Introduction

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.

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

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

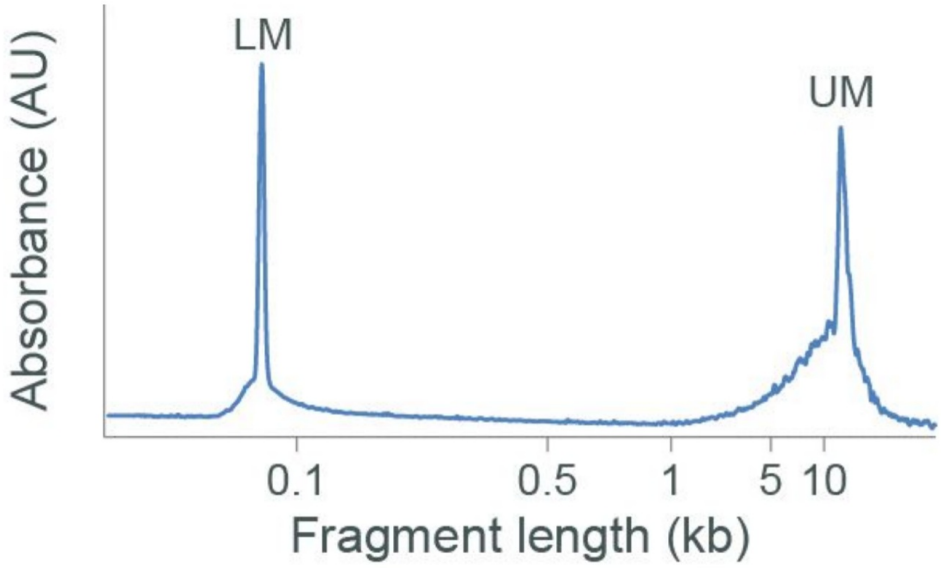
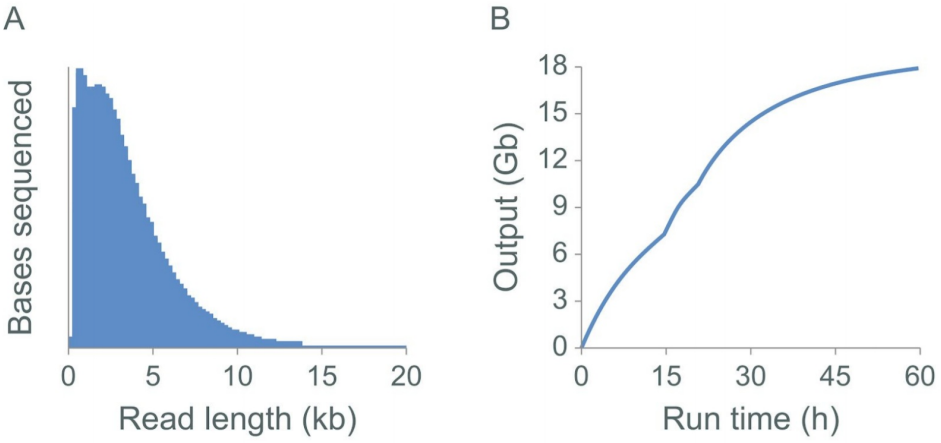


Figure 1. Agilent Bioanalyser DNA 12000 trace of non-size selected RE-Pore-C DNA extract.



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.