Introduction

This protocol describes a method to extract high molecular weight genomic DNA from *C. elegans*, strain SX3254 (descendent from N2 wild type). The worms were grown in NGM (nematode growth media) with *E. coli* as a food source, and were harvested with M9 buffer (0.85 M Na2HPO4, 0.4 M KH2PO4, 0.3 M NaCl, 1 mM MgSO4) as described this protocol (steps 1 and 2). The extraction was carried out using the QIAGEN Puregene Cell Kit and part of the genomic DNA was size-selected with the semi-selective DNA precipitation method. Sequencing performance was assessed using the MinION.

Materials

- Pellet from one plate of *C. elegans* worms, harvested as described here (steps 1 and 2) and stored at –20°C or –80°C
- QIAGEN Puregene Cell Kit
- QIAGEN RNase A
- Proteinase K
- Isopropanol
- 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
- Ice bucket with ice
- Refrigerated centrifuge and rotor for 15 ml tubes
- Incubator or water bath with capacity for 37°C and 50°C
- Sterile paper wipes
- Qubit dsDNA BR Assay Kit (ThermoFisher Scientific)
- 70% ethanol in nuclease-free water
- 2X “size selection buffer” (2.5% w/v PVP 360000 1.2 M NaCl, 20 mM Tris.HCl pH 8)

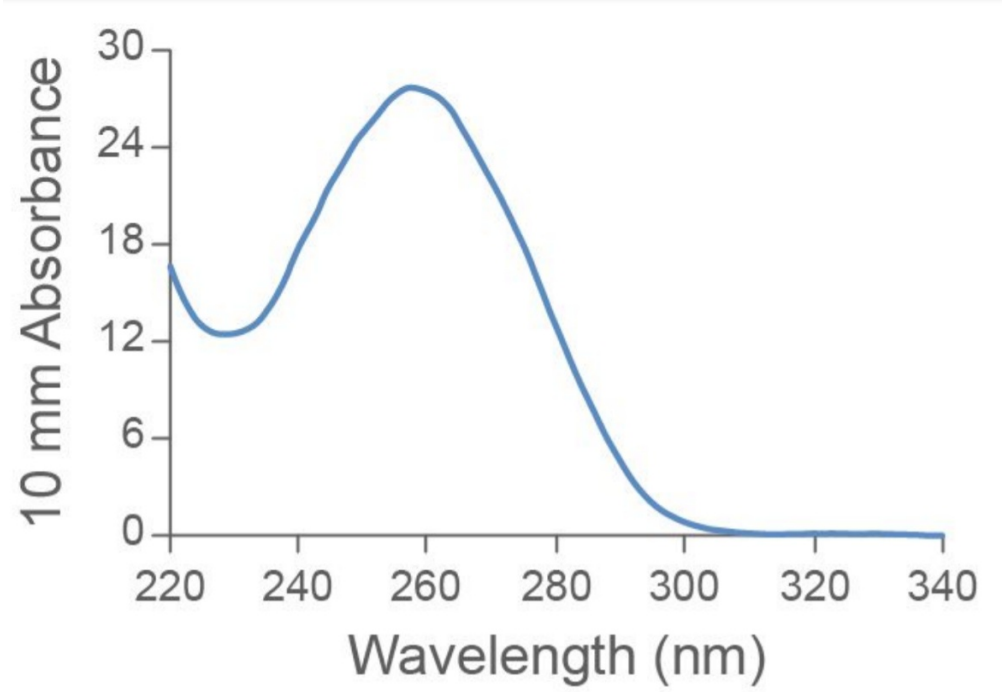
Methods

1. Add 3 ml of Cell Lysis Solution to the 15 ml Falcon tube containing the frozen worm pellet, and allow the pellet to thaw in the lysis buffer.
2. Add 15 µl of Proteinase K and resuspend the pellet by pipetting with a 1 ml wide-bore tip.
3. Incubate the resuspended worms at 50°C for 1 hour; if after 1 hour the lysate is not homogeneous (i.e. if whole worms or pieces of worms are still visible), extend the incubation to 2 hours. During this incubation, gently invert the tube 3 times every 30 minutes.
4. Add 15 µl of RNase A and mix by inverting the tube.
5. Incubate the tube at 37°C for 30 minutes.
6. Place the tube on ice for 2 minutes.
7. Add 1 ml of Protein Precipitation Solution and pulse-vortex three times for 5 seconds.
8. Centrifuge at 2000 x g for 10 minutes.
9. Add 3 ml of isopropanol to a fresh 15 ml Falcon tube.
10. Pour the supernatant from step 8 into the Falcon tube with isopropanol. Discard the pellet.
11. Gently invert the tube 50 times.

12. Centrifuge at 2000 x g for 5 minutes.
13. Discard the supernatant and add 3 ml of 70% ice-cold ethanol to the pellet. Gently invert the tube several times to mix.
14. Centrifuge at 2000 x g for 2 minutes.
15. Discard the supernatant and remove as much ethanol as possible using sterile paper wipes.
16. To maximize DNA yield, we recommend that the elution is performed overnight at room temperature in 600 µl TE buffer.
17. Take 3 µg of 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 ~40–50%.

Results

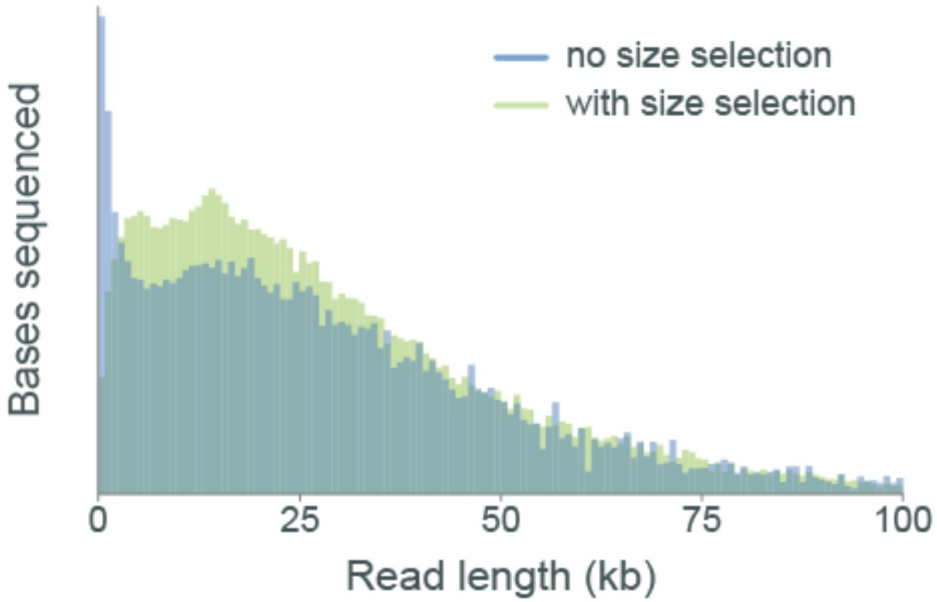
- **Yield:** 80–90 µg
- **OD260/280:** 2.15
- **OD 260/230:** 2.23



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



Change log

Version	Change
v3, December 2022	Updated Puregene name and link
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