

PCR Troubleshooting Guide – Thermo-Start *Taq* DNA Polymerase

Problem	Possible Causes	Actions
No Amplicon	Error in set up	Repeat the experiment, checking all reagents are added in correct volumes. Use master mix to ensure all components added correctly.
	Error in cycling	Check program is correct on thermal cycler and that cycling starts and finishes correctly
	Error in gel analysis	Check wells on gel loaded correctly, correct loading buffer was added to samples, EtBr is added to gel and UV settings are correct
	Incorrect annealing temperature	Run a temperature gradient in 2°C increments
	Incorrect MgCl ₂ concentration	Run a MgCl ₂ gradient of 0.5mM increments between 1.5 and 4.0mM
	Insufficient template	Increase template concentration
	Primer dimers	Increase temperature and/or decrease MgCl ₂ . Check self complementarity of primers on primer design software. Redesign primers.
	Primer design error	Blast primers. Check primer parameters on primer design software. Redesign primers
	DNA not clean or contains inhibitors	Check template is clean. Check all ethanol was evaporated from DNA extractions. If inhibitors are present diluting the template can improve the reaction.
	Secondary structure in template	Use Thermo-Start DNA Polymerase with High Performance Buffer
Low Yield	Enzyme not fully activated	Ensure activation step at 95°C for 15 minutes is carried out.
	Annealing temperature not optimal	Run a temperature gradient in 2°C increments
	MgCl ₂ concentration not optimal	Run a MgCl ₂ gradient of 0.5mM increments between 1.5 and 4.0mM
	Insufficient template	Increase template concentration
	Insufficient primers	Increase primer concentration
	Insufficient cycles	Increase amount of cycles
	GC-rich or difficult template	Use Thermo-Start DNA Polymerase with High Performance Buffer
	Extension time too short	For long products (>2kb), extension time (in mins) should be approximately equal to the number of kb in the amplicon.
	DNA not clean or contains inhibitors	Check template is clean. Check all ethanol was evaporated from DNA extractions. If inhibitors are present diluting the template can improve the reaction.
	Sample evaporating during cycling	Check levels in wells after cycling. Ensure screw-down lid is pressing firmly on plate. Use high quality adhesive seals and rigid PCR plates

Non-Specific Amplification – Multiple Products	Priming starting during set up	Use an incremental activation, to activate the enzyme in steps. For example, 2 minute incubations at 95°C for the first 7–8 cycles.
	Annealing temperature not optimal	Run a temperature gradient in 2°C increments
	MgCl ₂ concentration not optimal	Run a MgCl ₂ gradient of 0.5mM increments between 1.5 and 4.0mM
	Primers not specific	Blast primers to check specificity. Redesign primers.
	Overabundance of primer	Decrease primer concentration
	Overabundance of template	Decrease template concentration
	Annealing time too long	Decrease time of annealing step
	Contamination	Check no template control (NTC) for bands
Non-Specific Amplification – Smeared Product	Priming starting during setup	Use an incremental activation, to activate the enzyme in steps. For example, 2 minute incubations at 95°C for the first 7–8 cycles.
	Annealing temperature not optimal	Run a temperature gradient in 2°C increments
	MgCl ₂ concentration not optimal	Run a MgCl ₂ gradient of 0.5mM increments between 1.5 and 4.0mM
	Primers not specific	Blast primers to check specificity. Redesign primers.
	Overabundance of primer	Decrease primer concentration
	Overabundance of template	Decrease template concentration
	Annealing time too long	Decrease time of annealing step
	Template degraded	Minimize freeze thawing of DNA. Run template on agarose gel to check integrity.
Band in No Template Control (NTC) - Contamination	Contaminated reagents	Use a fresh aliquot of reagents
	Pipettes contaminated	Clean and sterilize pipettes. Use filter tips. Use different pipettes for pre- and post-PCR.
	Work area contaminated	Clean work bench or move areas. Use a different area for pre- and post-PCR.
	Aerosol contamination	Use a master mix to minimize pipetting steps, use filter tips, close lids on all tubes and expel reagents carefully. Change gloves regularly.
Wrong Size Band Amplified	Contamination	Check no template control for bands
	Wrong primers or template added	Check primers and template vials have been labeled correctly and selected correctly during setup.
	Different gene form	Check gene for isoforms or splice variants.
Reaction Not Reproducible or Reaction Stopped Working	Different cycling conditions	Use the same thermal cycler for optimization and all future experiments. Different cyclers can vary in ramping speeds and temperature.
	dNTPs degraded	dNTPs are very susceptible to freeze thawing. Replace with a fresh aliquot.
	Error in set up	Repeat - checking correct reagents added and correct thermal cycler program used.
	Change in component	Check any new components that have been added (eg. new batch of primers)
	Inhibitors in template	Decrease template concentration, dilute template or clean template.