Introduction

This protocol describes the preparation of a *C. elegans* sample to be processed with the restriction enzyme Pore-C (RE-Pore-C) protocol. The PD1074 strain of C. elegans were grown on solid agar media and fed with OP50 *Escherichia coli* (*E. coli*) over the course of 4–5 days at 21°C. The worms were predominantly harvested at the L1 larval stage following 1 day of starvation to reduce the presence of E. coli DNA in the worm gut. A worm pellet of approximately 2 ml worm pellet is used to yield approximately 5 ml of worm powder. This is sufficient for 5 preparations of Pore-C extract by following the RE-Pore-C protocol with approximately 1 ml of worm powder.

This protocol has been developed based on research by Oxford Nanopore Technologies and published literature: Jänes, J. et al. (2018) Chromatin accessibility dynamics across C. elegans development and ageing, *eLife*, **7**, pp. 1–24. doi: 10.7554/eLife.37344.

Materials

- Worms
- M9 salt solution supplemented with 1 mM MgSO4
- Crushed ice
- Liquid nitrogen
- 15 or 50 ml centrifuge tubes
- Mortar and pestle
- Temperature-controlled 50 ml centrifuge
- –80°C freezer storage

Collection of worms: 20 minutes hands-on-time

1. Gather sufficient worms by washing off the plate, to yield a pellet of approximately 2 ml following centrifugation in step 3.
   Note: fewer worms may be used but reduced volumes will be required for subsequent washes and resuspension.
2. Aspirate any supernatant above the worm pellet and then resuspend the ~2 ml worm pellet in 15 ml of M9 salt solution supplemented with 1 mM MgSO4.
3. Centrifuge at 800 g at 4°C for 5 minutes.
4. Aspirate the supernatant and store the pellet on ice.

Preparation of frozen worm balls: 10 minutes hands-on-time

Note: Pre-cool the mortar and pestle at –80°C for at least 30 minutes.

1. Resuspend the ~2 ml worm pellet in a minimal volume (~500 µl) of M9 salt solution supplemented with 1 mM MgSO4 final concentration, to achieve a worm slurry that can be pipetted.
   Note: It is important not to over-dilute the worm slurry as this will decrease the ratio of worm:solvent and result in an underestimation of sample input for the subsequent RE-Pore-C extraction.
2. Place the chilled mortar on ice and dispense a small volume of liquid nitrogen into the mortar.
3. Using a 1000 µl pipette tip, dispense the homogenous worm slurry dropwise into the liquid nitrogen to create worm balls of ~100 µl.
4. Use a spatula to collect the frozen worm balls and store in a large chilled centrifuge tube on ice.
5. Repeat steps 7–8 until all the worm slurry has been dispensed. Add more liquid nitrogen to the chilled mortar as required.
6. Snap freeze the tube of frozen worm balls in liquid nitrogen and store at –80°C.

Cryogrinding of frozen worm balls: 10 minutes hands-on-time

Note: pre-cool a mortar and pestle at –80°C for at least 30 minutes.

1. Place the chilled mortar and pestle on ice and add approximately 1 ml of frozen worm balls.
2. Carefully grind the balls into a fine powder, working quickly to minimise thawing.
3. Collect the worm powder into a chilled centrifuge tube on ice using a spatula.

RE-Pore-C extraction

1. Transfer approximately 1 ml cryo-ground worm powder to a 50 ml centrifuge tube and resuspend in 1 ml chilled 1X PBS.
2. Bring the volume of the re-suspended cryo-ground tissue to 10 ml in chilled 1X PBS.
3. Proceed with the RE-Pore-C protocol using the re-suspended cryo-ground tissue powder as input.

Results

Sample	DNA concentration, ng/µl	Total DNA mass, µg
C. elegans	8.62	1.29

Table 1. The yield of non-size selected RE-Pore-C DNA extract using NlaIII restriction enzyme.

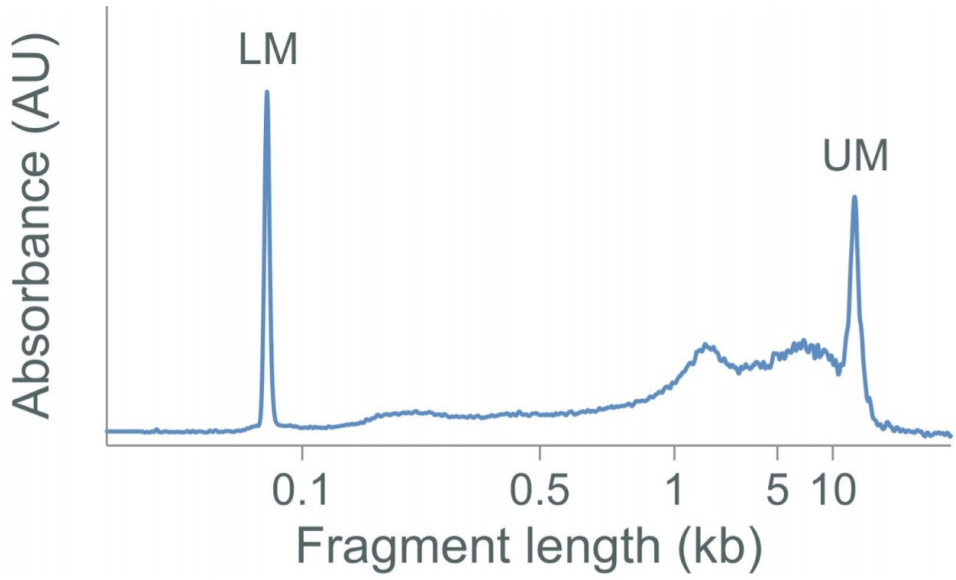
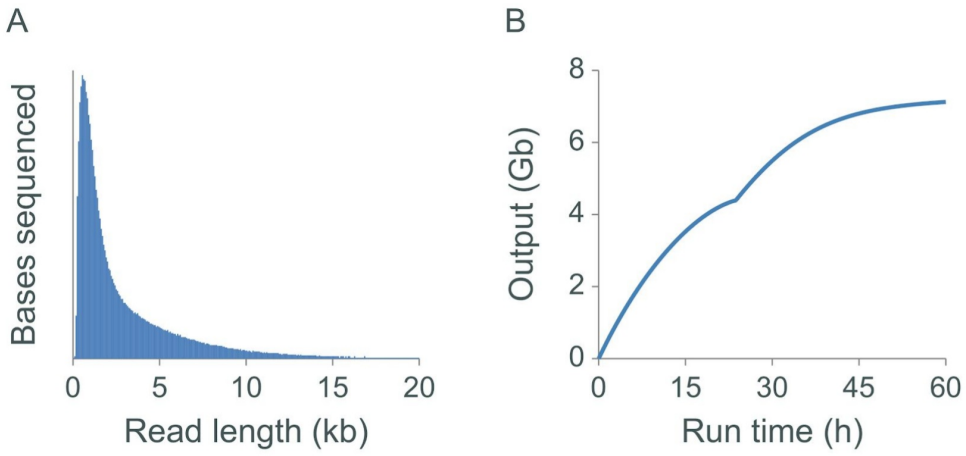


Figure 1. Agilent Bioanalyser DNA 12000 trace of non-size selected RE-Pore-C DNA extract.



C

Performance metric	Value
read N50 (kb)	~1-2
typical output (FLOMIN-106D)‡ (Gb)	~7-10
estimated mean monomer length (bp)	~700
estimated monomers/N50 read	~2
contacts/Gb	~1 million
cis contacts (%)	~45

‡ Nuclease flushes were performed to optimise flow cell output

Figure 2. The sequencing and Pore-C output for libraries assessed on GridION Mk1. Libraries were generated as described using Pore-C extracts prepared with NlaIII restriction enzyme. The read length distributions and output (Gbases) obtained from the library generated are shown in panels A and B, respectively. Panel C displays the Pore-C metrics obtained.

Change log

Version	Change
v2, 24th July 2020	Updated formatting and added re-suspension of cryo-ground tissue powder.