MicrobesNG - Genome Sequencing Service Methods

SAMPLE SUBMISSION

- Strain samples:

Pure cultures of each strain are grown in plates or broth. Cells are harvested and resuspended in a tube with cryoperservative (Microbank™, Pro-Lab Diagnostics UK, United Kingdom) or with DNA/RNA Shield (Zymo Research, USA) following MicrobesNG strain submission procedures. Strain containing tubes are sent to MicrobesNG for sequencing.

- DNA samples:

Extracted DNA is eluted in 10 mM Tris-HCl pH 8.0 or nuclease free water and sent to MicrobesNG for sequencing.

DNA EXTRACTION

Five to forty microlitres of the suspension are lysed with 120 µL of TE buffer containing lysozyme (final concentration 0.1 mg/mL) and RNase A (ITW Reagents, Barcelona, Spain) (final concentration 0.1 mg/mL), incubated for 25 min at 37°C. Proteinase K (VWR Chemicals, Ohio, USA) (final concentration 0.1mg/mL) and SDS (Sigma-Aldrich, Missouri, USA) (final concentration 0.5% v/v) are added and incubated for 5 min at 65°C. Genomic DNA is purified using an equal volume of SPRI beads and resuspended in EB buffer (Qiagen, Germany).

DNA is quantified with the Quant-iT dsDNA HS kit (ThermoFisher Scientific) assay in an Eppendorf AF2200 plate reader (Eppendorf UK Ltd, United Kingdom).

ILLUMINA SEQUENCING (SGS and EGS)

Genomic DNA libraries are prepared using the Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with the following modifications: input DNA is increased 2-fold, and PCR elongation time is increased to 45 s. DNA quantification and library preparation are carried out on a Hamilton Microlab STAR automated liquid handling system (Hamilton Bonaduz AG, Switzerland). Pooled libraries are quantified using the Kapa Biosystems Library Quantification Kit for Illumina. Libraries are sequenced using Illumina sequencers (HiSeq/NovaSeq) using a 250bp paired end protocol.

Reads are adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 [1]. De novo assembly is performed on samples using SPAdes version 3.7 [2], and contigs are annotated using Prokka 1.11 [3].

1. Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. http://doi.org/10.1093/bioinformatics/btu170
2. Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., ... Pevzner, P. A. (2012). SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology, 19(5), 455–477. http://doi.org/10.1089/cmb.2012.0021
3. Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics. 30(14):2068-9

ONT SEQUENCING (EGS only)

Long read genomic DNA libraries are prepared with Oxford Nanopore SQK-RBK004 kit and/or SQK-LSK109 kit with Native Barcoding EXP-NBD104/114 (ONT, United Kingdom) using 400-500ng of HMW DNA. Barcoded samples are pooled together into a single sequencing library and loaded in a FLO-MIN106 (R.9.4.1) flow cell in a GridION (ONT, United Kingdom).

Illumina reads are adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 [1]. Genome assembly is performed using Unicycler v0.4.0 [2]. The contigs are annotated using Prokka 1.11 [3]

1. Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. http://doi.org/10.1093/bioinformatics/btu170
2. Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Computational Biology 13(6): e1005595. https://doi.org/10.1371/journal.pcbi.1005595
3. Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics. 30(14):2068-9