

# Next Generation Sequencing: Applications of Oxford Nanopore Technologies to Juvenile Aphrophoridae Niche

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Blind DNA extraction from biological samples, including plants and animal tissue, microorganisms, and non-biological matrices, is a must-use for unconventional approaches or first approach to complex phenomena. In this context, Oxford Nanopore Technologies provides a comprehensive range of DNA library tools, giving streamlined access to long-read and real-time sequencing benefits. The library construction protocol covers over 86 procedures for step-by-step experimental guidance, and the specific recommendations for library preparation are based on sample type, input quantity, and experimental priorities.

In particular, MinION longer reads advantage the genome analysis, offering easier assembly, and higher accuracy in identifying and distinguishing repeating sequences. The raw genomic data produced by the device offer excellent chances for finding species but require significant downstream processing and analysis.

Our study was focused on the niche of juvenile spittlebugs: a self-produced liquid froth embedding the aquatic insects into minuscule habitats. The Meadow Spittlebug, *Philaenus spumarius*, has recently become famous for its primary role in the *Xylella fastidiosa* subsp. *pauca* ST53 transmission, the pathogen causing OQDS in Southern Italy. The Meadow Spittlebug is still the key pest of the Mediterranean olive orchard. We plated the froth (or foam) on a nutrient agar medium (Thermo Fisher Scientific, Waltham, MA, USA) by soaking sterile stabs and isolated four different bacteria entities based on color. We adopted a DNA extraction method with CTAB modified [1] to further accelerate the sample preparation process. Libraries were obtained by the Rapid Barcoding Sequencing protocol, with some modifications (magnetic particles, Mag-Bind Total Pure NGS, Omega Biotek), sequenced by means of the MinION device, and the output data analysed by means of *ad hoc* bioinformatic tools from the sequences quality checking and cleaning to their taxonomic profiling. The sequences obtained determined the presence of the genera *Microbacterium*, *Pseudomonas*, and *Agrobacterium*.

Here, we propose this new simple workflow for rapid Spittlebugs froth bacteria identification via MinION sequencing, reducing the time and providing a reliable method applicable to anaerobic and microaerobic conditions.

## References

[1] Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*, vol. 19(1): 11-15.