

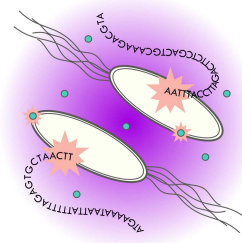
Feb 22, 2026

Version 2

DNA extraction for long-read sequencing of bacteria V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.4r3l2ox24v1y/v2>



Eby Sim^{1,2}

¹Sydney Infectious Diseases Institute (Sydney ID), Faculty of Medicine and Health, The University of Sydney;

²Centre for Infectious Diseases & Microbiology Public Health



Eby Sim

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.4r3l2ox24v1y/v2>

Protocol Citation: Eby Sim 2026. DNA extraction for long-read sequencing of bacteria . **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.4r3l2ox24v1y/v2>Version created by **Eby Sim**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: February 22, 2026

Last Modified: February 22, 2026

Protocol Integer ID: 243811

Keywords: DNA extraction, Long read sequencing, read sequencing of bacteria, complete bacterial genome, dna extraction, extracted dna, dna extraction method, read sequencing, bacteria, dna, liquid culture, sequencing

Abstract

Utilisation of long-read sequencing can reliably generate complete bacterial genomes. Here, we present a DNA extraction method which introduces minor modifications to the DNeasy® UltraClean® Microbial Kit (Qiagen) to generate DNA suitable for long-read sequencing. When sequenced, the extracted DNA should yield median read lengths greater than 7 kb. In addition, this protocol uses cultures growing on solid media as a starting point which will be useful for laboratories that do not routinely use liquid cultures.

Guidelines

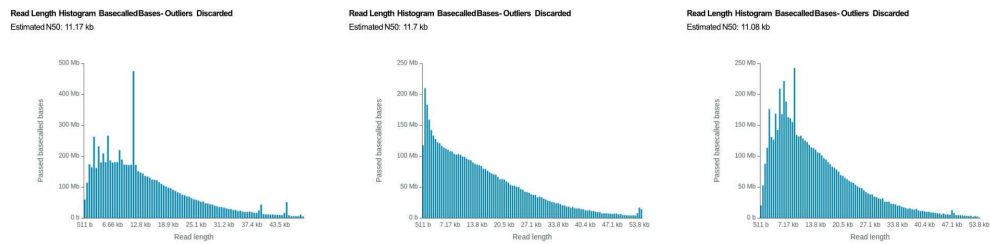
Users are reminded to be very gentle and deliberate in pipetting as this could shear DNA. Usage of wide-bore pipette tips (if accessible) will be preferable.

Materials

Please refer to the DNeasy UltraClean Microbial Kit Handbook for a list of all required equipment. No additional reagents are required.

Before start

Users should take note that this protocol uses both mechanical lysis and spin-columns for DNA extraction. This protocol should **not** be used if reads lengths of > 60 kb is desired. The following image shows the typical read length distribution obtained from this methodology.



Read length distributions of three different ONT runs on DNA extracted with this methodology. Libraries were prepared using the Rapid Barcoding Kit (SQK-RBK004) following manufacturer's instructions.



General bacteria culture

- 1 Streak bacteria of interest onto their respective, optimum solid media and incubate plates at optimal growth conditions.
- 2 On the day of DNA extraction, observe the plate to ensure purity. Do not attempt extraction if different colony morphologies are observed.

DNA extraction

- 3 To a clean 2 mL Powerbead tube, add  300 μ L Powerbead Solution and  50 μ L Solution SL .
- 4 Take a 1 μ L Inoculation loop and pick up 4 streaks from the first quadrant and dislodge the biomass into the Powerbead tube containing both the PowerBead solution and Solution SL.
- 5 Using a fresh 1 μ L Inoculation loop, pick up 4 streaks from the second quadrant and dislodge biomass into the Powerbead tube containing both the PowerBead solution and Solution SL.
- 6 Briefly vortex the Powerbead tube to mix reagents and bacterial biomass.
- 7 Carefully affix the Vortex adaptor onto the Vortex-Genie 2 vortex and place Powerbead tubes horizontally, with the cap facing inwards onto the Vortex adaptor. Vortex at maximum speed for  00:02:00 .

2m

Note

For Mycobacterium species, vortex at maximum speed for 10 minutes.

- 8 Remove Powerbead tubes from the Vortex adaptor and centrifuge at  10000 x g, Room temperature, 00:01:00 .

1m



- 9 Carefully aspirate  300 μL of supernatant without disturbing the pellet or picking up beads. Slowly dispense entire volume into a 2 mL collection tube.
- 10 Add  100 μL Solution IRS and gently finger-flick the collection tube to mix. If there is liquid stuck on the underside of the lid after mixing, give the tube a quick wrist-flick to collect the liquid.
- 11 Incubate the tube  On ice for  00:06:00 . 6m
- 12 Centrifuge the tube at  10000 x g, Room temperature, 00:02:00 . 2m
- 13 Carefully aspirate  300 μL of supernatant, without disturbing the pellet, and slowly dispense into a 2 mL collection tube.

Note

At this stage, no viable cells are expected in the supernatant. However, viability should be confirmed when working with higher containment microorganisms (e.g. *Mycobacterium tuberculosis*)

- 14 Add  600 μL Solution SB and gently invert tube to mix. If there is liquid stuck on the underside of the lid after mixing, pulse centrifuge collect the liquid.
- 15 Slowly aspirate  700 μL of the DNA mixture and dispense into a spin column and centrifuge at  10000 x g, Room temperature, 00:00:30 . Discard the flow through. 30s
- 16 Slowly aspirate the remaining  200 μL and dispense into the same spin column (as step 15) and centrifuge at  10000 x g, Room temperature, 00:00:30 . Discard the flow through and replace the collection tube with a new collection tube. 30s



- 17 Add  300 μ L Solution CB and centrifuge at  10000 x g, Room temperature, 00:00:30 . Discard the flow through and replace the collection tube with a new collection tube. 30s
- 18 Perform a dry centrifuge at  10000 x g, Room temperature, 00:01:00 to remove residual ethanol. Replace collection tube with a new 1.5 mL Lo-Bind tube. 1m
- 19 Add  50 μ L Solution EB to the centre of the membrane. Incubate at  Room temperature for  00:03:00 . 3m
- 20 Centrifuge the spin column at  10000 x g, Room temperature, 00:00:30 . Discard spin column 30s

DNA quality control

- 21 Assess quality on a Spectrophotometers (NanoDropTM (Thermofiseher) or equivalent). Key quality metrics are listed below. Samples that do not fall within this value should not be sent for long-read sequencing. While nucleic acid concentration is also measured by the spectrophotometer, it is not a key parameter at this stage.

Note

A260/A280: 1.8 - 2.0
A260/A230: 2.0 - 2.22

- 22 Assess the concentration of dsDNA on a fluorometer (Qubit or equivalent). Ensure that concentration and amount of extracted dsDNA meets the requirement of the sequencing technology.

Note

Due to the higher DNA input requirements for long-read sequencing, the broad range assay would be the most appropriate kit to use on the Qubit.

- 23 Assess the integrity of the extracted dsDNA via electrophoretic separation (either via a 0.6% (w/v) agarose gel electrophoresis or TapeStation) using an appropriately sized ladder.



Note

Ensure that majority of the dsDNA fragments are greater than 20 kb.

24 Store DNA at -80°C .

Note

Do not freeze DNA before finishing steps 21-23. Keep DNA in 4°C until all quality checks are done.