

## SUPPLEMENTAL METHODS

### Bacteria collection and DNA extraction

*A. pittii*, *E. faecium*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. enterica*, and *S. aureus* were isolated and purified from food or clinical samples using the methodologies outlined in prior publications.

To isolate and purify the *B. cereus* from marine water samples, firstly we weighed aseptically 5g sample into 45 ml PBS. Homogenized the sample thoroughly at room temperature using a stomacher and 1 mL of the supernatant was transferred to a tube with 9ml tryptic soy broth (TSB). Incubated the TSB tube overnight at 37 °C with continuous shaking at 250 rpm. Next, we spread 10 µL TSB broth onto mannitol egg yolk polymyxin (MYP) agar plates and incubated the plates at 30 °C overnight. Notable, the *B. cereus* colonies on the MYP agar would appear a characteristic pink-orange color, surrounded by a zone of precipitation. Then we purified the suspicious organism by streaking it on MYP agar plates at 30 °C for 18 hours or Brilliance™ *Bacillus cereus* Agar (oxid) plates, which will yield blue/green colonies at 37 °C for 18 hours. From each sample, we selected several typical colonies and subculture them.

To extract the genomic DNA, we used the PureLink Genomic DNA Mini Kit from Invitrogen (USA). Each DNA sample is divided into 6 equal portions for storage and subsequent sequencing in different platforms.

### Bacterial DNA library preparation and sequencing

The Falcon automation system was used for short-read sequencing library preparation and sequencing. The genomic DNA was fragmented into smaller fragments with an average size of 350 base pairs (bp) using the Frag enzyme. Subsequently, the DNA fragments underwent end-polishing, A-tailing, and ligation with adaptors for DNB sequencing, followed by PCR amplification. To facilitate the ligation step, a special molecule that was reverse-complemented to one specific strand of the PCR product was introduced. The single-strand molecule was then ligated using DNA ligase. The remaining linear DNA molecules were digested using an exonuclease, resulting in the formation of a single-strand circular DNA library. The library underwent quality control assessment for quantification and size distribution detection,

Quantification was performed using a Qubit fluorometer and read-time PCR, while size distribution was evaluated using a bioanalyzer. The quantified libraries were pooled in equal amounts to generate DNA Nanoballs (DNBs), which were then subjected to sequencing on a DNBseq-T7 platform.

The DNA extracted from each bacterial sample, totaling 400 ng, was diluted to a final volume of 12  $\mu$ L for the construction of Nanopore sequencing libraries. Native Barcoding Kit 24 V14 (SQK-NBD114.24) and Ligation sequencing gDNA Kit (SQK-LSK109) with Native Barcoding Expansion 1-12 (EXP-NBD104) were used for following the provided protocol. The repaired and end-prepared DNA from each sample was ligated with native barcodes and then sequencing adaptors were ligated with mixed samples containing the respective barcodes to finalize the library. Extracted DNA was also diluted to 100 pg and processed for whole genome amplification (WGA) according to our previous protocol (Ni et al. 2023). Each purified WGA product was diluted and used for library preparation with SQK-NBD114.24 or SQK-LSK109 with EXP-NBD104 kit. The libraries were loaded on R10.4.1 (FLO-MIN114) or R9.4.1 (FLO-MIN106D) flow cells, respectively, and sequenced using the MinION Mk1B sequencer for 72 hours.