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Sequencing Workshop Stellenbosch

22 – 26 January 2024

High-throughput FL-16S rRNA gene sequencing: rapid and accurate identification of bacterial species

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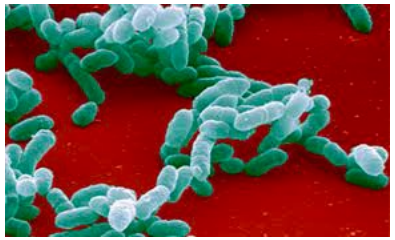
Centre for Food Safety
University College Dublin

Why sequence bacteria?

UCD School of Public Health,
Physiotherapy and Sports Science



Haemophilus influenzae

-First bacterium sequenced (Fleischmann *et al.*, 1995)
-Sanger sequencing (Sanger *et al.*, 1977)

Functionality

Food spoilage

Genus *Listeria*

16S ribosomal RNA Gene Sequencing

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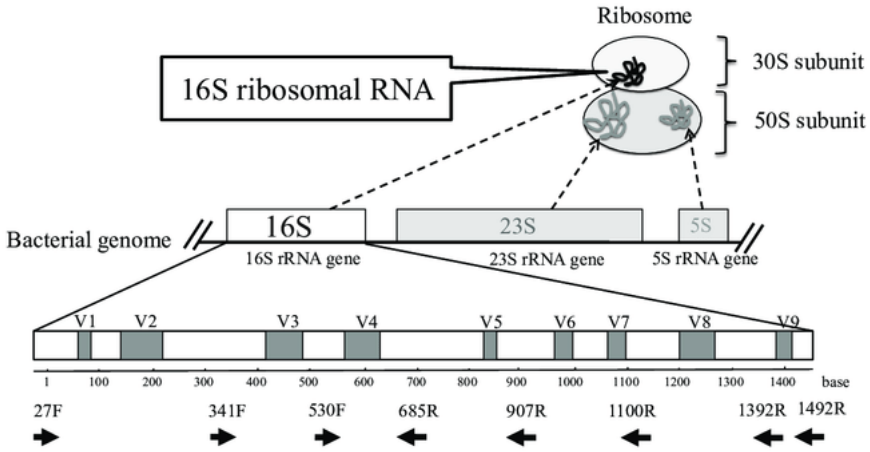
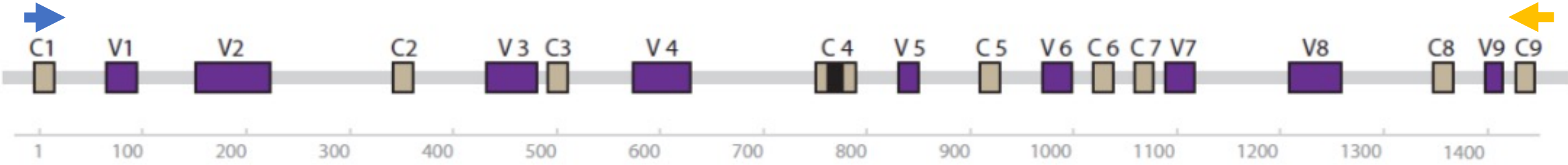
Species Identification



(Fukuda *et al.*, 2016)

Sanger Sequencing 16S rRNA gene

Full-length 16S ribosomal RNA (FL-16S rRNA) Gene Sequencing



Nanopore FL-16S rRNA Gene Sequencing

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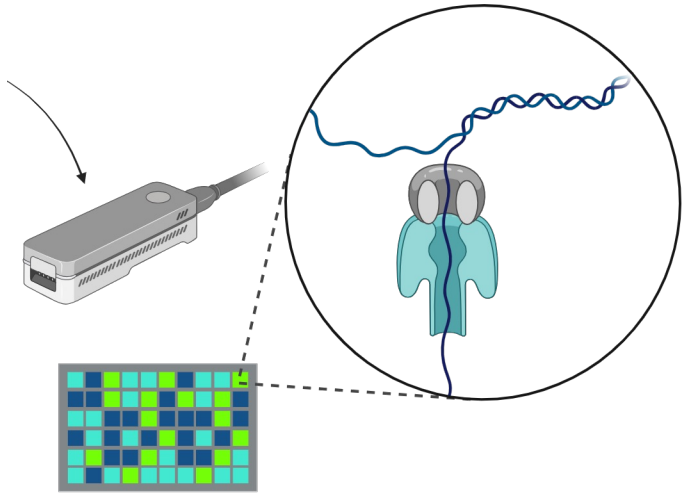
Oxford NANOPORE Technologies

FL-16S rRNA Gene Sequencing

-Cost-effective
-Timely
-Accurate



DNA Extraction

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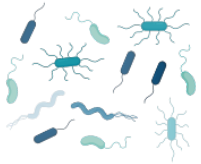
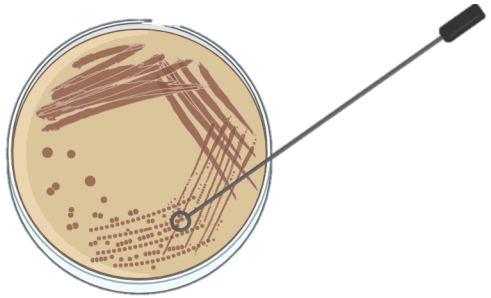


PowerFood® microbial kit



UltraClean® microbial kit

Library Preparation and Sequencing

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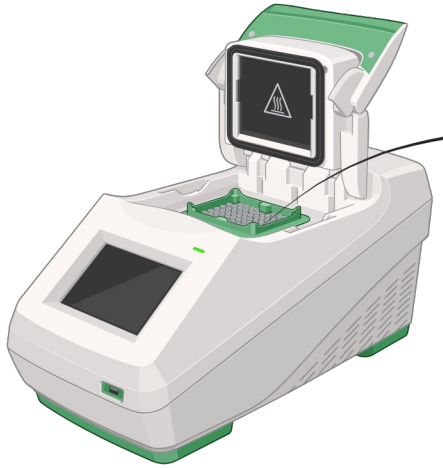
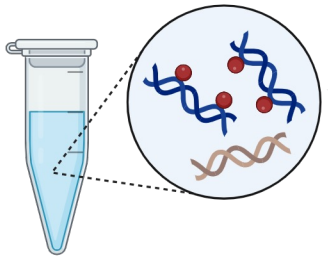
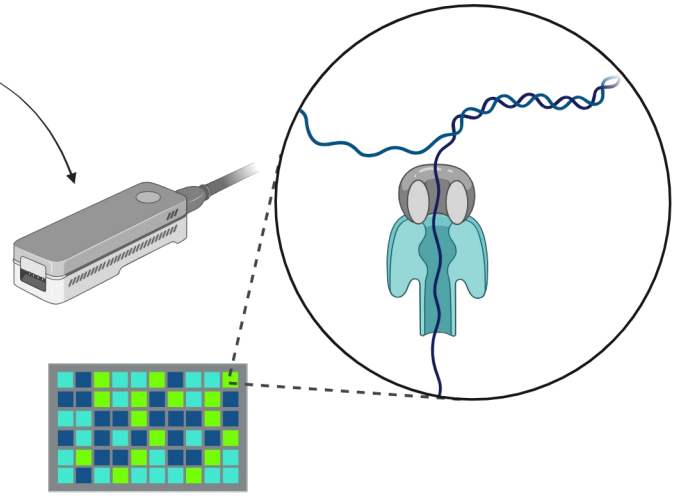


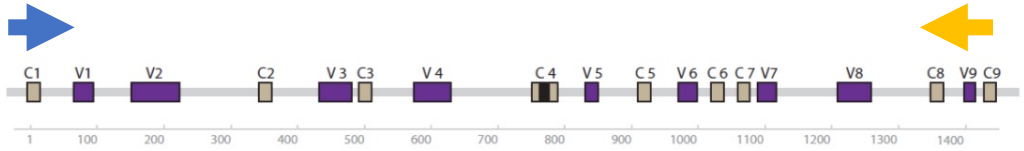
Full-length 16S ribosomal RNA gene (FL-16S rRNA) amplification using universal 16S primers



PCR clean-up and
Isolation of FL-16S rRNA gene
by AMPure XP beads



Sequenced FL-16S rRNA gene (~1.5 kb fragment) using Oxford Nanopore Technologies' Flongle flow cells



Oxford NANOPORE Technologies

Bioinformatic Analysis and Species Identification

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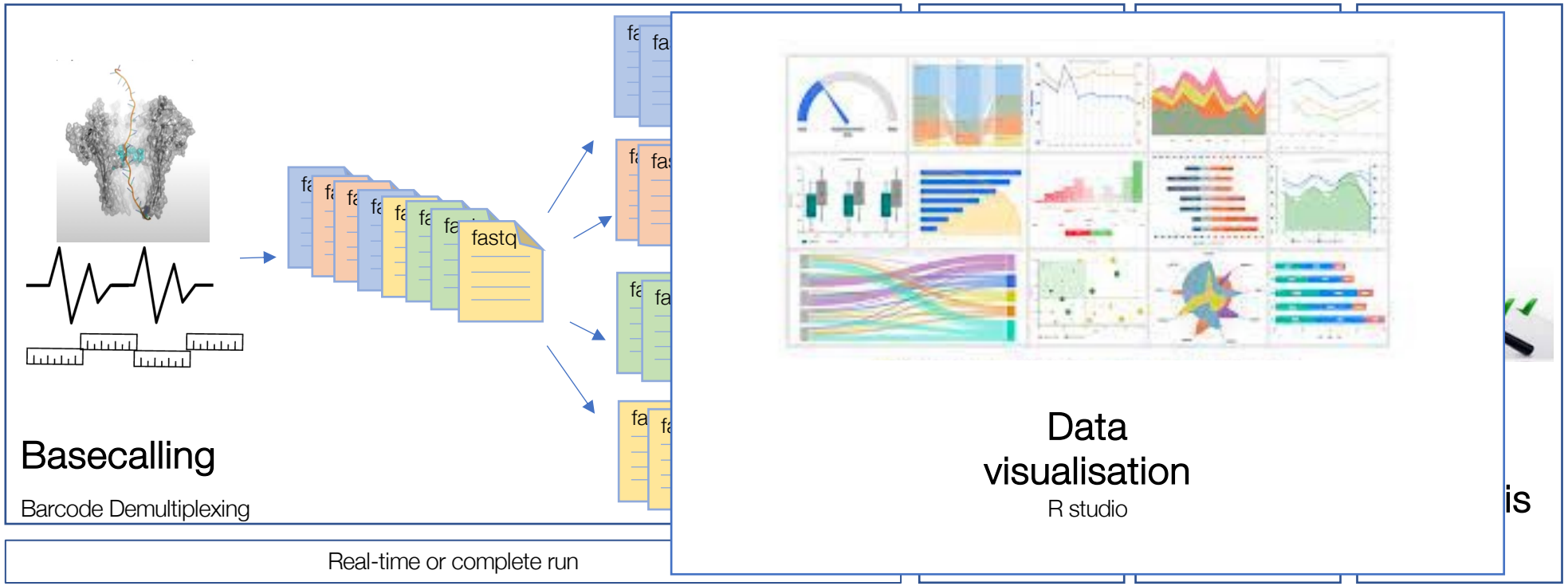

Acknowledgements

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UCD DUBLIN
UCD School of Public Health, Physiotherapy and Sports Science

Professor Séamus Fanning
Dr Guerrino Macori

Thank you

References

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Fukuda, K., Ogawa, M., Taniguchi, H. and Saito, M. (2016) 'Molecular Approaches to Studying Microbial Communities: Targeting the 16S Ribosomal RNA Gene', *J UOEH*, 38(3), 223-32, available: http://dx.doi.org/10.7888/juoeh.38.223.

Sanger, F., Nicklen, S. and Coulson, A.R. (1977) 'DNA sequencing with chain-terminating inhibitors', *Proc Natl Acad Sci U S A*, 74(12), 5463-7, available: http://dx.doi.org/10.1073/pnas.74.12.5463.