# High molecular weight gDNA extraction from worms (*Caenorhabditis elegans*)

August 19, 2019

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 $Na_2HPO_4$, 0.4 M $KH_2PO_4$, 0.3 M NaCl, 1 mM $MgSO_4$) as described in this protocol (steps 1 and 2). The extraction was carried out using the QIAGEN Gentra Puregene Cell Kit and part of the genomic DNA was size selected with the size selection of HMW DNA by semi-selective DNA precipitation protocol. Sequencing performance was assessed using the MinION Mk 1B.

## 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 Gentra Puregene Cell Kit
- QIAGEN RNase A
- Proteinase K
- 2x “size selection” (2.5% w/v PVP 360000, 1.2 M NaCl, 20 mM Tris.HCl pH 8)
- 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

# Method

## Step 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.

## Step 2

Add 15 µl of Proteinase K and resuspend the pellet by pipetting with a 1 ml wide-bore tip.

## Step 3

Incubate the resuspended worms at 50° C for one hour; if after one hour the lysate is not homogeneous (i.e. if whole worms or pieces of worms are still visible), extend the incubation to two hours. During this incubation, gently invert the tube three times every 30 minutes.

## Step 4

Add 15 µl of RNase A and mix by inverting the tube.

## Step 5

Incubate the tube at 37° C for 30 minutes.

## Step 6

Place the tube on ice for two minutes.

## Step 7

Add 1 ml of Protein Precipitation Solution and pulse-vortex three times for five seconds.

## Step 8

Centrifuge at 2000 x g for 10 minutes.

### Step 9

Add 3 ml of isopropanol to a fresh 15 ml Falcon tube.

### Step 10

Pour the supernatant from step 8 into the Falcon tube with isopropanol. Discard the pellet.

### Step 11

Gently invert the tube 50 times.

### Step 12

Centrifuge at 2000 x g for five minutes.

### Step 13

Discard the supernatant and add 3 ml of 70% ice-cold ethanol to the pellet. Gently invert the tube several times to mix.

### Step 14

Centrifuge at 2000 x g for two minutes.

### Step 15

Discard the supernatant and remove as much ethanol as possible using sterile paper wipes.

### Step 16

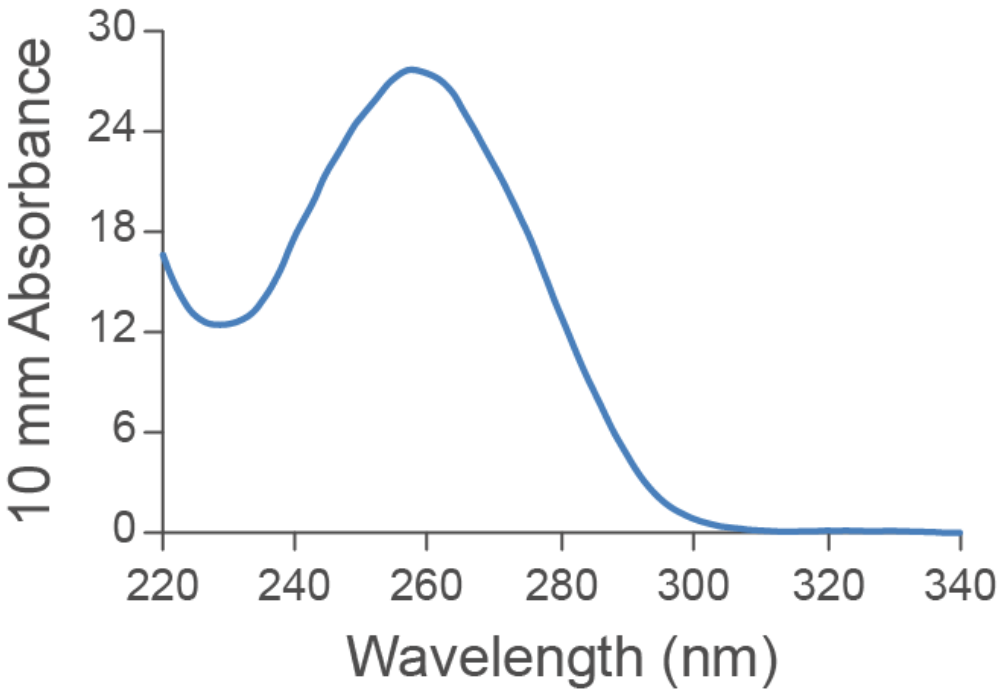
To maximize DNA yield, we recommend that the elution is performed overnight at room temperature in 600 µl TE buffer.

### Step 17

Take 3 µg of extracted DNA and perform a size selection using the size selection of HMW DNA by semi-selective DNA precipitation protocol. The expected DNA recovery after size selection is ~40–50%.

## Results

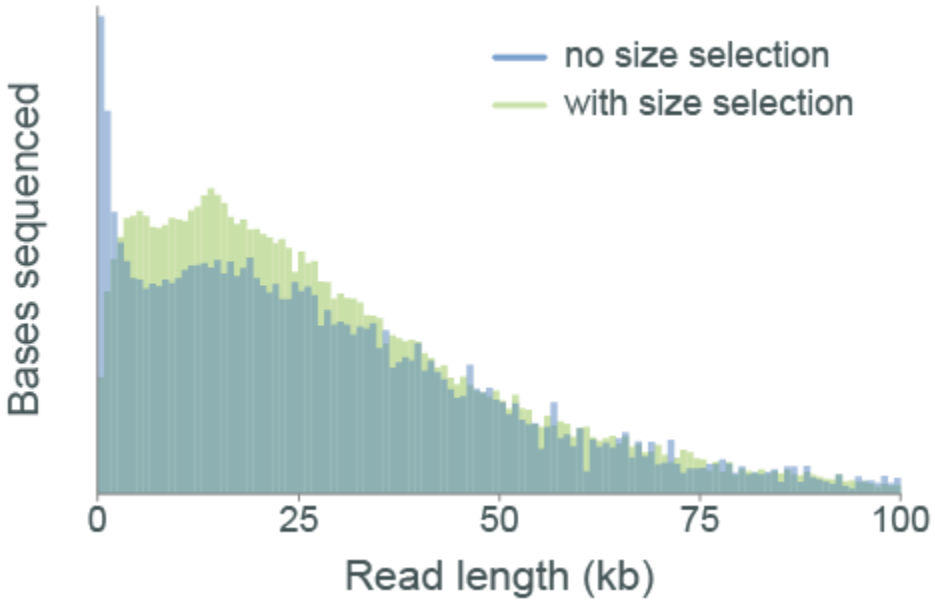
- **Yield:** 80–90 µg
- **OD 260/280:** 2.15
- **OD 260/230:** 2.23



## Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109):

- Typical throughput: ★★★ (8+ Gb in 48 h on FLO-MIN106D) for the Ligation Sequencing Kit, equivalent to the Lambda DNA supplied with the Control Expansion pack (EXP-CTL001).
- 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. |