

Comparison of Illumina and Oxford Nanopore Technology for genome analysis of *Francisella tularensis*, *Bacillus anthracis*, and *Brucella suis*

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Bacterial epidemiology requires understanding the spread of highly pathogenic bacteria like *Bacillus anthracis*, *Brucella* species, and *Francisella tularensis*. Whole genome sequencing (WGS) and genotyping are crucial for this purpose. While Illumina short-read sequencing is established, the suitability of Oxford Nanopore Technology (ONT) long-read sequencing remains unexplored.

This study evaluated Illumina, ONT flow cell versions 9.4.1 and 10.4 for six strains respectively, for each selected species. ONT produced ultra-long reads, while Illumina provided short reads with high accuracy. Flow cell version 10.4 improved accuracy. All technologies accurately identified the species and virulence markers. ONT allowed near closure assembly of chromosomes and virulence plasmids for *Ba. anthracis*.

All assemblies based on ONT, Illumina, and hybrid approaches correctly predicted canonical (sub-)clades for *Ba. anthracis* and *F. tularensis* and Multi-Locus Sequence Types (STs) for *Br. suis*. High-resolution genotyping showed comparable results between Illumina and ONT data for *F. tularensis*. *Ba. anthracis* aligned well only with flow cell version 10.4. *Br. suis* showed larger differences between Illumina and ONT.

In conclusion, combining ONT and Illumina data is feasible for high-resolution genotyping in *F. tularensis* and *Ba. anthracis*, but not yet for *Br. suis*. Improvements in nanopore technology may enable genotyping for all bacteria in the future.

Keywords

Bacillus anthracis; *Brucella*; *Francisella tularensis*; Genome sequencing; Illumina; Oxford nanopore technology; R10.

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Postdoc

Junior Scientist Status

No, I am not a Junior Scientist.

Primary author: LINDE, Jörg

Co-authors: Dr BRANGSCH, Hanka (Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute); Mrs THOMAS, Christine (Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute); Dr HÖLZER, Martin (Genome Competence Center (MF1), Methodology and Research Infrastructure, Robert Koch Institute, Berlin, Germany); Dr ELSCHNER, Mandy (Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute); Dr MELZER, Falk (Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute); Dr TOMASO, Herbert (Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute)

Presenter: LINDE, Jörg

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