

Technology Requirements for Molecular Biological Study of Chilean Phytoplankton Cells: Challenges and Applications

Robert Hatfield¹, David Ryder¹, Frederico Batista¹, Laura Latorre-Melín² & Alejandro Clément²

1: Centre for Environment Fisheries and Aquaculture Science, Weymouth, Dorset, UK, 2: Plancton Andino, Puerto Varas, Chile

Since the discovery of the DNA double helix by Watson and Crick in 1953, molecular tools have evolved rapidly, enabling increasingly precise and high throughput analysis. The development of polymerase chain reaction (PCR) in the 1980s by Kary Mullis enabled the detection of single molecules of DNA, and the subsequent discovery of thermostable Taq polymerase streamlined the process and reduced cost. These innovations laid the foundation for modern genetic assays and opened the door to high-throughput sequencing technologies.

Similarly, sequencing technologies have evolved through three distinct generations. First-generation Sanger sequencing, developed in the 1970s, provided the initial framework for reading DNA but was limited in scale and speed. Second-generation or “next-generation sequencing” (NGS), including platforms like Illumina, introduced massively parallel sequencing, dramatically increasing throughput and reducing costs but had limited read length. Most recently, third-generation long-read sequencing technologies, such as Oxford Nanopore and PacBio, have enabled real-time, portable, and high-resolution analysis of complex environmental samples.

These technological advances have the potential to revolutionise the detection and study of harmful algal blooms (HABs), ensuring public safety, successful aquaculture and monitoring marine ecosystem health. Traditional monitoring methods, such as light microscopy, are labor-intensive and often lack species-level resolution—especially for cryptic or morphologically similar taxa like *Pseudo-nitzschia* and *Alexandrium*.

To address these limitations, we have developed a suite of molecular assays including PCR and isothermal amplification—specifically recombinase polymerase amplification (RPA) with nanopore sequencing. These assays target barcode regions of ribosomal DNA, enabling species-specific identification of HAB organisms and aquatic pathogens directly from environmental samples. The RPA method offers several advantages over conventional PCR, including faster reaction times, reduced sensitivity to inhibitors, and compatibility with field-deployable platforms.

In addition to sequencing-based identification, lateral flow dipstick assays have been integrated to provide rapid, visual screening for key toxigenic species. This dual approach allows for both broad surveillance and targeted detection, making it suitable for routine monitoring and emergency response. Assays have been successfully developed for *Pseudo-nitzschia*, *Alexandrium*, and *Bonamia*, demonstrating the versatility of this technology across different biological threats.

The Chilean context presents unique challenges due to the diversity of phytoplankton species and the dynamic nature of coastal ecosystems. However, the tools described here offer scalable, sensitive, and real-time solutions that can be adapted to local needs. Furthermore, the expiration of key patents on RPA technology has opened the market to new suppliers, reducing costs and increasing accessibility for global monitoring programs.

In summary, the integration of modern molecular biology tools into HAB and pathogen detection represents a paradigm shift in marine monitoring. These technologies not only enhance our ability to detect and respond to biological threats but also pave the way for more comprehensive, data-driven management of aquatic environments.

Technology Requirements for Molecular Biological Study of Chilean Phytoplankton Cells: Challenges and Applications

Rapid and accurate identification of marine biological threats is essential for effective environmental monitoring and aquaculture management. This presentation explores the application of modern molecular biology tools including isothermal amplification and nanopore sequencing for species-specific detection of harmful algal bloom (HAB) organisms and aquatic pathogens. Assays developed for *Pseudo-nitzschia*, *Alexandrium*, and the oyster parasite *Bonamia* demonstrate the potential of recombinase polymerase amplification (RPA) combined with sequencing to deliver high-resolution data in real time. Additionally, lateral flow dipstick technology enables rapid screening in both laboratory and field settings. These approaches overcome limitations of traditional microscopy and offer scalable, sensitive solutions for monitoring a wide range of biological threats, including emerging diseases and parasites. The technology holds promise for integration into routine surveillance programs and emergency response strategies across diverse aquatic environments.