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Rapid Bacterial Isolate Whole Genome Sequencing

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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 **1×40s 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