

Template problems, if they are identified, can be solved by making fresh template preps or by cleaning up existing template.

Primer problems:

These can arise from a variety of causes. The first is poor primer design (non-matching T_m s, secondary structure problems, primer dimers, etc). If this problem is eliminated, by careful design, then there is the possibility that there was something wrong with the primer synthesis (you have little control over this, and can only try to order a fresh batch). The primer prep may also contain salts or other contaminants from the synthesis that inhibit your PCR reaction. Primers can be cleaned up easily if you suspect this is a problem. If this is a regular problem, find a different synthesis facility that actually does a good job of cleaning up the primers.

Cycling conditions:

This is the commonest source of problems. Annealing temperatures, magnesium concentrations and cycling times have to be carefully optimized for each template primer pair. This is an empirical process, though calculations can help you pick a starting annealing temperature.

Operator error:

Hey, pay attention!

Contamination:

Most of the problems discussed above relate to not getting amplification. If you have contamination of your template, primer or other reagents, you may end up with more amplification than you want. This may be in the form of additional bands, the wrong bands altogether or other uninterpretable results. The simplest way to avoid contamination is by being careful and by maintaining a clean workbench. Reagents should not be shared among various workers as far as possible, and all reagents should be aliquoted, so that if one aliquot gets contaminated, you can throw it away and use another.