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PCR Troubleshooting Quick-Check

Ten common PCR failure patterns, their most likely cause, and the first fix to try before changing anything else.

Symptom	Likely cause	First fix
No product	Template absent/degraded, or a reagent left out of the mix.	Run a positive control; check template concentration and purity first.
Multiple bands	Annealing temperature too low, primers bind partially complementary sites.	Raise annealing temperature in 2–3°C steps (gradient run if available).
Smear, no discrete band	Too much template, too many cycles, or degraded/contaminated template.	Dilute template 1:10 to 1:100 and/or reduce cycle count.
Primer dimers (fuzzy band below ~100 bp)	Complementary primer 3' ends, or annealing temperature too low for primer Tm.	Check primer design for 3' complementarity; raise annealing temperature.
Non-specific extra band alongside the correct one	Annealing temperature too low, or extension time too long.	Raise annealing temperature; shorten extension time to match amplicon length.
Low yield	Suboptimal Mg2+, too few cycles, or degraded enzyme/reagents.	Check Mg2+ concentration and reagent storage; add 2–3 cycles first.
Product is the wrong size	Mispriming on a related sequence, or contaminating template (e.g. genomic DNA in a cDNA reaction).	Check primer specificity in silico; add a no-template control (and no-RT control for RT-PCR).
Some samples in a batch fail while others work	Co-purified PCR inhibitors carried over from extraction.	Dilute the affected template 1:10 and re-test: inhibitors dilute out faster than template.
Amplification in the no-template control	Aerosol carryover contamination, or a contaminated reagent.	Prepare master mix in a dedicated pre-PCR area; swap reagents one at a time to isolate the source.
Inconsistent result between replicate wells	Poor master mix mixing, or a pipetting/calibration error.	Vortex and briefly spin the master mix before aliquoting; recheck pipette calibration.

Change one variable at a time and keep a positive control in every run: it is the fastest way to tell a reagent problem from a template problem.

Source: Innis et al., PCR Protocols: A Guide to Methods and Applications (Academic Press, 1990); NEB PCR Troubleshooting Guide (publicly published).

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For the theory behind PCR optimisation, see the Biotech Techniques Bible series.