Materials

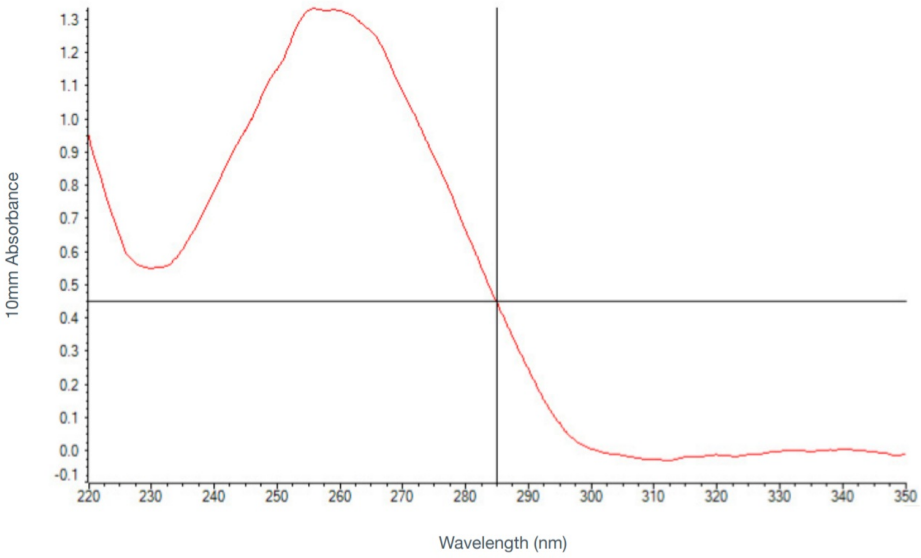
- 5 x 10^6 cells
- QIAGEN Puregene Cell Kit
- 70% ethanol in nuclease-free water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 1x phosphate buffered saline (PBS)
- Isopropanol
- 15 ml Falcon tubes
- 1.5 ml Eppendorf DNA LoBind tubes
- Wide-bore pipette tips
- Centrifuge and rotor for 15 ml Falcon tubes
- Incubator or water bath set at 37°C and 50°C
- Vortex mixer
- Inoculation loop or disposable tweezers for spooling DNA

Method

1. Harvest and pellet 5 x 10^6 cells by centrifugation at 300 x g for 3 minutes. If any liquid remains associated with the pellet, spin down the cells again and aspirate the remaining supernatant.
2. Add 200 µl of 1x PBS to the pelleted cells and centrifuge at 300 x g for 3 minutes. Aspirate and discard the supernatant.
3. Add 2 ml of Cell Lysis Solution to the washed cell pellet. Using a wide-bore pipette tip, resuspend the cells and transfer them to a 15 ml Falcon tube. If clumps of cells remain, gently invert the tube.
4. Incubate the sample at 37°C for 30 minutes.
5. Add 700 µl of the Protein Precipitation Solution to the lysed cells and mix by vortexing for three pulses of 5 seconds.
6. Centrifuge the sample at 2000 x g for 5 minutes.
7. Transfer the supernatant to a new tube and add 2.5 ml of room temperature isopropanol. Discard the pellet.
8. Mix by gently inverting the tube 50 times.
9. Spool the DNA using an inoculation loop or disposable tweezers.
10. Dip the spooled DNA in an Eppendorf tube containing 70% cold ethanol.
11. Remove the inoculation loop or tweezers with the spooled DNA from the ethanol tube, and allow it to air-dry for a few seconds.
12. Dip the DNA in a 1.5 ml Eppendorf DNA LoBind tube containing 250 µl TE (1 mM EDTA, pH 8.0) and allow the DNA to gently dislodge from the loop/tweezers.
13. Incubate the DNA pellet for 2 hours at 50°C, occasionally mixing the tube contents by gentle inversion. Note: The pellet may take some time to dissolve, so ensure the solution is homogenous before quantifying.
14. Take 3 µg of eluate and perform a SPRI size selection.

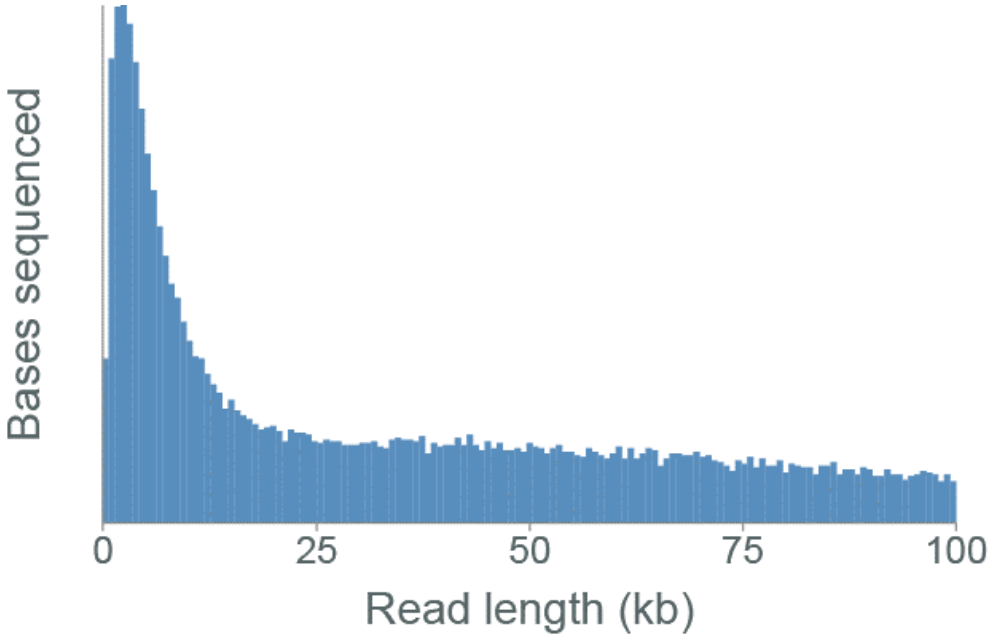
Results

- **Yield:** 20-30 µg
- **OD 260/280:** 1.99
- **OD 260/230:** 2.43



Sequencing performance

Libraries were prepared using the Ligation Sequencing Kit:



Version	Changelog
v3, December 2022	Updated step 1 to include centrifuge recommendations
V2, November 2022	Removed Gentra from QIAGEN Puregene kit name and link updated
V1	Initial publication