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Troubleshooting qPCR

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Noise - Ask TaqMan
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Reverse Transcription,
quantitative rt-PCR
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cluster separation
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optimization,
Calculating PCR

Efficiency *Selection of
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for PCR **Chapter 6**
**Polymerase Chain
Reaction PCR Part 1**
How to Optimize

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Multiplex qPCR

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TaqMan® Ep. 18 by

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Optimization and

troubleshooting in PCR

The use of polymerase
chain reaction (PCR) to

generate large amounts

of a desired product can

be a double-edged

sword. Failure to

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amplify under optimum conditions can lead to the generation of multiple undefined and unwanted products, even to the exclusion of the desired product. At the other extrem ...

Optimization and troubleshooting in PCR

It highlights the
significance of

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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

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g And explore the principles and strategies required for effective troubleshooting.

Guide

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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 for optimization
tips. Phusion DNA
Polymerase was

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developed by
Finnzymes Oy, now a
part of Thermo Fisher
Scientific.

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PCR Troubleshooting Guide | NEB

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Sample Preparation

PCR Protocols PCR

Troubleshooting Tips

Educational Resources

Pathway Maps Gene

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Info Cards Boster

Interviews--Expert Tips
on IHC Optimization

The Savvy Scientists'

Buffers Guide

PCR Troubleshooting Tips | Boster Bio

Lower the quantity to
reduce the generation of
nonspecific PCR

products. Poor integrity:
Degraded DNA may
appear as smears or lead

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to high background in gel electrophoresis. Minimize shearing and nicking of DNA during isolation. Evaluate the integrity of the template DNA prior to PCR by gel electrophoresis, if necessary.

PCR Troubleshooting Guide | Thermo Fisher Scientific - US

Summary — PCR

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Troubleshooting

Checklist. Check quality of sample (degraded material will cause erroneous results).

Check RT protocol is compatible with design (e.g., an Oligo-dT primed RT must have a qPCR assay in the 3' 1 kb of sequence). Check assay design. Check all controls. Check primers using SYBR green I

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dye/run a gel.

RT-PCR/RT-qPCR Troubleshooting | PCR Technologies Guide ...

PCR component concentrations and/or cycling conditions may not be sufficient for longer target sequences. Reoptimize your existing assay protocol and/or increase the duration of PCR steps,

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especially the extension step. Water was impure: Water could have been contaminated during prior pipetting events. Use fresh nuclease-free water. Not enough Mg²⁺

PCR Troubleshooting | LSR | Bio-Rad

PCR conditions.

Denaturation time
should be kept to a

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g And
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minimum to decrease depurination events. Use touchdown PCR; start at a higher annealing temperature and reduce by two degrees per cycle for several cycles. Design primers with melting temperatures (T_m) above 68°C . PCR polymerases. We offer several PCR polymerases optimized for long-range PCR.

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Optimizing your PCR - Takara Bio

OPTIMIZATION

PARAMETER

RECOMMENDATION

QPCR Plate It is recommended that opaque white PCR plates are used for QPCR analysis. The white color virtually eliminates cross talk and improves the efficiency

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of fluorescent detection
thereby increasing assay
sensitivity and well-to-
well consistency.

Guide

QPCR Optimization & Troubleshooting Guide

The Real-Time PCR

Doctor is here to help.

Unexpected

fluorescence data are

symptomatic of

problems with your real-

time PCR reaction

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components or
amplification protocol.

Click one of the
symptoms below to

learn about possible
causes and treatments.

Related Topics: What Is
Real-Time PCR?, How
Real-Time PCR Works,
and qPCR Assay Design
and ...

**Real-Time PCR
Troubleshooting | LSR**

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| **Bio-Rad**

from PCR

Troubleshooting: The
Essential Guide see also

PCR Troubleshooting
and Optimization: The
Essential Guide. PCR
Troubleshooting: Mg
Concentration

Magnesium is a required
cofactor for

thermostable DNA
polymerases. Mg²⁺ in
the PCR mixture

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stabilizes dsDNA and
raises the T_m .

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troubleshooting in pcr
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of a desired product can
be a double edged
sword failure to amplify
under optimum

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the generation of
multiple undefined and
unwanted products even
to the exclusion of the
desired product at the
other extrem

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