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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 □

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following guide can be used to

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troubleshoot PCR reactions. Use our
T_m calculator to help plan experiments
and click here for optimization tips.

Phusion DNA Polymerase was
developed by Finnzymes Oy, now a
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Lower the quantity to reduce the
generation of nonspecific PCR
products. Poor integrity: Degraded
DNA may appear as smears or lead to
high background in gel
electrophoresis. Minimize shearing

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

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Summary □ PCR Troubleshooting

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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

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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

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protocol and/or increase the duration of PCR steps, especially the extension step. Water was impure: Water could have been contaminated during prior pipetting events. Use fresh nuclease-free water. Not enough Mg²⁺

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PCR conditions. Denaturation time

should be kept to a 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

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polymerases. We offer several PCR polymerases optimized for long-range PCR.

Optimizing your PCR - Takara Bio
OPTIMIZATION PARAMETER
RECOMMENDATION QPCR PlateIt is
recommended that opaque white PCR

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plates are used for QPCR analysis. The white color virtually eliminates cross talk and improves the efficiency of fluorescent detection thereby increasing assay sensitivity and well-to-well consistency.

QPCR Optimization & Troubleshooting

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The Real-Time PCR Doctor is here to help. Unexpected fluorescence data are symptomatic of problems with your real-time PCR reaction components or amplification protocol. Click one of the symptoms below to learn about possible causes and treatments.

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Troubleshooting: Mg Concentration

Magnesium is a required cofactor for
thermostable DNA polymerases. Mg
2+ in the PCR mixture stabilizes
dsDNA and raises the T_m.

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