THERMAL CYCLERS Technical note: A01-002A

PCR Trouble shooting guide

ISSUE	CAUSE	SOLUTION
No amplification in any sample.	A reagent is missing.	Check correct reagents were added.
	No template added or template is degraded.	Use new template and repeat assay.
	Sequence not present in the sample.	To differentiate between true negative results and a false negative e.g. reaction component missing, always include a positive control.
	Incorrect reaction conditions (annealing temperature too high or too low).	Check thermal cycling program.
	Incorrect primer sequence.	Redesign primers.
	Reaction inhibitor present in template.	Ensure that the template is <10% of the assay volume. Purify template further.
	Error in gel analysis.	Check that the gel was loaded correctly and stained properly.
Some amplification but low yield.	Enzyme activation not long enough (or too long) or the temperature not high enough.	Check manufacturer's recommendations.
	Annealing temperature too high or too low.	Optimise the annealing temperature using a gradient if possible.
	Annealing or extension time too short.	Increase the hold times. For long products (>2kb) use incremented hold times on the extension step.
	Not enough cycles.	Increase the number of cycles.
	Not enough template.	Increase the amount of template.

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	Primer concentration is not optimal.	Optimise the primer concentration by performing a matrix of forward and reverse primer concentrations.
	$MgCl_2$ concentration is not optimal.	Start with the manufacturer's recommendation and increase in 0.5mM steps.
	Target has a high amount of secondary structure.	Ensure the template is fully denatured at the start of the run. Use touchdown PCR.
No template control (NTC) shows amplification.	Amplicon or template contamination of one of the reagents.	Repeat the assay with fresh reagents. Separate PCR set up from analysis. Use filter tips.
Non-specific products: primer-dimers	Short non-specific products amplified in preference to the target.	Reduce primer concentration; reduce $MgCl_2$ concentration; use a hot start enzyme; use a touchdown PCR protocol; re-design primers.
Non-specific products: smeared bands on gel	Degraded template and/or reactions conditions too permissive.	Check template integrity. Increase stringency of reaction – use a touchdown PCR method.
	Long products not amplified completely.	Increase extension time – use an incremented hold time on the step. Ensure the mix contains enough reagents such as dNTPs.
	Primers not specific	Check primer specificity.
Non-specific products: multiple bands	Reaction conditions too permissive.	Increase the stringency of reaction – use a touchdown PCR method. Redesign the primers.
	Contamination.	Check the NTC and positive control.
	Pseudo genes present.	Check primer design.
Reaction not reproducible.	Reaction was run on a different thermal cycler.	Reaction not optimized for the cycler. Use the original cycler or re-optimize for annealing temperatures, ramp rates and hold times.

	New batch of reagents or primers.	Compare the new batch with the previous batch in the same run.
	Inhibitor in the template.	Ensure that the template is <10% of the assay volume. Purify template further. Use a positive control in the reaction to check for inhibition.
Decreased volume in samples at end of a run.	Poor seal.	Ensure the sealing method is appropriate. Heat sealing is recommended.
	Sample volume too small.	Testing is recommended before using low sample volumes. Use low profile plates.
Condensation on tube lid.	Heated lid not on.	Ensure the heated lid is set to pre-heat before heating the sample.