Materials

- 3 ml of blood, collected in K2-EDTA
- QIAGEN Puregene Blood Kit
- 70% ethanol in nuclease-free water
- Isopropanol
- TE buffer (1 mM EDTA, pH 8.0)
- 1.5 ml Eppendorf DNA LoBind tubes
- 15 ml Falcon tubes
- Centrifuge and rotor for 15 ml Falcon tubes
- Incubator or water bath (set at 37°C and 50°C)
- Vortex mixer

Method

1. Perform cell separation and lysis according to the QIAGEN Puregene Handbook for 3 ml of blood (pages 19–20, steps 1–7):
   a. Dispense 9 ml of RBC Lysis Solution into a 15 ml Falcon tube.
   b. Add 3 ml of whole blood and mix by inverting 10 times.
   c. Incubate for 5 minutes at room temperature. Invert at least once during the incubation.
   d. Centrifuge for 2 minutes at 2000 x g to pellet the white blood cells.
   e. Carefully discard the supernatant by pipetting our pouring, leaving approximately 200 µl of the residual liquid and the white blood cell pellet.
   f. Vortex the tube vigorously to resuspend the pellet in the residual liquid. Vortexing greatly facilitates cell lysis in the next step.
   g. Add 3 ml of Cell Lysis Solution and pipette mix to lyse the cells or vortex for 10 seconds.

2. **Critical step:** Incubate the samples at 37°C for 30 minutes. If the sample is not homogenous, gently invert the tubes and extend the incubation for another 30 minutes.

3. Purify the lysate according to the QIAGEN Puregene Handbook for 3 ml of blood (pages 20–21, steps 8–17):

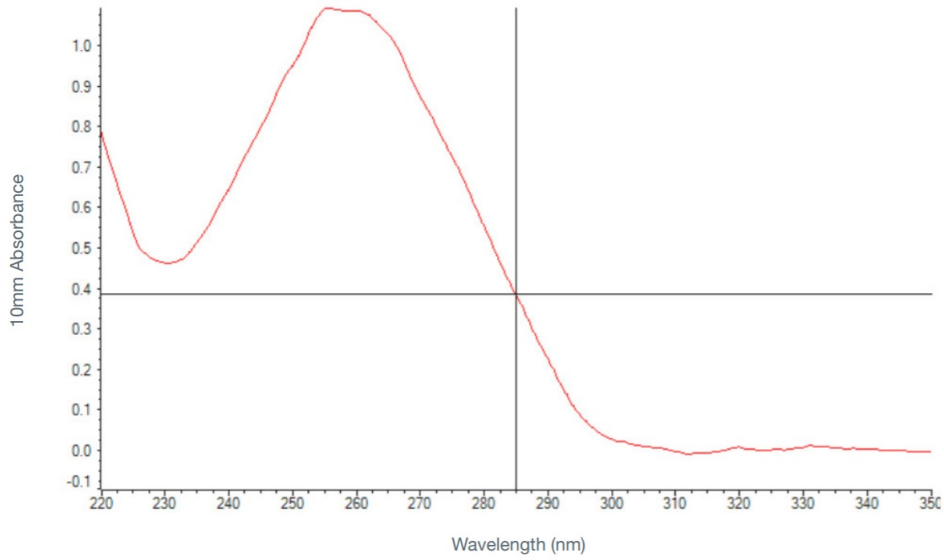
a. Add 15 µl of RNase A Solution and mix by inverting 25 times. Incubate for 15 minutes at 37°C. Then incubate for 3 minutes on ice to quickly cool the sample.
b. Add 1 ml of Protein Precipitation Solution and vorted vigorously for 20 seconds at high speed.
c. Centrifugre for 5 minutes at 2000 x g. The precipitated proteins should form a tight brown pellet. If the protein pellet is not tight, incubate on ice for 5 minutes and repeat the centrifugation.
d. Pipette 3 ml of isopropanol into a clean 15 ml Falcon tube and add the supernatant from the previous step by pouring carefully. Be sure that the protein pellet is not dislodged during pouring.
e. Mix by inverting 50 times until the DNA is visible as threads or a clump.
f. Centrifuge for 3 minutes at 2000 x g. The DNA may be visible as a small white pellet.
g. Carefully discard the supernatant and drain the tube by inverting on a clean piece of absorbant paper, taking care that the pellet remains in the tube.
h. Add 3 ml of 70% ethanol and invert several times to wash the DNA pellet.
i. Centrifuge for 1 minute at 2000 x g.
j. Carefully discard the supernatant. Drain the tube on a clean piece of absorbant paper, taking care that the pellet remains in the tube. Air dry the pellet for 5-10 minutes. The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet as the DNA will be difficult to dissolve.

1. **Critical step:** To maximize the DNA yield, we recommend that the elution is performed for 2 hours at 50°C, using 150 µl TE (1 mM EDTA, pH 8.0), occasionally mixing the tube contents by gentle inversion.

2. Take 3 µg of eluate and perform a SPRI size selection.

Results

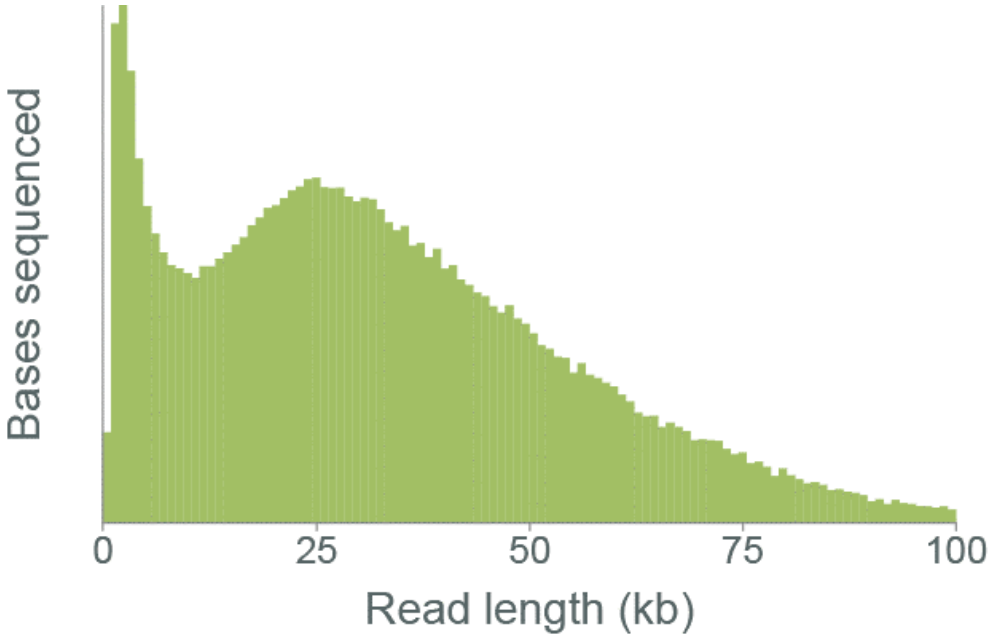
- **Yield:** 20-40 µg
- **OD 260/280:** 1.96
- **OD 260/230:** 2.36



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit.

- Read length profile:



Change log

Version	Change
v2, November 2022	Updated Puregene extraction kit name and link
v1, 27th July 2019	Initial protocol publication