Nanopore sequencing of bacterial *rpoB* and 16S rRNA gene amplicons for infectious disease diagnostics

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Background

Nanopore sequencing is a rapid approach, providing sequencing results within minutes with per-read accuracy of >99%. The bacterial 16S rRNA gene is commonly used as a target for amplicon sequencing in diagnostic microbiology. As 16S rRNA sequences are often too similar to distinguish between closely related species, *rpoB* represents an alternative target providing better discrimination within many clinically important taxa (1). The combination of more precise taxonomic markers like *rpoB* with rapid and accurate nanopore sequencing has the potential to improve diagnosis of complex samples.

The aim of the study was:

- to evaluate nanopore sequencing of both 16S rRNA and *rpoB* gene amplicons
- to design a novel pipeline for sequence analysis with clinically acceptable accuracy
- to assess the efficiency of 16S rRNA and *rpoB* nanopore sequencing for bacterial detection and identification in clinical settings

Workflow

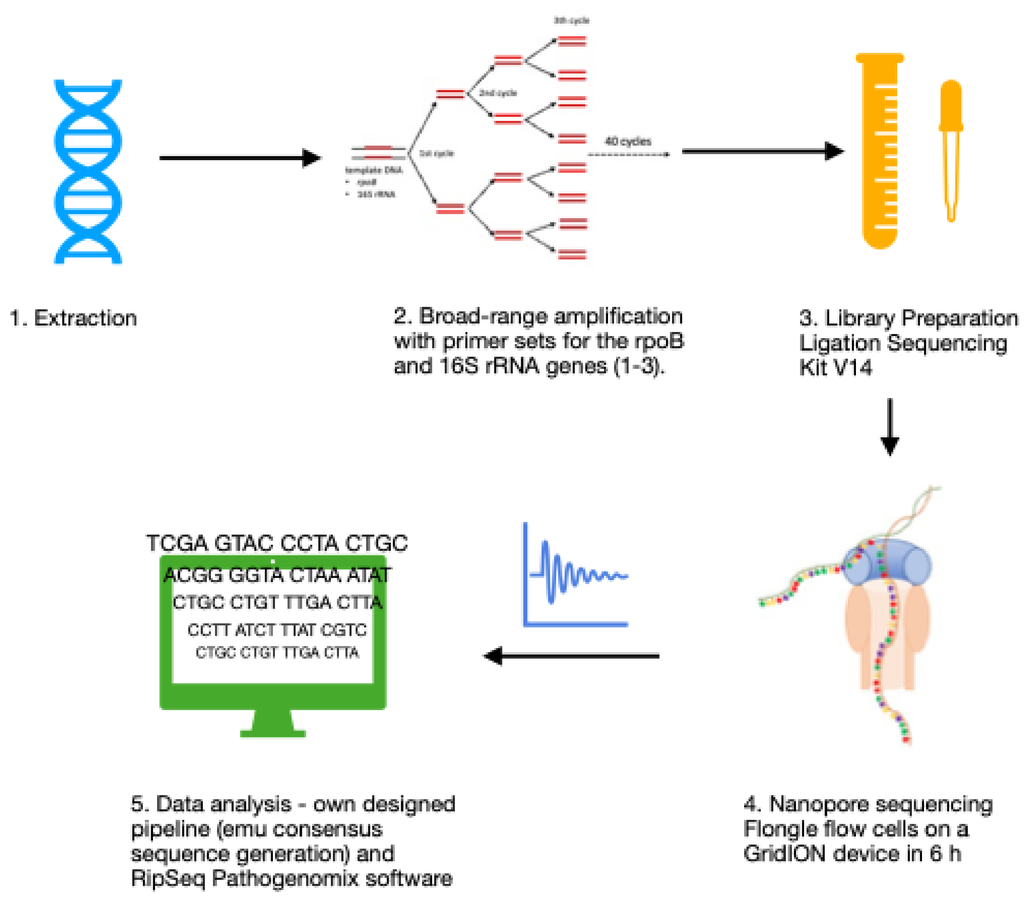


Figure 1. Workflow for the experiments

Results

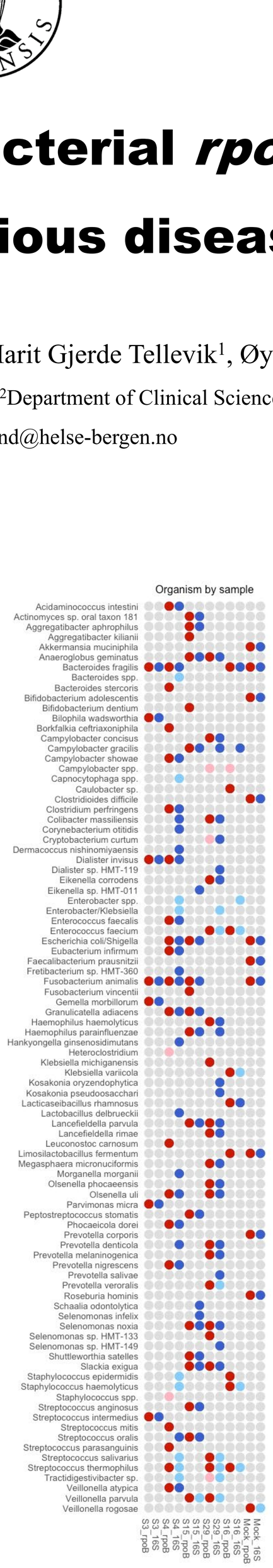


Figure 2. shows sequencing results for five polymicrobial samples and Mock community sample for both rpoB and 16S rRNA genes analysed in RipSeq (Pathogenomix). *rpoB* provided improved resolution between closely related bacteria with 94% of species level identification, as compared to 78% for the 16S rRNA gene. Genera with higher taxonomic classification level were, among others, *Enterococcus, Enterobacter*, *Klebsiella*, *Staphylococcus*, *Streptococcus* and *Veillonella.* We achieved 93 bacterial identification when using rpoB and 106 with the 16S rRNA gene. The average number of sequencing reads per samples was in total 311 629 for rpoB and 366 936 for the 16S rRNA gene, of which the average number of valid reads was 162 743 and 166 335, respectively.

Figure 2. results for the the rpoB and 16S rRNA gene nanopore sequencing. Colours indicate taxonomic classification level.

Conclusion

Nanopore sequencing of the *rpoB* and 16S rRNA genes has the potential to improve diagnosis of polymicrobial infections and can be easily implemented in clinical laboratories.

References:

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