Thermo
SCIENTIFIC

PCR Troubleshooting Guide

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 DNA can improve the reaction.
	Secondary structure in template	Use touchdown PCR, add adjuvant such as DMSO, BSA or Betaine or use a hot-start *Taq* DNA polymerase
Low Yield	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
	Buffer not optimal	Use a NH_4 based buffer instead of KCl based buffer for greater yield
	Insufficient template	Increase template concentration
	Insufficient primers	Increase primer concentration
	Insufficient cycles	Increase amount of cycles
	Secondary structure in template	Use touchdown PCR, add adjuvant such as DMSO, BSA or Betaine, or use a hot-start *Taq* DNA polymerase
	GC-rich template	Add adjuvant such as DMSO, BSA or Betaine, or 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.
	Long denaturation inactivating enzyme	Only use a 2 minute denaturation time for polymerases which do not require a hot-start.
	DNA not clean or contains inhibitors	Check template is clean. Check all ethanol was evaporated from DNA extractions. If inhibitors are present diluting DNA can improve the reaction.
	Sample evaporating during thermal 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.

Thermo SCIENTIFIC

Non-Specific Amplification – Multiple Products	Priming starting during set up	Set up reaction on ice or use a hot-start *Taq* DNA polymerase
	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
	Buffer not optimal	Use a KCl based buffer instead of a NH_4 based buffer for greater specificity
	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 set up	Set up reaction on ice or use a hot-start *Taq* DNA polymerase
	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
	Buffer not optimal	Use a KCl based buffer instead of a NH_4 based buffer for greater specificity
	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.
	Extension time too short	For long products (>2kb), extension time (in mins) should be approximately equal to the number of kb in the amplicon.
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 set up.
	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.