



Sample preparation of animal tissue for RE-Pore-C extraction

20th July 2020

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.

● Step 1

Gather 100 mg of animal tissue.

Note: If the material is $>1\text{ cm}^2$, dissect the sample into smaller pieces before proceeding to the next step.

● 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.

● Step 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.

● Step 4

Use a spatula to collect the tissue powder into a chilled centrifuge tube on ice.

RE-Pore-C extraction

● Step 5

Transfer approximately 100 mg of cryo-ground tissue to a 50 ml centrifuge tube and resuspend in 1 ml chilled 1X PBS.

● Step 6

Bring the volume of the re-suspended cryo-ground tissue to 10 ml in chilled 1X PBS.

● Step 7

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.

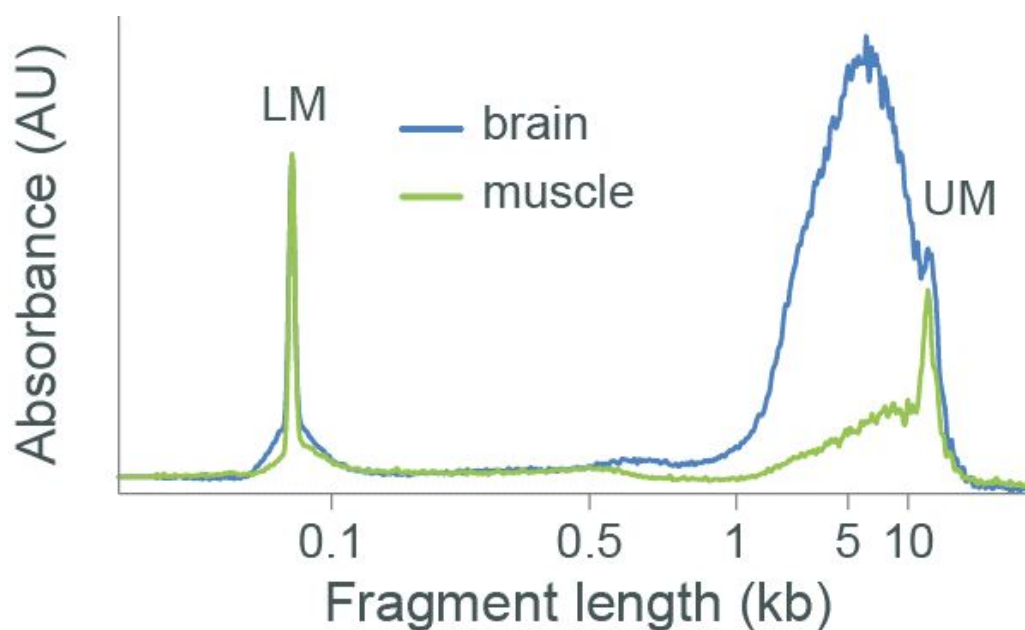


Figure 1. Agilent Bioanalyser DNA 12000 trace of non-size selected RE-Pore-C DNA extract.

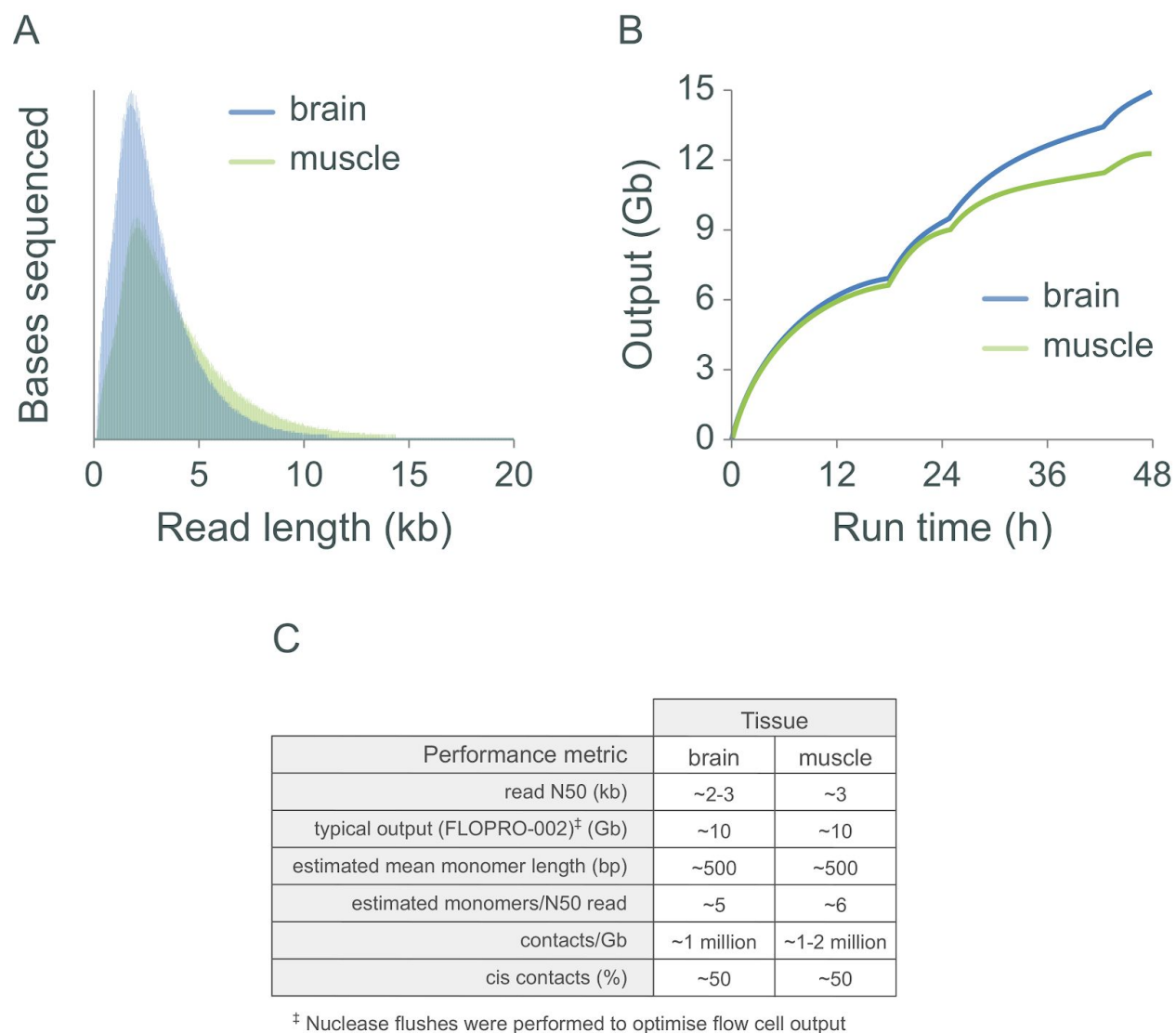


Figure 2. The sequencing and Pore-C output for libraries assessed on PromethION. Libraries were generated as described using Pore-C extracts prepared with the NlaIII restriction enzyme. The read length distributions and output (Gbases) obtained from the libraries generated are shown in panels A and B,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

Date	Changelog
28th July 2020	Title update