

Step 3

Add 4.5 ml of Buffer G2 and 19 µl of RNase A and mix by inverting the tube.

Step 4

Incubate the tube at 55 o C for 2 hours, with agitation at 150 rpm.

Step 5

Equilibrate a QIAGEN Genomic-tip 100/G column with 4 ml of Buffer QBT.

Step 6

Pour the lysate through the column.

Step 7

Purify the lysate according to the standard protocol (steps 3–6, pages 50–52).

Step 8

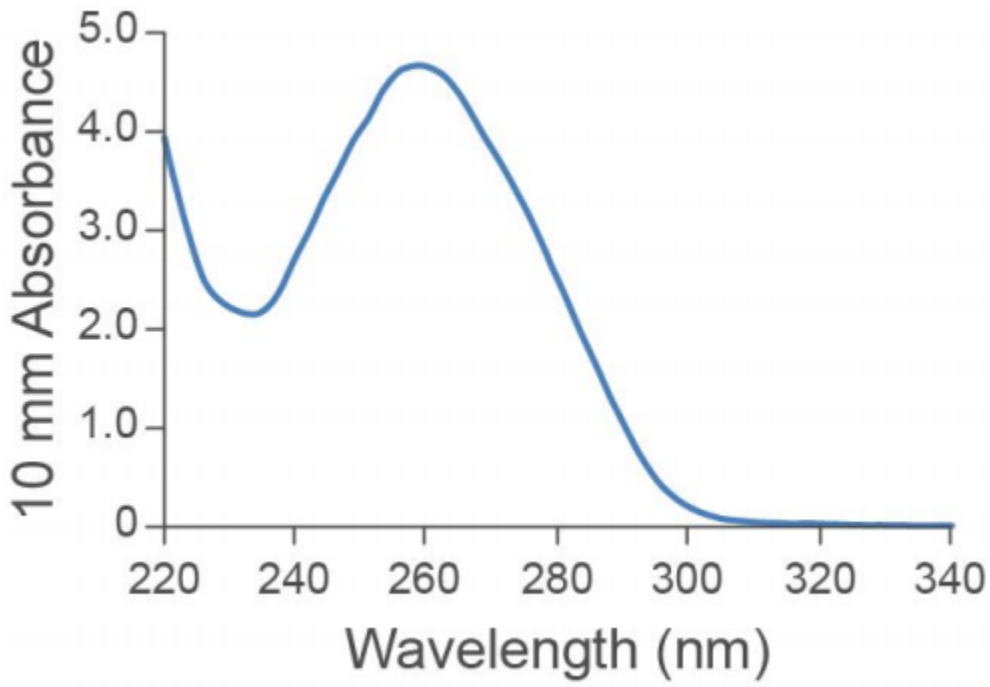
To maximize DNA yield, we recommend that the elution is performed overnight at room temperature in 200 µl TE buffer.

Step 9

Take the extracted DNA and perform a size selection using the "size selection of HMW DNA by semi-selective DNA precipitate" protocol. The expected DNA recovery after size selection is ~25-50%.

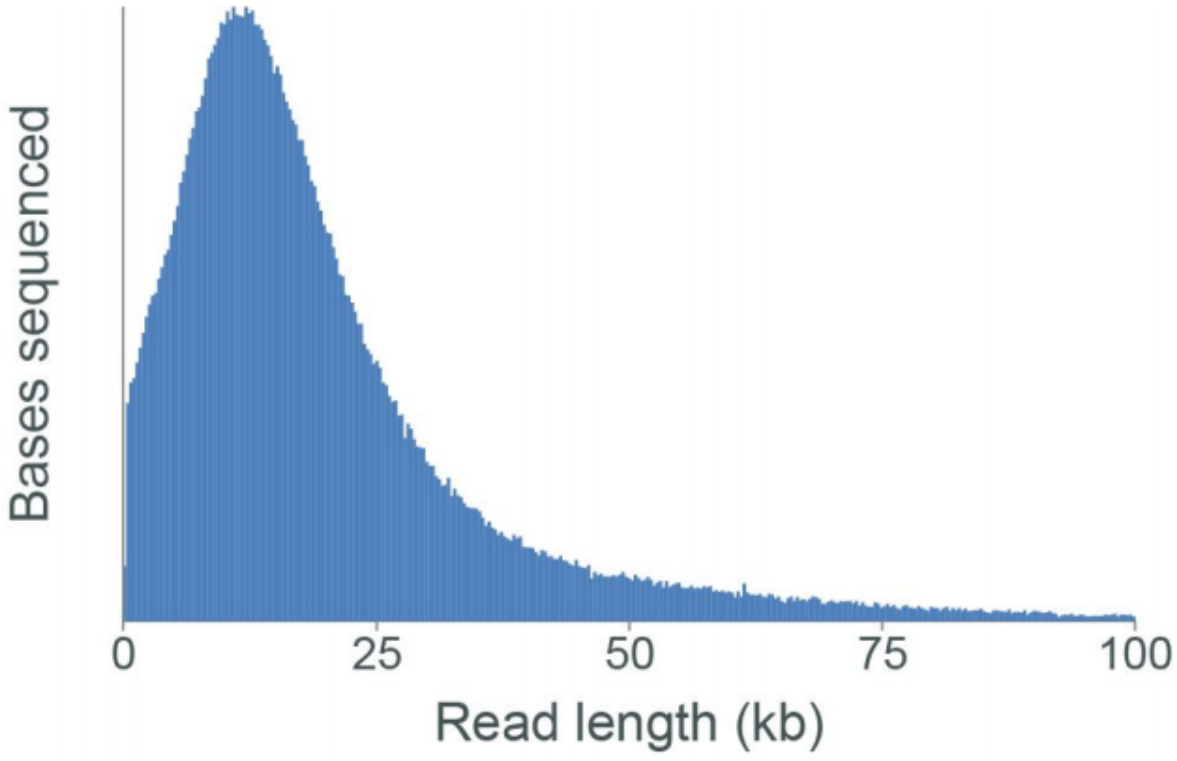
Results

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



Sequencing performance

- Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109):
- Typical throughput: ★★ (30–60 Gb from FLO-PRO002 flow cells). Throughput from the flow cell may be increased by performing a nuclease 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:



Date	Change note
September 2021	Updated protocol to size select DNA using the size selection of HMW DNA by semi-selective DNA precipitation protocol.