A complete nanopore-only assembly of an XDR *Mycobacterium tuberculosis* Beijing lineage strain identifies novel genetic variation in repetitive PE/PPE gene regions

Oxford NANOPORE Technologies

With over 10 million new cases reported each year and an increasing incidence of drug resistant infections, tuberculosis (TB) is one of the leading global threats to human health. To better understand the genomic changes that facilitate the emergence and spread of drug resistant *M. tuberculosis* strains (the predominant cause of TB), researchers at the University of Queensland, Australia, utilised nanopore sequencing to deliver a complete *de novo* genome assembly of a highly-transmissible XDR strain from Papua New Guinea[1].

The MinION™ allowed the full drug-resistance profile to be determined with complete phenotypic concordance. Novel insights were also gained on GC-rich and repetitive genes that are intractable to traditional short-read sequencing technologies.

Sample preparation

This sample preparation protocol, as described by Bainomugisa *et al*[1] for *M. tuberculosis*, can be applied to other bacterial species.

Sample preparation ~ 1.5 hours

Löwenstein-Jensen slope culture (4 weeks at 37°C).

PREPARATION

Culture loop added to 200 µl PrepMan Ultra sample reagent containing 5 (0.7mm) glass beads. Mix well.

CELL LYSIS

Dry heat incubate at 95°C for 10 mins. Bead beat at 6.0m/s for 40 sec using a mini-beadbeater-16 (BioSpec). Centrifuge – 10 mins at 13,000 rpm.

DNA PRECIPITATION

Transfer 40 µl to another vial. Add 45 µl NaOAc and 1 ml ice cold ethanol (96%). Centrifuge – 15 mins at max speed. Discard supernatant.

CLEAN-UP

Add 1 ml ethanol (70%) and incubate at RT for 1 min. Discard supernatant.

RESUSPENSION

Dry for 15 mins and resuspend in 40 µl nuclease-free water.

QUALITY ASSESSMENT

Quality and concentration assessed using NanoDrop and Qubit.

SIZE SELECTION

Size selection performed using 0.4X AMPure XP beads.

SIZE ASSESSMENT

Fragment distribution size assessed using Agilent 4200 TapeStation.

Library preparation

As per 1D Genomic DNA sequencing by ligation for the MinION device using SQK-LSK108* amendments[1].

Library preparation ~ 1.5 hours (single sample) ~ 2 hours (multiplex)

Purified gDNA 1–1.5 µg.

END-REPAIR, dA-TAILING

Standard end-repair, dA-tailing reaction but both incubation steps increased to 20 mins each. Purify using 0.7X AMPure XP beads.

ADAPTER LIGATION

Standard ligation step but incubation step increased to 20 mins at RT. Clean-up using 0.4X AMPure XP beads.

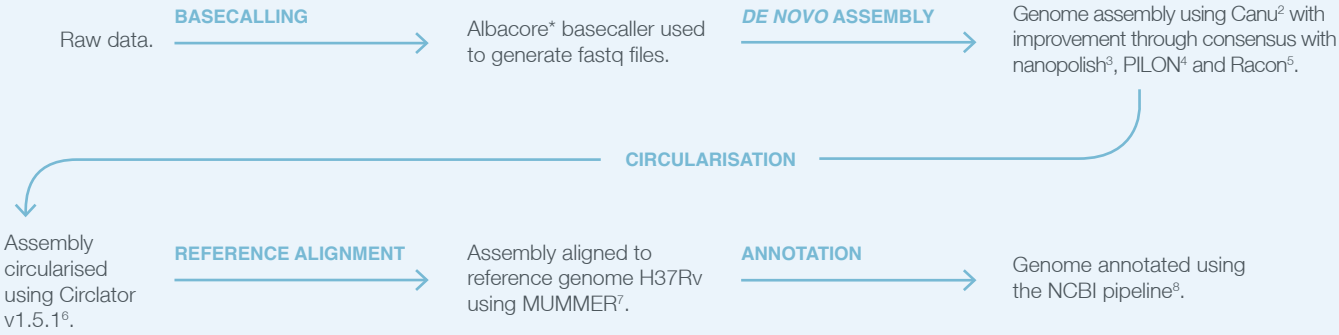
SEQUENCING

Oxford Nanopore also provides transposase-based library preparation kits, which offer a fast, 10-minute workflow. Both ligation- and transposase-based kits are compatible with barcoding, enabling cost-effective analysis of multiple samples on a single flow cell. For more information, visit www.nanoporetech.com.

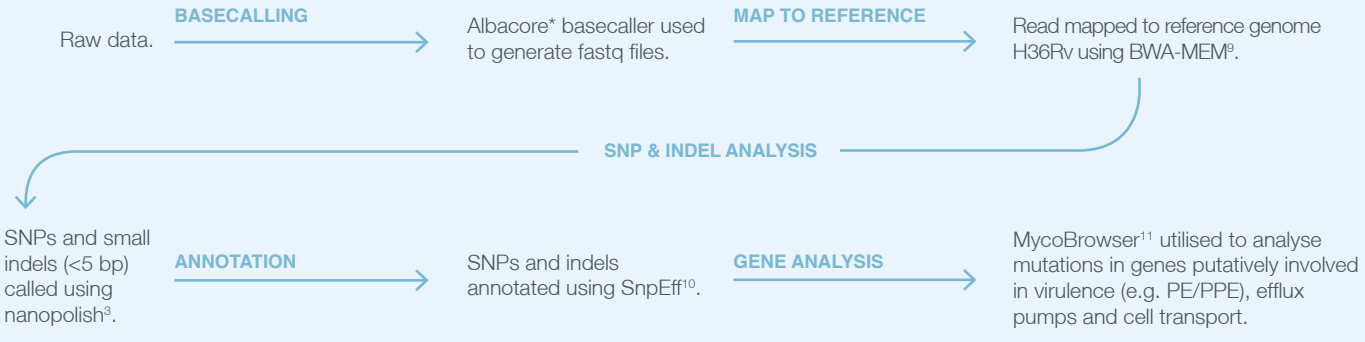
*SQK-LSK108 has since been superseded by SQK-LSK109.

Data analysis

De novo genome assembly



Variant analysis



In addition to the whole genome assembly and analysis tools used by Bainomugisa *et al*[1], the cloud-based EPI2ME data analysis platform offers a number of real-time analysis pipelines, including antimicrobial resistance profiling.

References

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Find out more about rapid, real-time bacterial sequencing and analysis at www.nanoporetech.com.

*Guppy has superseded Albacore as the production basecaller

WF_1023(EN)_V2_07May2019