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

Observation	Possible Cause	Solution
		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	Premature replication	Use a hot start polymerase, such as One*Taq* Hot Start DNA Polymerase

Observation	Possible Cause	Solution
OR NON-SPECIFIC PRODUCTS		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