# Automated high molecular weight gDNA extraction from blood

5th March, 2020

This protocol describes a method to extract high molecular weight genomic DNA from rabbit blood, as a proxy for human blood, using an automation device. The blood was collected in Greiner tubes and stored at -20°C until extraction. The extraction was performed with PerkinElmer® chemagic™ 360 with integrated chemagic™ dispenser automated device using the Standard Protocol (VD141118.che), which takes approximately 2 hours, and the Long Protocol (VD190723.che), which takes approximately 3.5 hours. The QIAGEN Gentra Puregene Blood Kit was used to manually extract DNA from blood as a control, using the linked extraction protocol. The Ligation Sequencing Kit (SQK-LSK109) was used to generate the sequencing libraries of the extracted DNA and the sequencing performance was determined by PromethION. In order to maximize the sequencing data obtained from the sample, users may find that their experiments would benefit from a flow cell wash, to allow sequential runs of the library on the same flow cell, using the Flow Cell Wash Kit.

## Materials

- 4 ml of blood collected in Greiner tubes and stored at -20°C or -80°C
- PerkinElmer® chemagic™ 360 with integrated chemagic™ dispenser automated device
- PerkinElmer® chemagic™ Prime DNA Blood 4k Kit H24 (catalog no. CMG-1074)

## Method

### Step 1

Set up the automation device according to manufacturer's recommendations.

### Step 2

Remove the blood sample from the freezer and thaw at room temperature.

### Step 3

Invert the tube 10 times and transfer the blood to the sample plate.

### Step 4

a) For the Standard Protocol (VD141118.che), add the blood sample to the plate well with no protease.

b) For the Long Protocol (VD190723.che), pipette 15 µl of protease into each well of the plate before adding the blood sample.

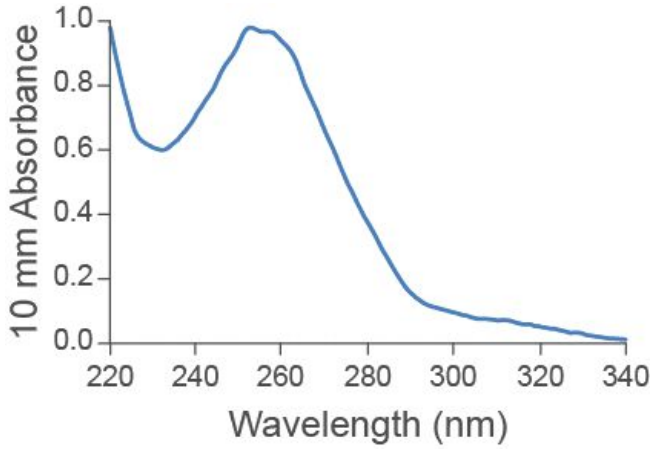
### Step 5

Select the protocol and follow the instructions provided by the software.

## Results

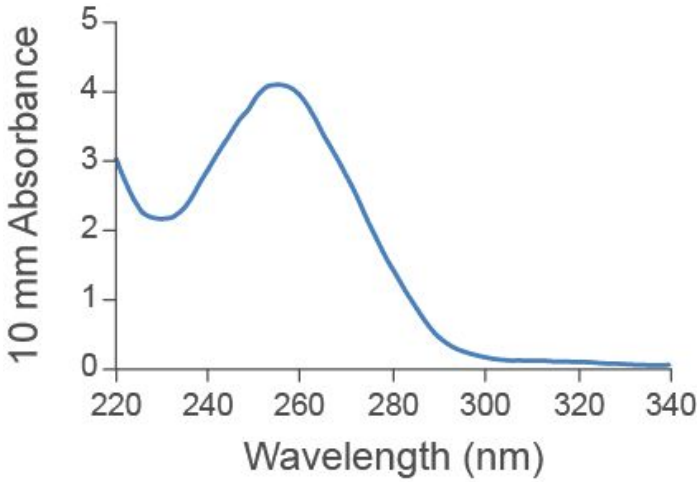
**Standard Protocol:**

- **Yield:** 10–50 µg
- **OD 260/280:** 2.01
- **OD 260/230:** 1.60




**Long Protocol:**

- **Yield:** 10–50 µg
- **OD 260/280:** 2.05
- **OD 260/230:** 1.60



## Sequencing performance

DNA was extracted from the blood samples using the standard protocol, the Long Protocol and the QIAGEN Gentra Puregene Blood Kit as a control. The Ligation Sequencing Kit (SQK-LSK109) was used to prepare DNA libraries from the extracted DNA samples before sequencing on a PromethION. After approximately 18 hours, the runs were paused and the flow cells washed with Flow Cell Wash Kit (EXP-WSH003) before re-loading additional library and re-starting the run. The wash step was repeated approximately 24 hours later.

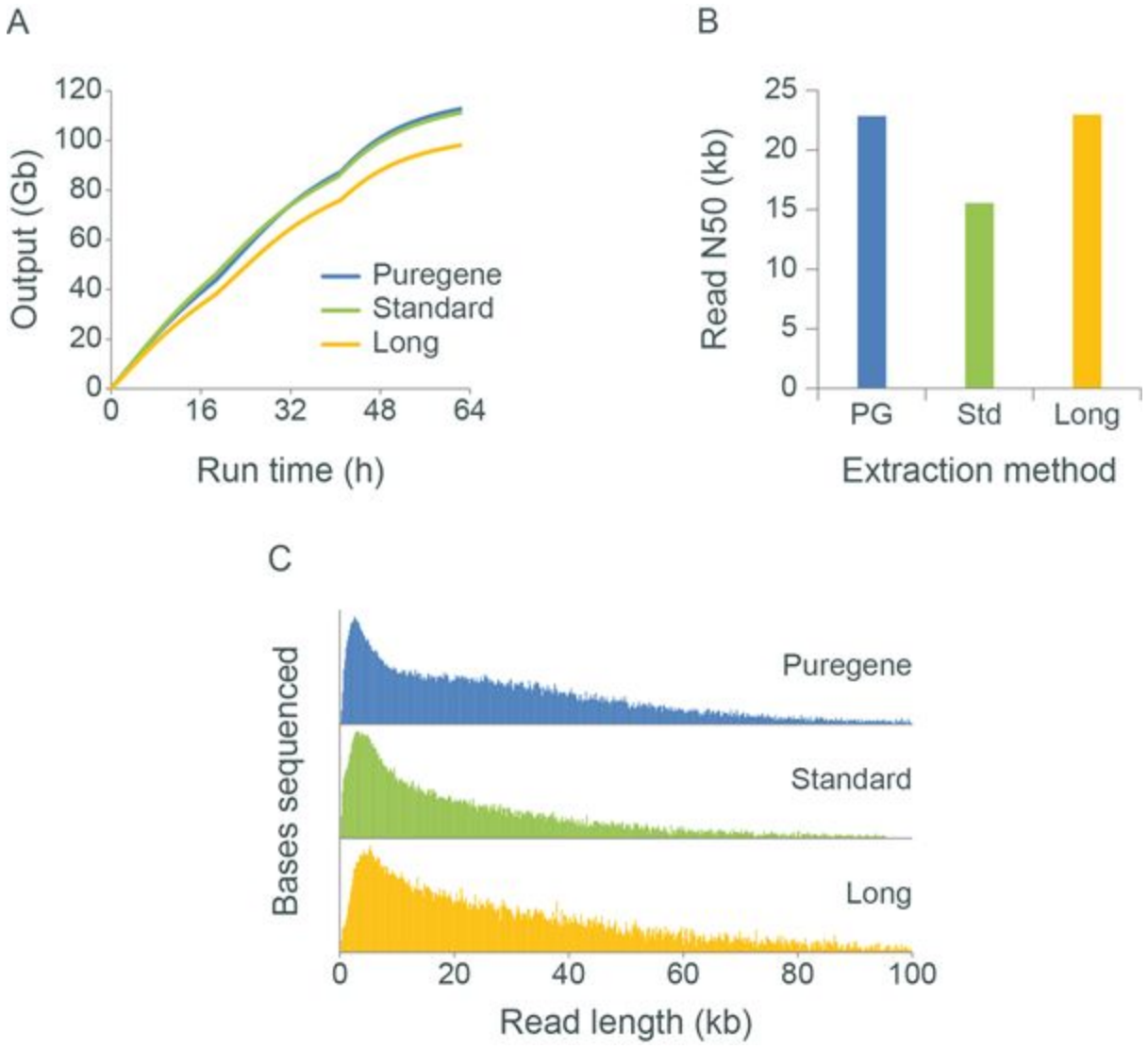



**Figure 1.** The sequencing performance of libraries generated using gDNA extracted from blood with the QIAGEN Gentra Puregene Blood Kit (manual extraction process) as control, and the

PerkinElmer® chemagic™ 360: **A)** flow cell output, **B)** read N50 values and **C)** read length distributions are shown. The Standard Protocol (PerkinElmer® chemagic™ 360) yielded similar flow cell output to the control extraction method, but with a reduced read N50. The read N50 was improved, relative to the standard method, by using the Long Protocol (PerkinElmer® chemagic™ 360), but this yielded a lower flow cell output.