Direct-from-colony microbial sequencing: rapid *Salmonella* serotyping with Oxford Nanopore sequencing

View the protocol

Salmonella species are one of the leading causes of food poisoning with over one million infections a year in the USA, resulting in ~26,500 hospitalisations and ~420 deaths[1–3]. Outbreaks of foodborne salmonellosis can originate from many kinds of foods, meaning that testing and tracing of contamination and/or outbreaks is of great importance to food safety and public health.

Whole-genome sequencing to identify and characterise isolated *Salmonella* organisms from contaminated food is becoming the gold standard for molecular typing[4]. Accurate genomic insights can be generated even with trace DNA samples[5] and this overview describes how to perform whole-genome sequencing directly from a single *Salmonella* colony in 10–20 hours by using PCR and multiplexed nanopore sequencing. This protocol eliminates the need for subculture into liquid broth, DNA extraction, and complex library preparation methods — offering a rapid, simple, and flexible end-to-end workflow.

Samples: single colonies of *Salmonella*

1 Prepare

Pick a single 2–3 mm *Salmonella* colony and suspend in 50 µl of 10 mM Tris buffer (pH 8)

2 Perform tagmentation

Perform DNA tagmentation with 3 µl of cell suspension and 1 µl of Fragmentation Mix (FRM) from the Rapid PCR Barcoding Kit V14

3 Multiplex samples

Multiplex up to 24 samples by amplifying the tagmented DNA using the Rapid Barcode Primers 01–24 (RPB01–24) from the Rapid PCR Barcoding Kit V14 and LongAmp Hot Start Taq 2X Master Mix (NEB)

4 Pool samples

Quench the reactions with 4 µl of EDTA and quantify 1 µl of each barcoded sample using a Qubit fluorometer

Pool 100 ng of each barcoded sample, purify using 0.6X AMPure XP Beads (AXP), and quantify 1 µl of purified DNA using a Qubit fluorometer

5 Attach sequencing adapter

Dilute the Rapid Adapter (RA) using the Adapter Buffer (ADB) from the Rapid PCR Barcoding Kit V14 and add 1 µl of diluted RA to 250 ng of pooled DNA in 11 µl of Elution Buffer (EB)

6 Sequence

Prepare the library for loading by combining 12 µl of the library with 37.5 µl of Sequencing Buffer (SB) and 25.5 µl of Library Beads (LIB)

Load 75 µl of the prepared library dropwise on to a MinION™ Flow Cell and set up sequencing on MinKNOW™ using the high accuracy (HAC) basecaller on a MinION or GridION™ device

Sequence to 50,000 reads per barcode — for a 24-plex run, this can be achieved by sequencing on one MinION Flow Cell for up to 12 hours

7 Analyse

Use the wf-bacterial-genomes workflow (which has SeqSero2[6] integrated) from EPI2ME™ to analyse basecalled data

The workflow performs genus/species identification, *Salmonella* serotyping, antimicrobial resistance profiling, and multilocus sequence typing

The workflow outputs an individual interactive isolate sequencing report in HTML format for each of the samples, as well as a combined summary report for all samples

For users preferring an easy-to-use graphical interface, this preconfigured workflow is free to access from the EPI2ME Desktop Application

For users with advanced bioinformatics experience, the workflow is simple to run in the command line

Both options can be run on local compute or in the cloud

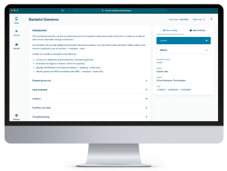
Kits, devices, and software

Library preparation

Rapid PCR Barcoding Kit V14

Sequencing

MinION Flow Cells on MinION or GridION devices



Analysis

EPI2ME wf-bacterial-genomes

View the end-to-end protocol:

nanoporetech.com/direct-from-colony-microbial-sequencing

References:

1. Centers for Disease Control and Prevention. *Salmonella*. https://www.cdc.gov/salmonella/index.html (2024) [Accessed 07 July 2025]
2. US Food and Drug Administration. Get the Facts about *Salmonella*. https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-about-salmonella (2023) [Accessed 07 July 2025]
3. World Health Organization. Food safety fact sheet. https://www.who.int/news-room/fact-sheets/detail/food-safety (2024) [Accessed 07 July 2025]
4. European Food Safety Authority. Whole-genome sequencing in foodborne outbreaks. https://www.efsa.europa.eu/en/topics/topic/whole-genome-sequencing-foodborne-outbreaks (2025) [Accessed 07 July 2025]
5. Wang, X. et al. *MedComm* (2020) 3(1):e116 (2022). DOI: https://doi.org/10.1002/mco2.116
6. US Food and Drug Administration. Foods program compendium of analytical laboratory methods. https://www.fda.gov/food/laboratory-methods-food/foods-program-compendium-analytical-laboratory-methods (2024) [Accessed 07 July 2025]

Oxford Nanopore Technologies

www.nanoporetech.com

phone +44 (0)845 034 7900

email support@nanoporetech.com

oxford-nanopore-technologies

@nanopore

@nanoporetech.com

WF_1265(EN)_V2_18Jul2025