Sample preparation of insect material for RE-Pore-C extraction

20th July 2020

This protocol describes the preparation of a sample of *Drosophila melanogaster* (*D. melanogaster*) to be processed using the restriction enzyme Pore-C (RE-Pore-C) protocol as an example of insect material. This protocol was developed using *D. melanogaster* white eye mutant variant W1118 at the adult stage.

Materials

- 100 mg of insect material
- 1X PBS pH 7.4
- Crushed ice
- Liquid nitrogen
- 15 or 50 ml centrifuge tubes
- Mortar and pestle
- –80°C freezer storage

Cryogrinding of insect material: 10 minutes hands-on-time

Note: Pre-cool the mortar and pestle at –80°C for at least 30 minutes. Fresh and frozen samples may be used.

Step 1

Gather 100 mg of insect material.

Note: If the sample of insect material is >1 cm^2, dissect the sample into smaller pieces before proceeding to the next step.

Step 2

Place the chilled mortar and pestle on ice. Pour a small volume of liquid nitrogen into the mortar and add the sample of insect material to freeze until the liquid nitrogen has evaporated.

Step 3

Carefully grind the frozen insect material into a fine powder, working quickly to minimise thawing. If the material starts to thaw, add another small volume of liquid nitrogen to the mortar.

Step 4

Use a spatula to collect the insect 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
D. melanogaster	31.8	4.77

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

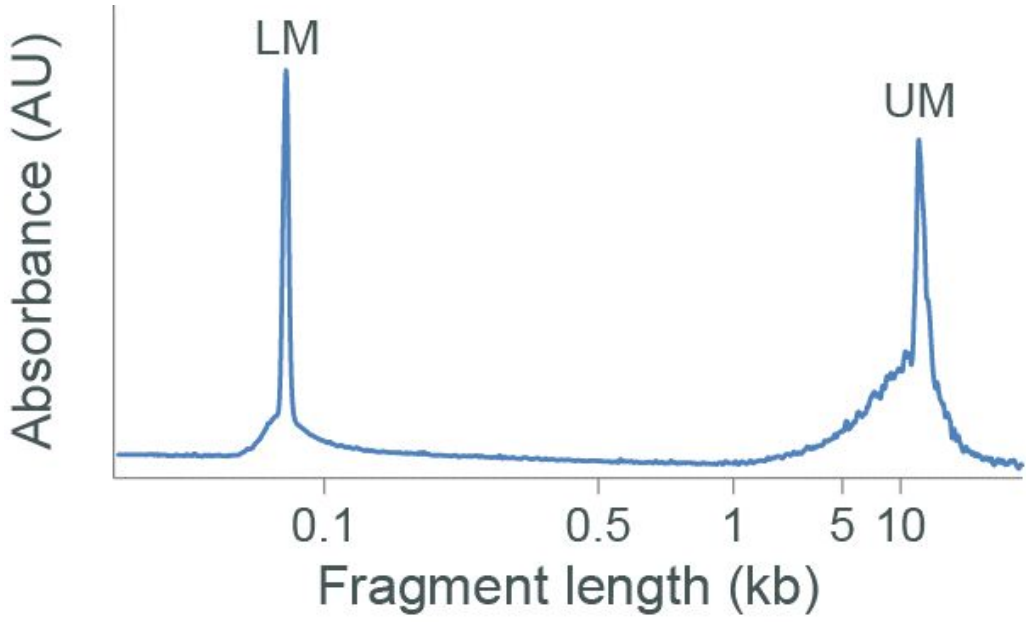
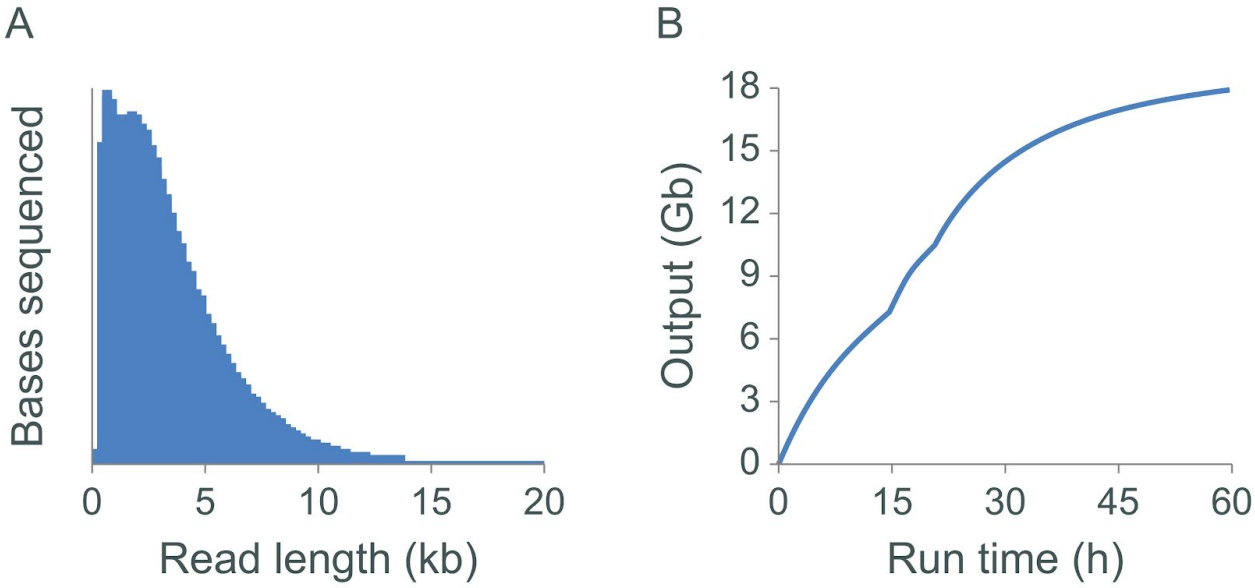


Figure 1. Agilent Bioanalyser DNA 12000 trace of non-size selected RE-Pore-C DNA extract.



C

Performance metric	Value
read N50 (kb)	~2-3
typical output (FLOPRO-002)‡ (Gb)	~15-20
estimated mean monomer length (bp)	~600
estimated monomers/N50 read	~4
contacts/Gb	~2 million
cis contacts (%)	~60

‡ 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 distribution and output (Gbases) obtained from the libraries generated are shown in panels A and B, respectively. Panel C displays the Pore-C metrics obtained.