Sep 11, 2024

Rapid Bacterial Isolate Whole Genome Sequencing

DOI
dx.doi.org/10.17504/protocols.io.kqdg32ndev25/v1

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Infectious research

DOI: dx.doi.org/10.17504/protocols.io.kqdg32ndev25/v1

Protocol Citation: Adela Alcolea-Medina, Luke Blagdon Snell, CIDR RESEARCH 2024. Rapid Bacterial Isolate Whole Genome Sequencing . **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg32ndev25/v1**

Protocol status: Working

Created: September 05, 2024

Last Modified: September 11, 2024

Protocol Integer ID: 106992

Abstract

Please cite the original publication where this process was described: Charalampous, T., Alcolea-Medina, A., Snell, L.B. *et al.* Evaluating the potential for respiratory metagenomics to improve treatment of secondary infection and detection of nosocomial transmission on expanded COVID-19 intensive care units. *Genome Med* **13**, 182 (2021). https://doi.org/10.1186/s13073-021-00991-y

Set-up and sample sheet

1

Sample number (IS1 etc or IS-N1 for negative control)	Lab number	Organism (Use 5 letter code – E. coli = ESCCOL)

Table for sample information

1.1 **Before beginning:**
Ensure that culture purity plate has been left for **48 hours** in the incubator and colonies have matured based on the amount of growth.

Isolate DNA extraction (bead-beating and bead wash):

2 **Bead-beating/mechanical lysis of bacterial isolates:**

2.1 For each isolate, add **500uL PBS** to a Lysing Matrix E 2mL (MP Biomedicals) bead-beating tube including one for negative control.

2.2 For each culture plate, take an **entire quadrant** using a 10uL loop and add to a bead-beating tube.

2.3 Start bead-beating at **1x40s 4m/s**.

2.4 Centrifuge at **14,000 RPM for 5 minutes**.

3 **Bead wash**

3.1 Transfer **150uL** of supernatant from each Lysing Matrix E tube to new 1.5mL DNA Lobind Tube, one per sample.

3.2 Add **75uL** of AMPure XP beads to each tube

3.3 Mix by flicking and incubate at RT for **5 minutes** with frequent agitation.

3.4 Pulse spin briefly.

3.5 Place tubes magnetic rack for **3 minutes** for beads to pellet.

3.6 Wash twice with **80% ethanol** whilst keeping tube on magnetic rack.

3.7 Pulse spin briefly.

3.8 Dry the beads for **1 minute.**

3.9 Take each tube from the magnetic rack, add 50uL of PBS to each tube, and allow DNA to elute during 5 minute incubation at room temperature.

3.10 Place each tube back on the magnetic rack, allow beads to pellet, and retrieve 35uL to 50uL of the eluted DNA into a new 1.5mL DNA Lobind Tube.

Library preparation and sequencing:

4 Library preparation for **SQK-RBK114.96**:

4.1 Qubit the samples and record the amount of DNA (found on page 4) with the BroadRange/BR Qubit reaction kit. Use 2uL of sample into 198uL of Qubit mastermix.

4.2 Add **7.5uL** of the extracted DNA for each sample to 2.5uL of RBK114.96 barcodes in 0.2mL PCR tubes, one unique barcode per sample.

4.3 Incubate the tubes on a thermocycler at **30°C for 2 minute** and **80°C for 2 minute***.

4.4 Pulse spin briefly.

4.5 Pool all barcoded samples together in a 1.5mL Lobind tube

4.6 Add equal amount of AMPure XP beads.

4.7 Mix by flicking till homogenise.

4.8 Incubate on hula-mixer for 5 minutes.

4.9 Pulse spin briefly.

4.10 Place on magnetic rack for 3 minute.

4.11 Remove supernatant.

4.12 Wash twice with 80% ethanol whilst keeping tube on magnetic rack.

4.13 Pulse spin briefly.

4.14 Remove remaining ethanol and air dry for 1 minute.

4.15 Remove tube from magnetic rack, add **11uL** EB to elute the DNA

5 Incubate for 5 minutes on the hula-mixer.

6 Place on magnetic rack for 1 minute

7 Transfer 10uL of eluate into a fresh 1.5mL DNA Lobind Tube to give the final library.

8 Use 1uL of final library from the original tube for Qubit measurement

9 Wait for 2 minutes, and record Qbit concentration of final library (found on page 4).

10 Create a RA + ADB mix: Add 1.5ul of RA to 3.5ul of ADB.

11 Add 1ml of RA+ADB mix to 10ml of final library.

12 Mix by flicking and pulse spin briefly

13 Incubate the mixture for 5 minutes at RT on the hula-mixer. Move to library sequencing steps 1-2 during incubation

14 In the tube, add as follows:
37.5uL Sequencing Buffer (SB)
25.5uL Library Beads (LIB)
11uL DNA library

15

Sample number	Barcode	Qubit concentration (ng/ul)	
		PCR	Final

Library sequencing:

16 Begin flowcell check on GridION for selected flowcell.

17 Record the number of pores.

18 Slide open the flow cell priming port and draw back a small volume to remove bubbles.

19 Add 800uL of flush buffer through the priming port

20 Wait for 5 minutes.

21 Open SpotON sample port cover.

22 Load 200uL of the flush buffer into the flow cell priming port (not the SpotON port).

23 Flick prepared library gently.

24 Add dropwise 75uL of the prepared library through the SpotON sample port.

25 Close all the ports and click start sequencing.

26 Input the run name in the following way:
<yymmdd>_<operator>_<isolate_run-number> and for sample name
<operator>_<isolate_run-number>.

27 Select the correct library kit: SQK-RBK114.96

28 Do not change any other settings and ensure FASTQ files are kept