

Introduction

This protocol describes a method to extract high molecular weight genomic DNA from fruit flies (*Drosophila melanogaster*), as an example of insects, using nuclear isolation followed by DNA extraction using the QIAGEN Blood and Cell Culture DNA Midi Kit. Sequencing performance was assessed using the MinION.

Materials

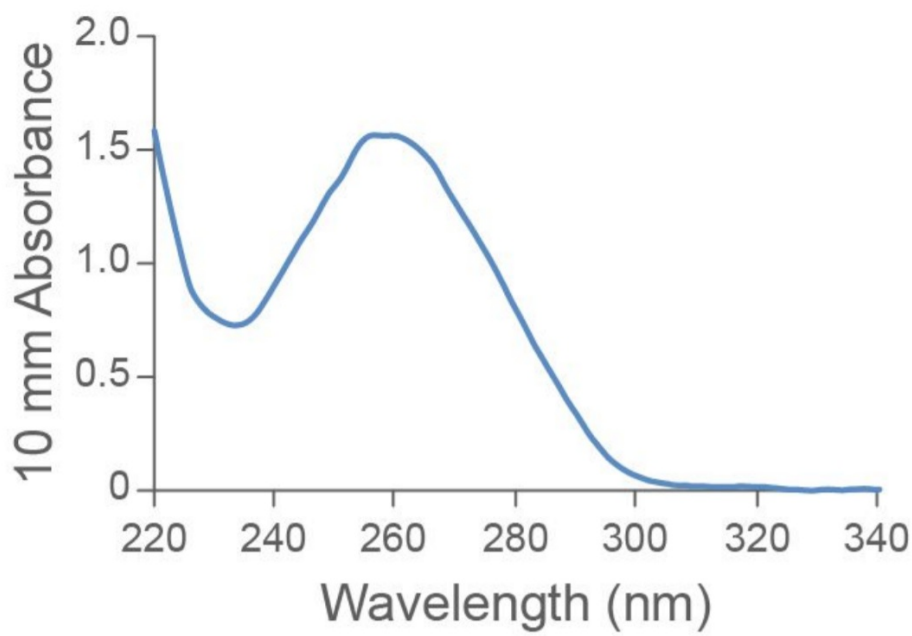
- ~100 flies, frozen at -80°C
- [QIAGEN Blood and Cell Culture DNA Midi Kit](#)
- [Sucrose](#)
- [EDTA](#)
- [Tris-HCl, pH 8.0](#)
- [Proteinase K](#)
- Isopropanol
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- [200 µm nylon mesh](#)
- [QIAGEN TissueRuptor II and probes](#)
- Vortex mixer
- 15 ml Falcon tubes
- Refrigerated centrifuge and rotor for 15 ml tubes
- Incubator or water bath with capacity for 50°C and agitation capability

Method

1. Prepare the nuclear isolation buffer (0.35 M sucrose, 0.1 M EDTA, 50 mM Tris-HCl).
2. Add 10 ml of the nuclear isolation buffer to a 15 ml Falcon tube and add approximately 100 frozen flies.
Note: we advise working quickly to avoid the flies thawing before being added to the buffer.
3. Homogenise the sample using TissueRuptor II with 2 x 15 second pulses on speed 2. No intact flies should be visible after homogenisation.
4. Place 2 layers of 200 µm nylon mesh into a fresh 15 ml Falcon tube. Using a 1 ml wide-bore tip, transfer the homogenised flies through the mesh into the Falcon tube.
5. Wash the nylon mesh with 2 ml of the nuclear isolation buffer. Repeat this wash step one more time. To avoid losing material, press the nylon mesh with a pipette tip to recover as much solution as possible. Discard the used mesh.
6. Centrifuge the filtered solution at 3500 x g for 15 minutes at 4°C. Discard as much supernatant as possible and retain the pellet.
7. Add 5 ml of Buffer G2 and 95 µl of Proteinase K to the pellet, and resuspend by pipetting up and down with a 200 µl wide-bore pipette tip.
8. Incubate at 50°C for 45 minutes with gentle mixing at 100 rpm. The lysate should be homogenous; if not, invert the tube 5 times and incubate for a further 15 minutes.
9. Equilibrate a QIAGEN Genomic-tip 100/G column with 4 ml of Buffer QBT.
10. Pour the lysate through the column.
11. Purify the lysate according to the [standard protocol](#) (steps 3–6, pages 50–52).
12. To maximize DNA yield, we recommend that the elution is performed overnight at room temperature in 150 µl TE buffer.

Results

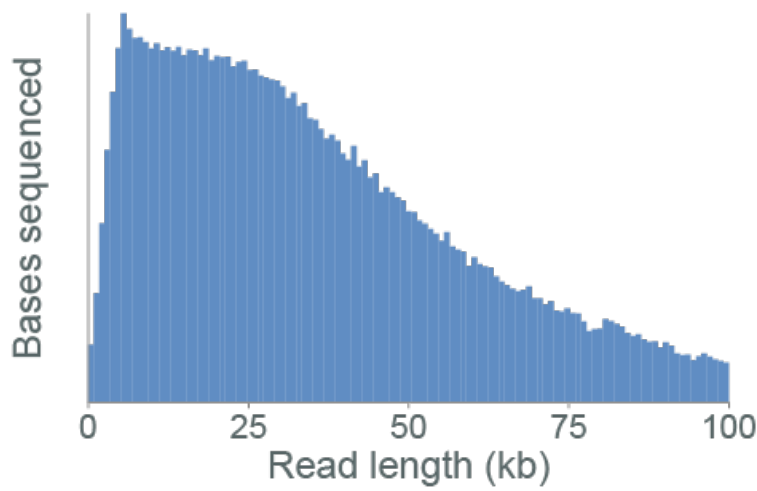
- **Yield:** 6–8 µg
- **OD260/280:** 2.01
- **OD 260/230:** 2.61



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



Change log

Version	Change
v1, 19th August 2019	Initial protocol publication