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PCR Troubleshooting 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

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amplification and detection
in one instrument, is a
rapid and accurate method
for nucleic acid detection
and quantification.

Optimization and
troubleshooting in PCR.

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PCR product has high GC content (>65%) GC-rich PCR products are difficult to amplify. To improve amplification, increase the annealing temperature. For greater accuracy, optimize the annealing temperature by

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using a thermal gradient.
DMSO or another secondary
structure destabilizer can
be added (do not exceed
10%) .

**Polymerase Chain Reaction:
Basic Protocol Plus ...**

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Polymerase chain reaction
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Optimization for efficiency,
precision and sensitivity of
PCR methodology. Essential
guidance on how to

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troubleshoot inefficient reactions. Design and optimization techniques, use of appropriate controls, the significance of standard curves and the principles and strategies required for effective troubleshooting.

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Guide PCR Troubleshooting and Optimization: The Essential Guide

Experts in PCR describe design and optimization techniques, discuss the use of appropriate controls,

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explain the significance of standard curves, and explore the principles and strategies required for effective troubleshooting.

PCR Troubleshooting and
Optimization: The Essential

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If standard PCR conditions do not yield the desired amplicon, PCR optimization is necessary to attain better results. The stringency of a reaction may be modulated such that the

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specificity is adjusted by altering variables (e.g., reagent concentrations, cycling conditions) that affect the outcome of the amplicon profile.

PCR Troubleshooting Guide -

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OPTIMIZATION STRATEGY. Raise the maximum and minimum annealing temperatures (i.e., shift the range upward) in the TD PCR program. Decrease the total number of cycles by

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eliminating some cycles from
the bottom of the range
and/or from the terminal
constant temperature cycles.

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The polymerase chain reaction (PCR) is a commonly used molecular biology tool for amplifying DNA, and various techniques for PCR optimization which have been developed by molecular biologists to improve PCR

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PCR Troubleshooting | LSR |
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from PCR Troubleshooting:
The Essential Guide see also
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Guide. PCR Troubleshooting:
Inadequate dNTPs An
incorrect concentration of
deoxynucleotidetriphosphates
(dNTPs) can cause problems
for the PCR procedure. The
usual dNTP concentration is

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between 40?M and 200?M of
EACH of the four dNTPs.

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Thermo Fisher Scientific -
US

Quantitative PCR (qPCR):
Hallmarks of an Optimized

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SYBR Green I Assay This tutorial describes the characteristics of an optimized SYBR ® Green I quantitative PCR (qPCR) assay. qPCR assays must be optimized to ensure results that are biologically and

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statistically significant.

Topics include a brief review of qPCR chemistry, with an emphasis on SYBR Green I reactions, and definitions of the four main characteristics, or hallmarks, of an optimized

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Real-Time PCR

Troubleshooting | LSR | Bio-
Rad

Optimization and
troubleshooting in PCR. Roux
KH. The use of polymerase
chain reaction (PCR) to

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generate large amounts of a desired product can be a double-edged sword. Failure to amplify under optimum conditions can lead to the generation of multiple undefined and unwanted products, even to the

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exclusion of the desired
product .

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In addition, proper
optimization of PCR

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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

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how to interpret the
information they provide.

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Review recommendations on

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the amount of DNA polymerase to use in PCR, and optimize as necessary. Increase the amount of DNA polymerase if the reaction mixture contains a high concentration of an additive (e.g., DMSO, formamide) or

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inhibitors from the sample
sources. Optimize Mg²⁺
concentration for maximum
PCR yields.

PCR Troubleshooting and
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...

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PCR troubleshooting guide.

In molecular cloning, after the synthesis of cDNA from mRNA molecule templates, a PCR program must be designed to amplify the gene of interest, as well as add additional elements such as

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restriction sites or
detection/purification tags.

9781904455721: PCR
Troubleshooting and
Optimization: The ...
The polymerase chain
reaction (PCR) is a

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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

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and quantification. Although PCR is a very powerful technique, the results achieved are valid only if the appropriate controls have been employed.

Amazon.com: PCR

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Troubleshooting and
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PCR Troubleshooting Guide.
The following guide can be
used to troubleshoot PCR
reactions. Use our T_m
calculator to help plan
experiments and [click here](#)

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for optimization tips.

Choose a higher fidelity polymerase such as Q5® (NEB #M0491), Phusion® (NEB #M0530) DNA Polymerases.

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When troubleshooting, test the primers at 100 nM, 500 nM and 900 nM final concentration and/or T a between 55 °C to 70 °C (using a temperature gradient) to identify whether the assay will

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improve with further
optimization.

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