Título: Pcr Troubleshooting And Optimization: The Essential Guide

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The polymerase chain reaction (PCR) is a fundamental tool in scientific research and clinical testing. Real-time PCR, combining both amplification and detection in one instrument, is a rapid and accurate method for nucleic acid detection and quantification. Although PCR is a very powerful technique, the results achieved are valid only if the appropriate controls have been employed. In addition, proper optimization of PCR conditions is required for the generation of specific, repeatable, reproducible, and sensitive data. This book discusses the strategies for preparing effective controls and standards for PCR, when they should be employed, and how to interpret the information they provide. It highlights the significance of optimization for efficiency, precision, and sensitivity of PCR methodology and provides essential guidance on how to troubleshoot inefficient reactions. Experts in PCR describe design and optimization techniques, discuss the use of appropriate controls, explain the significance of standard curves, and explore the principles and strategies required for effective troubleshooting. The book highlights the importance of sample preparation and quality, primer design, controlling inhibitors, avoiding amplicon and environmental contamination, optimizing reagent quality and concentration, and modifying the thermal cycling protocol for optimal sensitivity and specificity. In addition, specific chapters discuss the history of PCR, the choice of instrumentation, the applications of PCR in metagenomics, high resolution melting analysis, the MIQE guidelines, and PCR at the microliter scale. The strategies, tips and advice contained in this concise volume will enable the scientist to optimize and effectively troubleshoot a wide range of techniques, including PCR, reverse transcriptase PCR, real-time PCR, and quantitative PCR. It will be an essential book for anyone using PCR technology.