Introduction

This protocol describes the preparation of *Rattus norvegicus* (*R. norvegicus*) tissue samples to be processed using the restriction enzyme Pore-C (RE-Pore-C) protocol as an example of animal tissue. This protocol was developed using brain and muscle tissues isolated from the Hsd:Sprague Dawley (SD) strain of *Rattus norvegicus domestica* subspecies.

Materials

- 100 mg tissue (e.g. brain or muscle)
- 1X PBS pH 7.4
- Crushed ice
- Liquid nitrogen
- Scalpel
- 15 or 50 ml centrifuge tubes
- Mortar and pestle
- –80°C freezer storage

Cryo-grinding of animal tissues: 20 minutes hands-on-time

Note: Pre-chill a mortar and pestle at –80°C for at least 30 minutes. Both fresh or frozen samples may be used.

1. Gather 100 mg of animal tissue.
   Note: If the material is >1 cm2, dissect the sample into smaller pieces before proceeding to the next step.
2. Place a chilled mortar and pestle on ice. Add a small volume of liquid nitrogen into the mortar and add the animal tissue to freeze until the liquid nitrogen has evaporated.
3. Carefully grind the frozen animal tissue into a fine powder, working quickly to minimise thawing. If the animal tissue starts to thaw, add a small volume of liquid nitrogen to the mortar.
4. Use a spatula to collect the tissue powder into a chilled centrifuge tube on ice

RE-Pore-C extraction

1. Transfer approximately 100 mg of cryo-ground tissue 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
Muscle	79.4	1.19
Brain	14.9	2.24

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

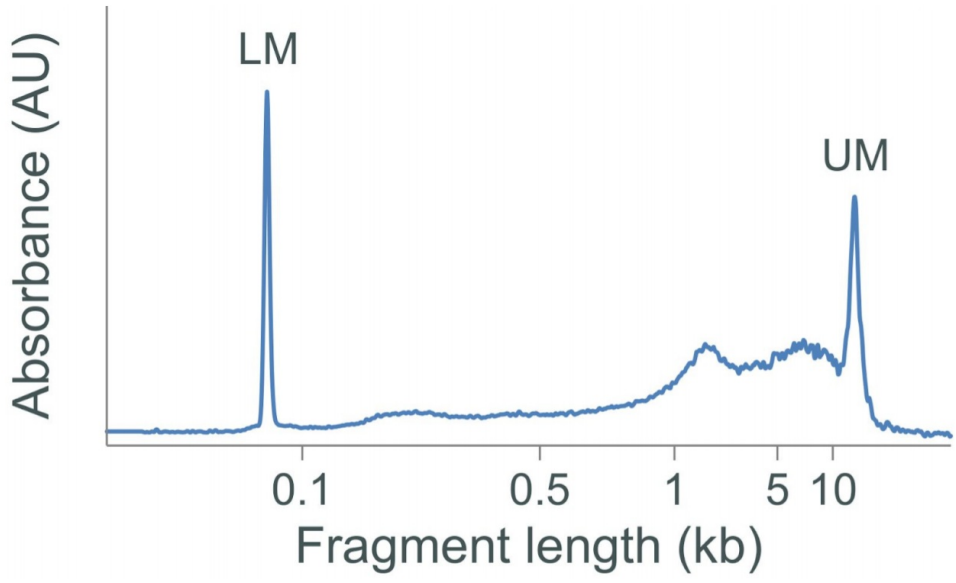
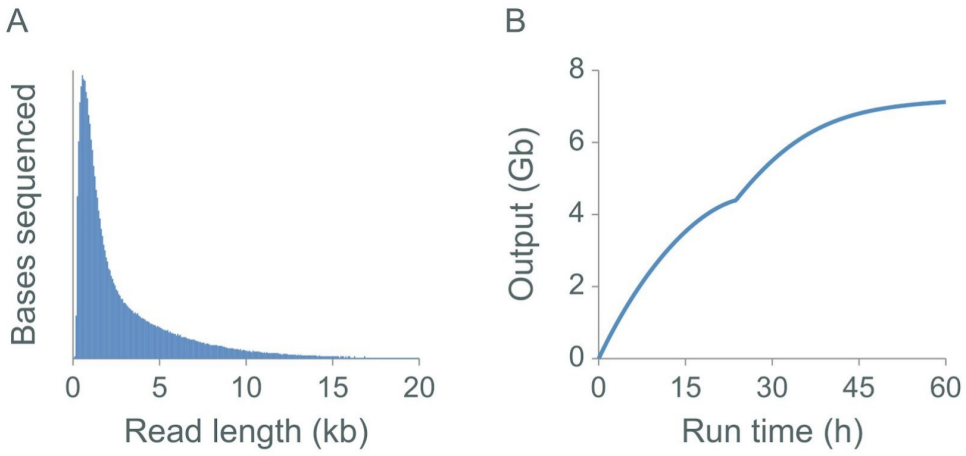


Figure 1. Agilent Bioanalyser DNA 12000 trace of non-size selected RE-Pore-C DNA extract.



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

Change log

Version	Change
v2, 28th July 2020	Title update

Version	Change
v1, 20th July 2020	Intial protocol publication