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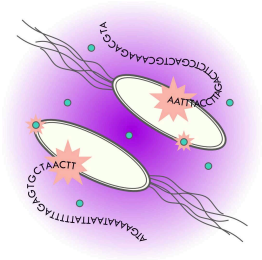
DNA extraction for long-read sequencing of bacteria



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Protocol status: Working
We use this protocol and it's working

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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.

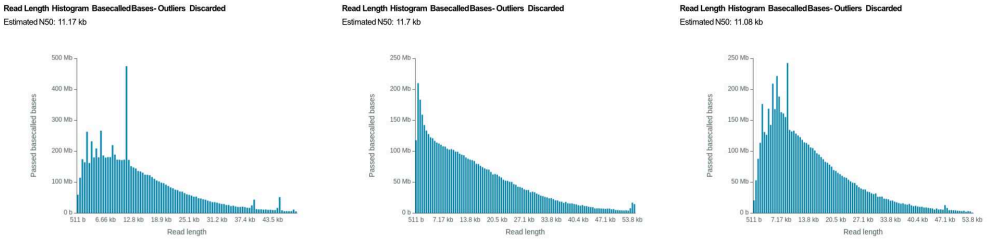
Materials

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

Troubleshooting

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 µL Powerbead Solution and 50 µL Solution SL .

4 Take a 1 µL 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 µL 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

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.

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 µL 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 µL 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 (NanoDrop™ (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^{O}C until all quality checks are done.