PCR Troubleshooting Guide

The following guide can be used to troubleshoot PCR reactions. Use our Tm calculator to help plan experiments and click here for optimization tips.

Observation	Possible Cause	Solution
SEQUENCE ERRORS	Low fidelity polymerase	▪ Choose a higher fidelity polymerase such as Q5™ (NEB #M0491), Phusion® (NEB #M0530) DNA Polymerases
	Suboptimal reaction conditions	▪ Reduce number of cycles ▪ Decrease extension time ▪ Decrease Mg^{++} concentration in the reaction
	Unbalanced nucleotide concentrations	▪ Prepare fresh deoxynucleotide mixes
	Template DNA has been damaged	▪ Start with a fresh template ▪ Try repairing DNA template with the PreCR® Repair Mix (NEB #M0309) ▪ Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired sequence may be toxic to host	▪ Clone into a non-expression vector ▪ Use a low-copy number cloning vector
INCORRECT PRODUCT SIZE	Incorrect annealing temperature	▪ Recalculate primer Tm values using the NEB Tm calculator
	Mispriming	▪ Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{++} concentration	▪ Adjust Mg^{++} concentration in 0.2–1 mM increments
	Nuclease contamination	▪ Repeat reactions using fresh solutions
NO PRODUCT	Incorrect annealing temperature	▪ Recalculate primer Tm values using the NEB Tm calculator ▪ Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor primer design	▪ Check specific product literature for recommended primer design ▪ Verify that primers are non-complementary, both internally and to each other ▪ Increase length of primer
	Poor primer specificity	▪ Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	▪ Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions
	Missing reaction component	▪ Repeat reaction setup
	Suboptimal reaction conditions	▪ Optimize Mg^{++} concentration by testing 0.2–1 mM increments ▪ Thoroughly mix Mg^{++} solution and buffer prior to adding to the reaction ▪ Optimize annealing temperature by testing an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair

	Poor template quality	▪ Analyze DNA via gel electrophoresis before and after incubation with Mg^{++} ▪ Check 260/280 ratio of DNA template
	Presence of inhibitor in reaction	▪ Further purify starting template by alcohol precipitation, drop dialysis or commercial clean up kit ▪ Decrease sample volume
	Insufficient number of cycles	▪ Rerun the reaction with more cycles
	Incorrect thermocycler programming	▪ Check program, verify times and temperatures
	Inconsistent block temperature	▪ Test calibration of heating block
	Contamination of reaction tubes or solutions	▪ Autoclave empty reaction tubes prior to use to eliminate biological inhibitors ▪ Prepare fresh solutions or use new reagents and new tubes
	Complex template	▪ Use Q5 High-Fidelity (NEB #M0491) or One*Taq*® DNA Polymerases (NEB #M0480) ▪ For GC-rich templates, use Q5 High-Fidelity (NEB #M0491) or One*Taq*® DNA Polymerases. Include the appropriate GC enhancer. ▪ For longer templates, we recommend LongAmp® *Taq* DNA Polymerase or Q5 high-Fidelity DNA polymerase or Q5 Hot-Start High-Fidelity DNA Polymerase (NEB #M0493)
MULTIPLE OR NON-SPECIFIC PRODUCTS	Premature replication	▪ Use a hot start polymerase, such as One*Taq* Hot Start DNA Polymerase ▪ Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
	Primer annealing temperature too low	▪ Increase annealing temperature
	Incorrect Mg^{++} concentration	▪ Adjust Mg^{++} in 0.2–1 mM increments
	Poor primer design	▪ Check specific product literature for recommended primer design ▪ Verify that primers are non-complementary, both internally and to each other ▪ Increase length of primer ▪ Avoid GC-rich 3´ ends
	Excess primer	▪ Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions.
	Contamination with exogenous DNA	▪ Use positive displacement pipettes or non-aerosol tips ▪ Set-up dedicated work area and pipettor for reaction setup ▪ Wear gloves during reaction setup
	Incorrect template concentration	▪ For low complexity templates (i.e. plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction ▪ For higher complexity templates (i.e. genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.
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