Effective use of PCR introduces considerable challenges. The amplification process, which relies on the presence of a specific target sequence, can be affected by various factors such as template concentration, primer design, and reaction conditions. In some cases, PCR can yield nonspecific products, leading to false positives. Moreover, PCR is particularly susceptible to errors due to the use of short primers, which can lead to primer dimer formation and other artifacts.

The accuracy of PCR is further compromised by the presence of contaminants in environmental samples. These contaminants can includeDNA from dead cells, which can interfere with the amplification process. Despite these limitations, PCR remains a powerful tool for detecting and quantifying target molecules in complex samples.

To improve the accuracy of PCR results, it is important to consider the potential for contamination and to implement appropriate controls. This can include the use of negative controls, which are samples that are known to be free of the target molecule of interest. These controls can help to identify and correct for any potential contamination.

Furthermore, it is important to carefully select primers and reaction conditions to minimize the risk of nonspecific amplification. This can include the use of degenerate primers, which can be designed to amplify a wider range of targets, or the use of nested PCR, which involves a second round of amplification using primer sets that are specific to the target sequence.

In conclusion, while PCR-based methods have revolutionized the field of environmental biology, it is important to be aware of the potential pitfalls and limitations of this approach. By implementing appropriate controls and carefully selecting primers and reaction conditions, it is possible to improve the accuracy and reliability of PCR-based results. This will be crucial for advancing our understanding of the complex communities that inhabit the natural environment.
are actually present in the environmental sample. Steps 4 and 5 also give additional valuable information about the relative and absolute abundances of the respective sequences in the environmental sample and depending on the sample preparation even on their spatial organization [10, 11]. Only closing the full circle approach by following all 5 steps represents a fully controlled experimental setup.

Environmental interpretations of PCR based sequence datasets from environmental samples do almost always require a quantitative aspect of data analysis. Microscopic examinations of environmental samples did already radically correct the ecological conclusions drawn from PCR based results [12]. To avoid erroneous conclusions from environmental sequences data sets, we have to make sure, that the sequences of interest are actually of quantitative relevance in environmental samples. Complementing PCR based sequence retrievals from environmental samples with well documented microscopic proof will ensure the accuracy of future interpretations of such datasets. This is a demanding challenge for experimental setups as well as for reviewers in the future. It will require a combined and interdisciplinary effort to guarantee the necessary accuracy in the performance and documentation of the methodological spectrum required for the full circle approach. However what is the good practice standard in molecular ecology since at least 1999 has to finally become the standard in present day publications as well.

References


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